Changes in Aqueous Immunoglobulin and Albumin Levels following Penetrating Keratoplasty

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The feline model of induced rejection of corneal allografts was employed to define the changes in the concentrations of immunoglobulins and albumin in the anterior chamber prior to, and concomitant with, the rejection of the transplanted cornea. Fourteen animals received unilateral exchange corneal allografts. Aqueous humor obtained by anterior chamber paracentesis at regular intervals prior to and following the performance of the penetrating keratoplasties was analyzed for IgG, IgM and albumin concentrations using the micro enzyme-linked immunosorbant assay (ELISA). Two patterns of anterior chamber protein modulation were observed. Eight of the animals demonstrated a biphasic pattern in which both immunoglobulin and albumin concentrations were elevated two- to five-fold above presurgical values 14 days postkeratoplasty, returning to preoperative values by day 42. Three to 5 weeks after corneal rejection was induced increases in protein concentrations were observed that correlated with the appearance of clinical signs of rejection. A second, monophasic pattern of anterior chamber protein modulation following keratoplasty was observed in four of the animals. It was distinguishable from the biphasic pattern in that levels did not return to baseline values after the initial rise following keratoplasty until the rejection process was completed. The monophasic response was found to be characteristic of more rapid and vigorous corneal rejection. Examination of albumin to immunoglobulin ratios suggested that all changes in protein levels following keratoplasty were a result of increased influx of serum proteins into the anterior chamber, rather than due to local immunoglobulin synthesis.


A sizeable body of evidence indicates that the participation of cell-mediated immunity is required for corneal allograft rejection.1-8 The humoral immune response to corneal allografts, while not as well characterized,9-16 also may participate in corneal allograft rejection. However, relatively few studies exist where quantitative changes in immunoglobulin levels, either local (anterior chamber) or systemic, have been reported following cornea transplantation and during a rejection episode.1,17-26

This study was undertaken with the aim of monitoring changes in immunoglobulin levels in feline aqueous humor following penetrating keratoplasty and subsequent induced corneal allograft rejection. We specifically wished to determine: (1) normal concentrations of immunoglobulins M (IgM) and G (IgG) and albumin in the aqueous humor using modern microanalytic methods; (2) the quantitative changes in immunoglobulin and albumin concentrations that occurred following corneal allografting and induced allograft rejection; and, based on the above, (3) to determine whether there was evidence for the local synthesis of immunoglobulins consequent to corneal allografting or during rejection.

We selected the feline model of corneal transplantation for our studies because the feline cornea has a large surface area, a relatively ample volume of aqueous humor, and a corneal endothelium that exhibits limited mitotic potential,27 the latter feature being similar to the regeneration potential of human corneal endothelium.28-38 In addition, cats exhibit corneal endothelial cell loss following allografting similar to that reported in human transplantation,39-42 as well as demonstrating a similar frequency of spontaneous allograft rejection.43-46 In this model, corneal graft rejection can be reliably induced by the application of a full thickness skin graft from the cornea donor.41 The process of graft rejection is characterized morphologically by a cellular inflammatory reaction directed against the donor endothelium.45

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Supported by research grant EY-03573 from the National Eye Institute, Bethesda, Maryland.

Submitted for publication: February 19, 1988; accepted August 9, 1988.

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Materials and Methods

Animals

Adult cats were purchased from a licensed, local distributor. The general health, housing, and maintenance of the animals were supervised by The University of Michigan Unit for Laboratory Animal Medicine (ULAM). Animals exhibiting any signs symptomatic of external ocular infection or irritation were excluded from the study. Upon arrival, oral tetracycline, intravenous fluids and distemper vaccine were administered to all animals as needed. The provisions of the ARVO Resolution on the Use of Animals in Research were followed.

Experimental Protocol

Fifteen penetrating keratoplasties were performed on 15 outbred adult cats. Fourteen animals received unilateral exchange corneal allografts. One animal received a unilateral rotational autograft that served as a control for the surgical procedure. Two additional unoperated animals functioned as controls. The unoperated eye in each animal receiving a unilateral penetrating keratoplasty served as a control for local (anterior chamber) inflammatory events possibly associated with paracentesis, the keratoplasty procedure, or the induced corneal allograft rejection.

Blood and aqueous humor samples were obtained from each animal prior to surgery in order to establish immunologic and hematologic baselines. Thereafter, postsurgical samples were obtained on days 14, 28, 42, and then weekly commencing with day 49 through day 77 (Fig. 1).

