Effect of Alloantibodies on Corneal Allograft Survival

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PURPOSE. The precise role of antibodies in corneal transplantation is ambiguous, with evidence to support as well as repudiate their involvement in graft rejection. Accordingly, this study was undertaken to investigate the direct contribution of donor-specific antibodies to corneal graft rejection.

METHODS. Serum samples from CB6F1 rejectors of orthotopically grafted C3H/Hej corneas were tested by ELISA for elevated levels of donor-specific alloantibody. Orthotopic corneal allograft rejection was also examined in B-cell-deficient mice. In a prospective study, naive BALB/c T-cell-deficient nude mice and complement-depleted nude mice were passively infused with immune donor-specific serum and grafted with fully allogeneic C57BL/6j corneas. The incidence and speed of graft rejection were observed in each case. The susceptibility of corneal cells to antibody-mediated lysis was tested in vitro.

RESULTS. Seventy percent of the CB6F1 hosts that rejected the C3H/Hej corneal allografts possessed significantly elevated levels of alloantibody in serum. Although BALB/c corneal allografts were rejected by B-cell-deficient mice at the same incidence as wild-type control mice, their mean survival time (MST) was significantly longer than that of their wild-type counterparts. Serum of BALB/c mice immunized against C57BL/6j alloantigens produced complement-dependent cytolytic activity against C57BL/6j corneal cells in vitro. Passive transfer of this alloantiserum to T-cell-deficient BALB/c nude mice produced complement-dependent corneal lesions, resulting in significantly increased opacity of C57BL/6j corneal grafts, compared with the relatively clear grafts in control hosts.

CONCLUSIONS. Alloantibody, although not necessary for corneal graft rejection, can produce extensive injury to corneal allografts in a complement-dependent manner. (Invest Ophthalmol Vis Sci. 2002;43:1012–1018)

Since the first successful human corneal transplant was performed in 1905, the cornea has attained the status of the commonly transplanted human tissue or organ, with more than 40,000 keratoplasties performed each year in the United States alone.1 HLA typing is not performed routinely, and systemic immunosuppression is not used, except in the case of high-risk individuals who have either received a previous corneal transplant or who have prevascularized graft beds. Immunosuppression is normally restricted to topical steroid treatment, which is administered after surgery. It is rather remarkable that typical 2-year survival rates for initial grafts onto avascular graft beds are in excess of 90%.1 In spite of the high success rate of corneal transplantation, approximately 4000 corneal grafts fail each year in the United States because of immunologic rejection. Moreover, the risk of graft rejection increases to 65% in individuals who have undergone rejection of a previous corneal transplant.1

Although immune rejection is the leading cause of corneal allograft failure, the immunologic mechanisms leading to graft rejection remain poorly understood. A large body of evidence suggests that corneal allograft rejection is a cell-mediated process.2–9 However, various clinical studies have also implicated antibody.10–15 Several investigators have detected circulating donor-specific antibodies in patients who have had keratoplasty,10–15 with one group discovering a correlation between the appearance of antibodies directed against donor major histocompatibility (MHC) class I antigens and graft rejection.15 Other investigators have argued that neither the presence nor absence of antibodies, either before or after corneal transplantation, has any clinical predictive value for corneal transplant survival.16,17

There has been very little experimental research investigating the role that antibodies play in orthotopic corneal allograft rejection. Goslings et al.18 reported that corneal allograft rejection occurs in B-cell-deficient mice and C5-deficient mice, which suggests that neither antibody nor complement-mediated mechanisms are necessary for the destruction of corneal allografts. However, a common property of solid organ transplants is the redundancy in the immune mechanisms capable of mediating graft rejection. We suspected that although corneal graft rejection could occur in the absence of antibody, it remains possible that in some circumstances antibody is capable of mediating corneal graft destruction. The present study was undertaken to directly investigate the role that antibodies play in orthotopic corneal allograft rejection. Prospective studies were conducted in which hyperimmune donor-specific serum was passively transferred to corneal transplant recipients, and the effect on the rate and speed of graft rejection was observed. These experiments were performed in a fully allogeneic donor-host combination that is disparate at the entire major histocompatibility complex (MHC) and at multiple minor histocompatibility (H) loci and thus parallels the histoincompatibility that normally occurs in human patients who undergo keratoplasty.

