Corneal Transplantation in Antibody-Deficient Hosts

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it is likely that T lymphocytes are involved in the induction of Behçet's disease. The activated T cells may release MIF in the early stage of Behçet's disease. It is thought that MIF is an inflammatory cytokine that induces the release of other cytokines. Indeed, tumor necrosis factor α, interferon-γ, IL-10, IL-12, and tumor necrosis factor receptor, which are involved in various inflammatory processes, were detected at high levels in Behçet's disease. It seems to us that the elevation of the MIF level in the sera is involved in the induction of various inflammatory symptoms in Behçet's disease including uveitis.

Acknowledgments

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References


Corneal Transplantation in Antibody-Deficient Hosts

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Purpose. To examine the role of donor-specific antibodies, with or without complement, in rejection of orthotopic corneal transplants by using mice as recipients in which the genes for the heavy chain of immunoglobulin or the third complement component have been eliminated by homologous recombination.

Methods. BALB/c corneas were transplanted into eyes of B-cell-deficient (n = 17) or wild-type control C57BL/6 (n = 30) mice and into eyes of complement (C3)-deficient (n = 15) or wild-type control 129-C57BL/6 (n = 13) mice. After surgery all grafts were evaluated over 8 weeks in a masked manner by biomicroscopy for signs of rejection.

Results. The rates of corneal transplant rejection were similar among B-cell-deficient and C3-deficient mice compared with rejection rates in their respective wild-type control subjects. This similarity applied to the time course of rejection and to cumulative survival rates.


Evidence that antibody and complement can contribute to allograft rejection has emerged from several recent studies of solid organ transplantation in laboratory animals. In rats immunosuppressed with cyclosporin-A or anti-CD4 monoclonal antibodies, the passive transfer of immune serum causes acute rejection of renal and cardiac allografts. Similar, repeated transfer of immune serum to severe combined immunodeficiency mice provoked chronic vascular lesions in cardiac allografts that were characterized by antibody and complement deposition and macrophage infiltration. Finally, rats deficient in complement component C6 rejected cardiac allografts 2 weeks later than did wild-type rats.

Despite this evidence in solid organ transplantation, the role for antibody and complement in the rejection of corneal allografts remains unclear. Cornea-specific and donor-specific antibodies have been detected in host serum after clinical⁶ and experimental⁷ corneal grafting, but it is not certain whether these antibodies contribute to graft rejection. Hutchinson et al.⁸ have reported that although donor-specific serum alloantibodies are evoked by orthotopic corneal transplants, the antibodies are not detected until after the grafts have been rejected, and recently published data in Hedge et al.⁹ indicate that passive transfer of donor-specific antibodies fails to cause
corneal allograft rejection in mice. However, keratoplastics performed in high-risk eyes of humans have been reported to evoke donor-specific HLA class I antibodies, which have been found to be associated with graft rejection.\(^6\)

In an effort to contribute to resolution of the question of whether antibodies, or complement, or both contribute to corneal allograft rejection, we examined the survival of allogeneic corneas transplanted orthotopically into naive mice that were genetically deficient in B cells or the third complement component (C3). The findings support the view that neither antibody nor complement contributes to acute corneal graft rejection.

**METHODS**

Six- to 10-week-old BALB/c (H-2^k^) mice (Taconic Farms, Germantown, NY) were used as donors. Recipients consisted of same-age C57BL/6 (H-2^b^) and B-cell-deficient C57BL/6Igh-6 mice (Jackson Laboratory, Bar Harbor, ME). All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Each animal was deeply anesthetized with an intramuscular injection of 3 mg to 4 mg ketamine and 0.1 mg xylazine before all surgical procedures.