Rejection of exchanged corneal allografts between paired animals was induced by performing reciprocal, full-thickness skin grafts four weeks (day 28) following cornea transplantation.

Penetrating Keratoplasty

Details of the penetrating keratoplasty procedure have been reported previously.40,45 Briefly, transplant recipients were fasted for 12 hr prior to performing penetrating keratoplasty. Atropine sulfate (0.04 mg/kg) was administered subcutaneously 30 min before surgery in order to reduce ocular secretions and to dilate the pupil. Anesthesia was induced by the intramuscular administration of xylazine (1 mg/kg), followed 10 min later by an intramuscular injection of ketamine hydrochloride (5–8 mg/kg). Additional ketamine was administered during the operative procedure to maintain an adequate anesthesia level.

Surgery was performed using aseptic technique, standard microsurgical instruments and a surgical microscope. Two surgeons performed simultaneous exchange corneal allografts between paired animals (average operating time of 60 min per eye). All exchange allograft and rotational autograft buttons were 8.0 mm in diameter. Grafts were sutured into an 8.0 mm diameter bed using interrupted, alternating 10-0 and 9-0 nylon suture material. The rotational autograft button was excised, rotated 180°, and resutured. Ophthalmic antibiotic ointment (bacitracin) and atropine (1%) were applied to the operated eye. Two hundred and fifty microliters of cephalosporin (50 mg in water) was injected into the nictitating membrane at the conclusion of the keratoplasty procedure.

Postoperative Care

Upon completion of surgery, animals were placed in protective cages during recovery from anesthesia and maintained under close observation. In order to avoid irritation during the postoperative period (first week), no topical medications were administered. However, the animals did receive chloramphenicol (100 mg/day) in their drinking water during the first week. Systemic and topical antibiotics administered both at the time of surgery and after weekly examinations effectively prevented corneal and other external ocular infections. At the conclusion of the study, animals were euthanized by the administration of an overdose of intravenous pentobarbital.

Induction of Corneal Allograft Rejection

Corneal allograft rejection was induced by performing reciprocal, 3.0 × 3.0 cm, full-thickness skin grafts between paired cornea donors 4 weeks following cornea transplantation. The grafts were positioned onto a graft bed prepared on the upper back and sutured into place using discontinuous 5-0 silk sutures. Animals received oral chloramycetin (100 mg twice a day) commencing 1 day preoperatively and continuing for 5 days subsequent to skin grafting.

[EXPERIMENTAL TIMELINE]

**Fig. 1.** Experimental timeline.
Postoperative Clinical Evaluation

The operated eye of each animal received a daily penlight examination to assess graft position, depth of the anterior chamber, gross clarity of the host and graft cornea, and evidence of ocular inflammation. Further examination of questionable grafts was accomplished while the animal was under anesthesia. At the conclusion of each weekly examination, animals received Garamycin® (20 mg; Schering, Kenilworth, NJ) intramuscularly and bacitracin ointment topically.

Anterior Chamber Paracentesis

The anterior chamber of the feline eye contains approximately 2.2–2.5 ml of aqueous humor. The periodic withdrawal of 0.8–1.0 ml samples occasioned no untoward effects on either the eye or the animal. The anterior chamber was fully restored within 1–1.5 hr of the sampling. Aqueous humor from both eyes of all animals was obtained biweekly for 6 weeks following keratoplasty and then weekly through the conclusion of the experiment. No signs of inflammation were observed that could be attributed to paracentesis. Corneal endothelial cell counts (central cornea) did not change as a consequence of four paracenteses (data not shown).

Working with the surgical microscope, the limbal conjunctiva was first grasped with fine-toothed forceps, and the anterior chamber then entered with a 30-gauge needle on a tuberculin syringe. The syringe contained 0.05 ml heparin (1000 units/ml in balanced salt solution) to prevent fibrin clots. The aqueous humor was gently aspirated. Bacitracin antibiotic ointment was applied to each eye at the conclusion of the procedure. Samples were then centrifuged at 15,000 g in a microcentrifuge for 2 min to remove host inflammatory cells, coded, and stored at −70°C for later study.