METHODS

Animals

C3H/Hej (H2b), CB6F1 (H2b/b), BALB/c (H2b), and DBA/2 (H2d) mice were obtained from our breeding facility. C57BL/6j (H2b), BALB/c ByJ (H2b), nude (BALB/cByJ-H2b), and B-cell-knockout (B6-lyg-cd11c) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and maintained in pathogen-free animal facilities. The B–cell knockout mouse strain has been described previously as not having the membrane form of the heavy chain of IgM (m chain), resulting in a deficiency of mature B cells, because B cell development is arrested at the pre-B cell stage.19 All animals used were female, 8 to 12 weeks of age. All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Surgical Technique

Full-thickness penetrating orthotopic corneal grafts were performed as previously described\textsuperscript{23} with a few modifications. Mice were anesthetized systemically with an intraperitoneal (IP) injection of 1.33 × 10\textsuperscript{-1} mg/kg ketamine HCl (Fort Dodge Laboratories, Fort Dodge, IA) and 6.68 × 10\textsuperscript{-3} mg/kg xylazine (Bayer Corp., Shawnee Mission, KS). Proparacaine HCl ophthalmic solution USP (0.5%; Alcon Laboratories, Fort Worth, TX) was used as a topical anesthetic. Donor grafts and recipient graft beds were scored with 2.5- and 2.0-mm trephines, respectively, and the corneas were excised with Vannas scissors. Donor grafts were sewn into place with 12 interrupted (running sutures) were used for mice on C57BL/6 backgrounds) 11-0 nylon sutures (Ethicon, Somerville, NJ), and sutures were removed on day 7 after transplantation. Topical antibiotic (Akorn, Decatur, IL) was applied immediately after surgery and immediately after removal of sutures. Immunosuppressive drugs were not used, either topically or systemically.

Clinical and Histopathologic Evaluation of Grafted Corneas

Corneal grafts were examined two to three times a week with a slit lamp biomicroscope (Carl Zeiss, Oberkochen, Germany). Graft opacity was scored using a scale of one to four, as previously described.\textsuperscript{21} Corneal grafts were considered rejected on two successive scores of 3. Rejected corneal grafts were embedded in paraffin, stained with hematoxylin and eosin (H&E), and examined by compound microscopy by two masked observers. One hundred inflammatory cells were randomly counted in five to six high-power fields (×250 magnification) on each section, and the number of mononuclear versus polymorphonuclear inflammatory cells was ascertained by two masked observers.

Hyperimmunization against Donor Antigens

Naïve recipients received an intramuscular (IM) injection of 10\textsuperscript{7} donor splenocytes, together with complete Freund’s adjuvant (CFA; Sigma, St. Louis, MO) on day 0. Recipients were boosted with a subcutaneous (SC) injection of 10\textsuperscript{7} donor splenocytes without CFA on day 14. Hyperimmune lymphocytes and serum were harvested on day 28.

Adoptive Transfer

Naïve graft recipients examined an IP injection of one donor equivalent of hyperimmune donor-specific splenocytes (5 × 10\textsuperscript{7} cells) 2 hours before transplantation of an orthotopic corneal graft.

Passive Transfer

Naïve graft recipients received an intravenous (IV) injection in the tail vein of 0.5 mL hyperimmune serum (one donor equivalent) 2 hours before receiving an orthotopic corneal graft. Graft recipients received a second IV injection of 0.5 mL hyperimmune serum on day 14 after transplantation.

Corneal Cell Cultures

Tissue-cultured C3H/HeJ (H-2\textsuperscript{b}), BALB/c (H-2\textsuperscript{d}), and C57BL/6j (H-2\textsuperscript{b}) corneal epithelial and endothelial cells were used as target cells for complement-mediated cytotoxicity assays, rather than the usual lymphoid cells, because corneal cells are the relevant target cells in vivo during corneal allograft rejection. Furthermore, it has been shown that the corneal epithelial and stromal cell layers express MHC class I antigens, with little to no expression on the corneal endothelial cell layer.\textsuperscript{22,23} Whereas lymphoid cells express high levels of MHC class I antigens, cell cultures were established as described previously.\textsuperscript{24} Briefly, cultures were established from freshly dissected corneal explants\textsuperscript{25,26} and propagated in minimum essential medium (MEM; BioWhittaker, Walkersville, MD) supplemented with 10% heat-inactivated fetal bovine serum (FBS; HyClone Laboratories, Logan, UT). Once primary cultures were established, the cells were immortalized with human papilloma virus genes E6 and E7, using the disabled recombinant retroviral vector pLXSNI166/E6/7.\textsuperscript{27} The transformed corneal cells proliferate indefinitely, maintaining their original morphologic characteristics and expressing the same histocompatibility antigens as their nontransformed counterparts.\textsuperscript{28} Cell lines were maintained in complete MEM containing 10% heat-inactivated PBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 2 mM MEM vitamins, and 1% penicillin-streptomycin-fungizone solution (all from BioWhittaker).