B-cell-deficient mice are unable to mount antibody responses as a result of a targeted disruption of the membrane exon of the immunoglobulin μ chain gene.\(^7\) B-cell development is arrested at the stage of pre-B-cell maturation. As a consequence, mature B cells, IgM- or IgD-expressing cells, or serum IgM are not detected in these mice. The ability of wild-type C57BL/6 mice to produce antibodies against donor antigens was examined by testing serum from C57BL/6 mice that had rejected BALB/c corneas in a complement-mediated cytotoxicity assay\(^10\) with BALB/c lymphocytes as target cells. In addition, same-age C3-deficient and wild-type 129-C57BL/6 (H-2^k^) mice served as recipients. These mice were constructed by homologous recombination in embryonic stem cells.\(^11\) The C3-deficient mice have no detectable C3 protein in their serum, analyzed by enzyme-linked immunosorbent assay, and have no functional activity in a C3 hemolytic assay.\(^11\)

Corneal transplantation was performed as described previously.\(^12\) Seventy-seven corneal transplants were performed, of which 75 were deemed technically acceptable for long-term follow-up (i.e., anterior segment integrity was maintained with no signs of wound leak, infection, or hyphema). All 75 transplants confronted recipients with a full range of both major and minor histocompatibility antigens: Corneas from BALB/c donors were grafted into normal C57BL/6 (n = 30), B-cell-deficient C57BL/6Igh-6 (n = 17), normal 129-C57BL/6 (n = 13), and C3-deficient 129-C57BL/6 (n = 15) eyes. Graft sutures were removed in all cases on day 7.

Transplants were evaluated by slitlamp biomicroscopy twice weekly over 8 weeks. At each time point, grafts were scored for opacification. A previously described scoring system\(^12\) was used to measure the degree of graft opacification between 0 and 5 (+), clear and compact graft; 1+, minimal superficial opacity; 2+, mild deep [stromal] opacity with pupil margin and iris details visible; 3+, moderate stromal opacity with anterior chamber visible; 4+, intense stromal opacity with total obscuration of the anterior chamber. Grafts with an opacity score of 2+ or higher after 3 weeks were considered to be rejected (immunologic failure); grafts with an opacity score of 3+ or higher at 2 weeks that never cleared were also regarded as rejected. Grafts with an opacity score of 2+ or higher at any time point after 2 weeks were considered to have a rejection reaction, regardless of the opacity score at 8 weeks. We constructed Kaplan-Meier survival curves to compare the probability of overall graft survival over the follow-up period in all groups studied.

**RESULTS**

Seventy-five transplants were performed in the various crosses described above. Corneas from BALB/c donors were grafted...
In line with recent reports from Hutchinson et al. and Hedge et al., the results of our current studies lead us to conclude that neither antibody nor complement plays a significant role in mediating rejection of allogeneic corneas grafted into low-risk healthy eyes of naive mice. Although wild-type animals were able to mount antibody responses against donor antigens as could be seen in C57BL/6 mice which had rejected their cornea, animals that were deficient in B cells or the complement C3 component (and therefore both complement pathways) rejected corneal grafts in a manner similar to their respective wild-type control subjects. In fact, the rejection of corneal allografts in recipients devoid of B-cell or complement activity was, if anything, marginally swifter than that observed among their respective wild-type control subjects.

We are intrigued by the inability to find a role for antibody and complement in rodent model systems, because several studies in humans have, at least indirectly, implicated donor-specific antibodies in corneal allograft failure. In clinical settings, however, corneal grafting often occurs in vascularized (high risk) eyes, which may allow antibody and complement to cause damage to corneal grafts that are normally avascular and thus are relatively resistant to antibody- and complement-mediated damage. To that end, posttransplant antibodies directed against donor class I HLA antigens in high-risk patients have been associated with graft rejection. In addition, Hahn et al. reported that corneal grafts fare more poorly when transplanted into patients with preformed anti-class I HLA antibodies as a consequence of previous corneal transplants or blood transfusions. These reports raise two important issues that we intend to explore experimentally. First, the human studies suggest that antibodies may contribute to graft failure if the recipient has been sensitized previously to donor antigens. Second, grafts placed in high-risk eyes may be more susceptible to antibody-mediated rejection than grafts placed in low-risk (normal mouse) eyes. Because our study did not involve grafts placed in experimental “high-risk” eyes of mice with recipient bed vascularization, nor did it involve hosts pre sensitized to donor alloantigens, we cannot conclude that antibody and complement play no role in all forms of corneal graft rejection. Finally, we are mindful that in clinical practice (unlike our rodent model systems) local or systemic immunosuppressive pharmacotherapy is routinely used. It is altogether possible that these therapies may undermine cellular immune responses so that antibody-mediated responses may act at later stages in a more chronic rejection reaction.