Blood Collection

Samples of blood for hematologic (2 ml) and immunoglobulin (5 ml) determinations were obtained from the external jugular veins of all animals concurrent with the aqueous humor sampling (Fig. 1). Anesthetized animals were positioned with the neck hyperextended. The hair over the external jugular vein was shaved, and the skin was moistened with 70% ethanol. After the vein was distended by application of digital pressure at the sternal notch, the vein was carefully entered with a 21-gauge butterfly catheter. Samples for immunoglobulin determinations were collected in sterile red-top tubes (Vacutainer, Becton-Dickinson, Rutherford, NJ) and allowed to clot at room temperature for 30 min. Hematologic evaluations (hematocrits, WBC counts and WBC differential counts) were performed with blood collected in sterile tubes containing EDTA (Vacutainer). Sera for immunologic analyses were aliquoted, coded, and frozen at −70°C. The clinical status of the animals was unknown to the laboratory performing the assays.

Immunoglobulin Quantitation

Immunoglobulin (IgG, IgM) and albumin concentrations in the sera and aqueous humor were determined by use of the micro enzyme-linked immunosorbent assay (microELISA). Wells of flat-bottom polystyrene microtiter plates (NUNC Immunoplate II, GIBCO, Grand Island, NY) were sensitized with 0.1 ml of heavy chain-specific, affinity-purified antibodies against feline IgG, IgM, or albumin (Pel-Freeze, Rogers, AK, or Zymed, South San Diego, CA) diluted to 5 μg/ml in 0.05 M sodium carbonate coating buffer (pH 9.6) containing 0.02% sodium azide. The plates were sealed and incubated for three hours at 37°C in a closed chamber with high humidity. The plates were then washed five times with phosphate-buffered saline containing 0.05% polyoxyethylene (20)-sorbitan monolaurate (Tween-20). Sera, aqueous humor, and standards were diluted in PBS-Tween, and 0.1 ml of each dilution was added, in duplicate, to sensitized wells. Following a 3 hr incubation at room temperature, the plates were again washed as described. Alkaline phosphatase, conjugated to heavy chain-specific, rabbit anti-feline immunoglobulin was diluted 1:1000 in PBS-Tween, and 0.1 ml was added to each well. After incubation at room temperature for 16 to 20 hr, the plates were washed four times with PBS-Tween, once with substrate buffer, and 0.1 ml of alkaline phosphatase substrate (Sigma 104, Sigma Chemical Co., St. Louis, MO) at 1 mg/ml was added to all the wells. After 20 to 45 min at room temperature the absorbance was measured with a Titertek Multiskan spectrophotometer (Flow Laboratories, McLean, VA).

The concentration of protein (IgG, IgM or albumin) in each test sample was calculated by comparing experimental values to absorbance curves generated with immunoglobulin or albumin standards. Feline immunoglobulin standards for use in the micro-ELISAs were prepared from pooled normal cat sera by precipitation of nonimmunoglobulin proteins with caprylic acid. Subsequent immunoglobulin fractionation and purification was performed by use of DEAE-Sepharose column chromatography. Purity
of each immunoglobulin standard was verified by SDS polyacrylamide gel electrophoresis. Serum albumin (fraction V) was isolated from pooled feline serum by the ethanol precipitation method.48

Statistical Analysis

The significance of the changes in protein concentrations at successive timepoints were assessed by analysis of variance using the Scheffe analysis. The significance of changes between the treated and untreated eyes at each time point was assessed by paired t-tests.

Results

Clinical Progression of Feline Corneal Allograft Rejection

Penetrating keratoplasty was performed successfully and without complications in all but two animals. Two corneal allografts exhibited wound dehiscence following suture removal. Both dehiscent grafts rapidly became opaque and were excluded from the study. The successful corneal grafts remained thin and clear for at least 6 weeks, after which inflammatory manifestations of induced cornea rejection became apparent. Moderate inflammation (2+ cells and flare) was noted in the anterior chambers of all operated eyes 14 and 28 days following keratoplasty. In 13 of 14 eyes, deep stromal vessels had reached the graft margin by the 28th day, while one eye never developed cornea vascularization, even after induced allograft rejection.

The onset of skin graft-induced corneal allograft rejection, commencing 14 to 28 days after skin grafting (day 42 to 56 post-keratoplasty), was characterized by the appearance of evenly distributed, fine keratic precipitates over the posterior surface of the graft. The keratic precipitates became progressively larger and more numerous until they were almost confluent covering the endothelial surface. Occasional small keratic precipitates were present on the host endothelium immediately adjacent to the wound margin in eyes where corneal grafts were nearly covered by keratic precipitates. However, the host cornea never exhibited signs of endothelial decapsulation. Increased cornea thickness and stromal opacity usually did not occur until after the graft corneal endothelium had become extensively decorated by keratic precipitates. This was most frequently observed to occur 1 to 3 weeks after the onset of rejection. The induced corneal allograft rejection progressed until the transplant exhibited stromal edema, graft opacity and bullous keratopathy. However, the grafts did not degrade further, and the eyes exhibited minimal signs of intraocular or extraocular inflammation. The specificity of the host response to the corneal allograft was conspicuous. The rejected allograft appeared as an opaque disc surrounded by the thin and clear host cornea (Fig. 2A, B). During rejection, external ocular erythema was mild, and the aqueous humor reaction was characterized by mild to moderate cell and flare.

Contralateral, unoperated eyes remained free of signs of anterior chamber inflammation throughout the course of the experiment. No aqueous cell or flare was apparent, even while a severe rejection process was occurring in the opposite eye.

There were no recognizable differences between experimental or control animals with regard to WBC total and differential counts. No evidence of anemia resulting from the blood sampling regimen was observed.

Fig. 2. (A) Photograph of a rejected corneal graft induced by skin grafting. The failed graft is opaque, while the host cornea between the graft margin and limbus (arrows) is thin and clear. (B) Lateral view of a failed corneal allograft. The host reaction has specifically affected the graft, while the remainder of the cornea (indicated by the bar) between the graft margin and limbus (arrow) is clear. One of a number of small blood vessels traversing the cornea to the graft is indicated by the small arrow.
Table 1. Concentrations of IgG, IgM and albumin in normal feline aqueous humor and serum compared to reported human values*

<table>
<thead>
<tr>
<th></th>
<th>IgG (μg/ml)</th>
<th>IgM (μg/ml)</th>
<th>Albumin (μg/ml)</th>
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</thead>
<tbody>
<tr>
<td><strong>Aqueous humor</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feline</td>
<td>53.5 ± 3.3</td>
<td>2.8 ± 0.2</td>
<td>314.2 ± 16.4</td>
</tr>
<tr>
<td></td>
<td>(23.6-128.4)</td>
<td>(1.1-5.1)</td>
<td>(177.8-578.8)</td>
</tr>
<tr>
<td>Human</td>
<td>70.024</td>
<td>0.0</td>
<td>290</td>
</tr>
<tr>
<td></td>
<td>23.023</td>
<td>0.0</td>
<td>—</td>
</tr>
<tr>
<td><strong>Serum</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feline</td>
<td>36,400 ± 1600</td>
<td>3397 ± 1500</td>
<td>50,871 ± 5682</td>
</tr>
<tr>
<td></td>
<td>(21,700-53,400)</td>
<td>(1840-4880)</td>
<td>(28,000-152,400)</td>
</tr>
<tr>
<td>Human</td>
<td>907024</td>
<td>7,100</td>
<td>18,380</td>
</tr>
<tr>
<td></td>
<td>14,75024</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>[Aq. humor]/serum ×100</td>
<td>0.15</td>
<td>0.08</td>
<td>0.62</td>
</tr>
<tr>
<td>Feline</td>
<td>0.77</td>
<td>—</td>
<td>1.58</td>
</tr>
<tr>
<td>Human</td>
<td>0.16</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* Human values determined by radial immunodiffusion.  † Range of values observed in parentheses.

Normal Levels of Immunoglobulin and Albumin in the Cat

Feline aqueous humor and serum, removed from control animals and taken from presurgical samples from previous studies, provided assay material for establishing baseline information concerning the content of immunoglobulins and albumin of the sera and aqueous humor. Measurement of albumin levels in the aqueous humor provided a means for gauging whether any observed fluctuations in immunoglobulin levels reflected local (anterior chamber) response to the antigenic challenge of the allograft, or rather, represented an influx of systemic serum components induced by surgical trauma and nonspecific inflammation.

Aqueous humor obtained from 40 eyes was assayed and mean values for IgG, IgM and albumin were determined. As shown in Table 1, IgG was present in the aqueous humor at a concentration of 53.5 μg/ml and in significantly higher quantities in the sera (36.4 mg/ml). IgM content was 2.8 μg/ml and 3.4 mg/ml in the aqueous humor and sera, respectively. The IgM value was ≤10% of that for IgG in both serum and aqueous. Based on the general relationship [aqueous]/[serum] × 100, a greater proportion of albumin was present in the aqueous (0.62%) than either of the immunoglobulins. This is probably due to its relatively small molecular size, allowing it to permeate the blood-aqueous barrier with greater ease than the immunoglobulins. It was the dominant protein, at 314 μg/ml in the aqueous and 50.9 mg/ml in the sera.