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References


Cultured Bovine Corneal Epithelial Cells Express a Functional Aquaporin Water Channel

Fengying Kang,1 Kunyan Kuang,1 Jun Li,1 and Jorge Fischbarg1,2

Purpose. Given recent physiological and in situ hybridization evidence for the presence of a water channel in corneal epithelium, this study was conducted to investigate its expression and characteristics using cultured bovine corneal epithelial cells (CBCEPCs).

Methods. CBCEPCs were grown in DMEM containing 2 ng/ml fibroblast growth factor and 6% fetal bovine serum. To determine their osmotic permeability \( (P_f) \), cells were passaged onto rectangular glass coverslips, and anisotonically induced volume changes were monitored by light scattering. To investigate expression, poly(A+) RNA from CBCEPCs was injected into Xenopus laevis oocytes, and the \( P_f \) of the oocytes was determined.

Results. For CBCEPCs challenged with a 10% hypotonic solution at 37°C, the kinetic constant of volume change was \( k = 0.52 \pm 0.04 \) seconds\(^{-1}\), and the calculated \( P_f \) was 72 ± 6 \( \mu \)m/sec (n = 16). The \( P_f \) of oocytes injected with water was 14 ± 1.8 \( \mu \)m/sec \( (n = 4) \); injection with poly(A+) RNA from CBCEPCs increased \( P_f \) to 77 ± 6 \( \mu \)m/sec \( (n = 6) \). This increase in \( P_f \) was inhibited by 72% (reduced to 22 ± 1 \( \mu \)m/sec) by 0.3 mM HgCl\(_2\) and inhibited by 56% to 58% by co-injection with aquaporin (AQP)5 antisense oligonucleotide.

Conclusions. The comparatively high \( P_f \) determined for CBCEPCs, the presence of mRNA encoding water channels, and sensitivity to mercurial agents are typical of the expression of functional water channels. The predominant message is for AQP5, although the evidence was consistent with the presence of additional water channels. These findings bring renewed support for the notion that the epithelium can contribute to corneal hydration homeostasis.

Although the barrier function of the stratified corneal epithelium is well established, the epithelial role in the regulation of stromal hydration has remained controversial. Still, this epithelium has been recognized to have high osmotic permeability. In addition, corneal epithelial transport has been linked to corneal hydration homeostasis, and isolated reports have appeared showing evidence of some fluid transport by corneal epithelium in rabbit and frog preparations. The subject gained in clarity more recently when it became known that chloride activates water permeability in the frog corneal epithelium, the mRNA of frog corneal epithelium encodes a water channel, and the water channel AQP5 was located in rat corneal epithelium by hybridization immunohistochemistry. We have investigated this matter further using cultured bovine corneal epithelial cells (CBCEPCs). We have confirmed the presence of AQP5 in corneal epithelium and have determined that it is functional and that its message seems to be the predominant one for these cells.

Materials and Methods

Cultured Bovine Corneal Epithelial Cells

The primary culture methods used were established in Cubitt et al. Bovine corneas were isolated, and the endothelium along with part of the stroma was removed by peeling. The resultant preparation was incubated with neutral protease (Dispase II; Gibco, Grand Island, NY) at 25 units/ml at 37°C for 40 minutes to separate the epithelium from its basement membrane, after which Dulbecco’s modified Eagle’s medium containing 6% fetal bovine serum (Atlanta Biologics,