Immunologic Features of Feline Aqueous Humor during the Course of Allograft Rejection

Two distinct patterns of immunoglobulin-albumin changes following penetrating keratoplasty were observed. The first, biphasic patterns, observed in eight of the 12 animals that received sequential corneal and dermal allografts are shown in Figure 3A–C. As shown, both immunoglobulin and albumin concentrations of the operated eyes were elevated two- to five-fold above presurgical values 14 days postkeratoplasty. By day 42, immunoglobulin and albumin concentrations returned to near preoperative levels. Three to 5 weeks following skin grafting (days 49–63 of the experiment) concurrent increases in the concentrations of immunoglobulin and albumin were observed that correlated with the appearance of clinical signs of corneal graft rejection. Levels declined once again toward baseline values as the rejection process was completed.

A second pattern of postkeratoplasty modulation of immunoglobulin and albumin levels, observed in four of the allografted eyes, was monophasic in character (shown in Fig. 4A–C). As found in the biphasic pattern, the immunoglobulin and albumin concentrations of the allografted eyes increased two- to five-fold by day 14. However, the concentrations of these proteins remained elevated with little or no shift back to baseline levels. After skin grafting, immunoglobulin and albumin concentrations continued to be elevated up to 42–49 days after keratoplasty. Thereafter, all protein levels began returning toward baseline levels. A review of clinical observations of these four
Aqueous Humor IgM Levels Following PK (Biphasic Pattern)

A

![Graph showing IgM levels following PK with significant differences indicated.]

Aqueous Humor Albumin Levels Following PK (Biphasic Pattern)

C

![Graph showing albumin levels following PK with significant differences indicated.]

Aqueous Humor IgG Levels Following PK (Biphasic Pattern)

B

![Graph showing IgG levels following PK with significant differences indicated.]

Fig. 3. The "biphasic" modulation of the concentrations of IgM (A), IgG (B) and albumin (C) in the aqueous humor following the performance of penetrating keratoplasty (day 0). Reciprocal exchange skin grafts were performed at day 28. In all cases, the penetrating keratoplasty was performed on the right eye, and the left eye served as the control for nonspecific effects of the procedures. This figure indicates the mean (±SEM) responses of eight animals. The box below the graph indicates significant changes (95% confidence level) between intervals (horizontal arrows) and between treated and untreated eyes at each interval (vertical arrows).
Aqueous Humor IgM Levels Following PK (Monophasic Pattern)

Fig. 4A.

Aqueous Humor Albumin Levels Following PK (Monophasic Pattern)

Fig. 4C.

Aqueous Humor IgG Levels Following PK (Monophasic Pattern)

Fig. 4B.

Fig. 4. The "monophasic" modulation of the concentrations of IgM (A), IgG (B) and albumin (C) in the aqueous humor following the performance of penetrating keratoplasty (day 0). Reciprocal exchange skin grafts were performed at day 28. In all cases, the penetrating keratoplasty was performed on the right eye, and the left eye served as the control for nonspecific effects of the procedures. This figure indicates the mean (±SEM) responses of four animals. The box below the graph indicates significant changes (95% confidence level) between intervals (horizontal arrows) and between treated and untreated eyes at each interval (vertical arrows).
eyes revealed that the onset of graft rejection, as indicated by anterior chamber inflammation and keratic precipitates on the graft endothelium, occurred more rapidly, subsequent to skin graft exchange, than it did in eyes with the other (biphasic) pattern (Table 2). Within 14 days of skin grafting, the four “monophasic” eyes demonstrated clinical signs of developing corneal allograft rejection, progressing over the following weeks to graft failure with edema. Clinically, there was only a brief period (prior to skin grafting) when the eyes were free of inflammation. The levels of immunoglobulins and albumin in the aqueous humor remained elevated until the rejection process was completed and the inflammation had resolved. As shown in Table 2, the monophasic response represented a more rapid rejection of the transplant than was characterized by the biphasic pattern.

The unoperated eyes of all animals exhibited no signs of inflammation (most cat eyes have some cells in the aqueous [1+], but no other signs of inflammation). Aqueous humor from the rotational autograft contained elevated immunoglobulin and albumin concentrations by day 14, postkeratoplasty. Minor oscillations in concentration occurred thereafter. As seen in Figure 5, while the IgM levels in the aqueous humor followed a pattern quite similar to both the mono- and biphasic responses, the IgG and albumin changes were quite meager in comparison.

Finally, the ratio of IgG to albumin and IgM to albumin was examined throughout the course of these studies. There were no significant changes in the proportions of either IgG to albumin or IgM to albumin, thus indicating that the elevated levels of IgG and IgM were derived from the sera, rather than from local synthesis of specific antibody (data not shown). Additional support of this conclusion, aqueous and serum samples were obtained by examining the sera and aqueous humor for specific antibodies using a micro ELISA sensitized with feline corneal endothelial cells. In no instance could antibodies be detected (data not shown).

### Discussion

Corneal allografts were reliably induced to reject by performing reciprocal, full-thickness skin grafts 4 weeks after performance of penetrating keratoplasty. Within 14 to 28 days after skin grafting, a mild aqueous cell and flare reaction was observed. This was accompanied by the formation of keratic precipitates on the donor endothelium. Seven to 14 days following the onset of rejection (28–35 days post skin graft), the allograft endothelium became heavily studded with keratic precipitates. Stromal thickening and opacification began, due to stromal hydration, the result of endothelial cell failure. While rejection events progressed in the grafted eye, the contralateral,

### Aqueous Humor Levels (Rotational Autograft)

![Fig. 5. The modulation of the concentrations of IgM (top panel), IgG (middle panel) and albumin (bottom panel) in the aqueous humor following the performance of a rotational autograft (Day 0). The autograft was performed on the right eye (OD, open box), and the left eye (OS, black diamond) served as the control for nonspecific effects of the procedure. This figure indicates the response of a single animal.](image-url)
unoperated eye remained free of clinical signs of inflammation. Findings in this study closely agree with the clinical course of induced allograft rejection reported in our previous studies.\(^{40,45}\) In addition, the rejection process of this model correlates well with those described for rabbit and human corneal allograft failure,\(^{46-51}\) however, the precise mode of endothelial cell destruction and its relation to the host cellular immune response still remains to be clarified.\(^{52-56}\)

The establishment of normal baseline values for immunoglobulins (IgG, IgM) in both serum and aqueous humor provided a working picture of the ranges of concentrations that could be expected during the initial stages of the experiment. From these data, various indices were established, based on ratios existing between the two immunoglobulins and between the immunoglobulins and albumin, a ubiquitous, nonimmune protein. A significant departure from these values during the experiment would have been suggestive of local immunoglobulin class-specific synthesis. Thus, albumin served as the key indicator for gauging whether increases in immunoglobulin concentrations in the anterior chamber were due to local synthesis, or represented influxes of immunoglobulin from outside the eye, presumably entering the eye from the circulatory system. The latter proved to be the case, since the ratio of IgG/albumin and IgM/albumin remained relatively constant throughout the study.

Two major points stand out in surveying the temporal data. First, there was an initial two- to five-fold increase in immunoglobulins (IgG, IgM) recorded in the aqueous humor of all animals immediately after surgery. This peak represented the nonspecific (ie, nonimmune) inflammation associated with penetrating keratoplasty. Its inflammatory origin is characterized by the sharp rise in albumin levels concurrent with those of IgG and IgM, a finding indicative of increased vascular permeability. As further corroborated that this initial rise is an effect of surgery, this pattern also held for the surgical control animal, which received a rotational autograft.

Second, the induction of cornea rejection set up inflammatory events characterized by two distinct patterns of changing immunoglobulin and albumin levels in the aqueous humor of cornea recipients, but not in the surgical control. The “monophasic” pattern was distinguishable by a sustained elevation of protein levels, including albumin, until the graft failed. The “biphasic” pattern presented an aqueous profile marked by a brief respite from the inflammatory effects of induced cornea rejection. The monophasic modulation pattern appeared to be associated with both an earlier onset of rejection and a more vigorous rejection episode. There was no evidence of class-specific immunoglobulin response to corneal allograft rejection observed.

This study demonstrates for the first time the temporal effects of penetrating keratoplasty on the anterior chamber immunoglobulin content. These changes occur independent of detectable antibodies reactive with donor cells, suggesting that antibodies do not play a major role in the primary rejection of transplanted corneas. Of equal importance is the observation that unoperated, contralateral eyes maintain a normal homeostatic environment, free of sympathizing effects produced by either penetrating keratoplasty or the process of corneal allograft rejection.

Key words: immunoglobulin, albumin, penetrating keratoplasty, aqueous humor, feline

Acknowledgments

We are indebted to Barbara J. Robinson for both her assistance during surgical procedures and maintenance of the animals before and after surgery. We are grateful for the expert technical assistance of Heidi Dare. We also extend thanks to Csaba L. Martonyi for performing the specular photography.

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