Complement-Mediated Cytotoxicity Assay

This is a modified form of the cytotoxicity assay described previously.\textsuperscript{29} Briefly, heat-inactivated (56°C for 30 minutes) serum samples were incubated at a 1:100 dilution with \textsuperscript{51}Cr-labeled (sodium chromate solution, 1 mCi/mL; Amersham Pharmacia Biotech, San Francisco, CA) donor strain corneal target cells (1 × 10\textsuperscript{5} cells) in a 96-well U-bottomed microtiter plate (Corning Inc., Corning, NY) for 15 minutes at 37°C. Rabbit complement (1:6.75 dilution; Cederlane Laboratories, Hornby, Ontario, Canada) and complete MEM were added for a total volume of 200 μL/well. The plate was centrifuged at 500 rpm for 3 minutes before incubation at 37°C for 2 hours. The plate was centrifuged at 800 rpm for 5 minutes, and 100 μL of the supernatant from each well was harvested and counted on a gamma counter (Tracor Analytical, Atlanta, GA). Cytotoxicity was evaluated by the amount of \textsuperscript{51}Cr released by the target cells, and the percentage of specific lysis was calculated as

\[
\text{Percent Specific Lysis} = \frac{(\text{Experimental cpm}) - (\text{Spontaneous release cpm})}{(\text{Maximum release cpm}) - (\text{Spontaneous release cpm})} \times 100
\]

Enzyme-Linked Immunosorbent Assay

BALB/c corneal epithelial and endothelial cell lines cultured in our laboratory were used as a source of antigen. Briefly, a 1:1 mixture of the two cell lines was washed and resuspended in phosphate-buffered saline (PBS). The cell suspension was rapidly frozen (at −70°C) and thawed (at 37°C) three times, and the protein concentration of the lysate was determined by the Bradford assay.\textsuperscript{29} ELISA plates (Corning, Inc.) were coated with the antigen (8.50 μg in 50 μL PBS per well) and incubated overnight at room temperature. Plates were washed four times with PBS containing 0.01% Tween-20 (wash buffer; Sigma), blocked with 300 μL/well 5% bovine serum albumin (BSA) in PBS containing 0.5% Tween-20 (blocking buffer) at 37°C in 10% CO\textsubscript{2}, for 1 hour, washed four times with wash buffer, and incubated with serum (1:100 dilution in blocking buffer, 50 μL/well) for 2 hours at 37°C in 10% CO\textsubscript{2}. Plates were washed four times with wash buffer, and blocked with 300 μL/well blocking buffer for 10 minutes, washed four times with wash buffer, and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibody in blocking buffer at 100 μL/well (1:500 dilution; Accurate Chemical Company, Westbury, NY) at 37°C in 10% CO\textsubscript{2} for 45 minutes. Plates were washed three times with wash buffer, and developed with 100 μL/well of ABTS solution (0.1 M 2,2’-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid with 0.1 M citric acid monohydrate; pH 4.2) containing 10 μL 30% H\textsubscript{2}O\textsubscript{2} in 50 μL ABTS, in the dark at room temperature for 30 minutes. The reaction was stopped with 100 μL/well 5% SDS (Sigma), and the plates were then read on a microplate reader (Molecular Devices Corp., Sunnyvale, CA) at 405 nm.

In Vivo Complement Depletion

Mice were depleted of complement in vivo as previously described.\textsuperscript{30} Naïve graft recipients received an IP injection of 0.4 mg/kg cobra venom factor, Naja naja kaouthia (CVF; Calbiochem-Novabiochem Corp., San Diego, CA) immediately after receiving an orthotopic corneal graft, and every 4 days thereafter until day 28 after transplantation.

Statistical Analysis

The Mann-Whitney test was used to compare the median survival times (MSTs) between groups, whereas the graft rejection rates between
groups were compared using the $\chi^2$ test. Student’s $t$-test was used in all other cases. In each case, $P < 0.05$ was considered to be significant.

RESULTS

Effect of Elevated Levels of Alloantibody on Rejection of MHC and Minor H Mismatched Corneal Allografts

Experiments were first performed to determine whether, as demonstrated in human studies, there is a correlation between the appearance of alloantibodies and corneal graft rejection in a mouse model of orthotopic corneal transplantation. We selected a donor–host combination that experienced a 100% corneal allograft rejection rate and thereby allowed us to evaluate alloantibody responses in a larger sample group of rejector animals. Accordingly, C3H/Hej corneal allografts were transplanted to naïve CB6F1 hosts. This donor–host combination exhibits disparity at both the MHC and multiple minor H loci. C3H/Hej corneas were transplanted orthotopically to normal CB6F1 mice, and serum was collected from rejecters within 3 to 5 days of graft rejection and assessed for IgG antibody directed against C3H/Hej alloantigens. The results of a representative ELISA are shown in Figure 1 and indicate that 7 of the 10 rejecter animals generated alloantibody at significantly elevated levels over normal control serum. In a separate experiment, BALB/c corneal allografts were transplanted to DBA/2 hosts, a donor–host combination mismatched at multiple minor H loci only. In this case, none of the 25 graft recipients that either rejected ($n = 9$) or retained ($n = 16$) the corneal grafts displayed detectable IgG antibodies to BALB/c alloantigens (data not shown).

![Figure 1: Alloantibody responses in corneal graft rejecters. Data are the mean of triplicate wells and are reported as mean optical density ± SE. The experiment was repeated three times, and representative data are shown. Each bar represents an individual graft-recipient animal with data compared with that of serum from a naïve animal. Naive CB6F1 serum (N) served as a negative control, and CB6F1 anti-C3H alloantiseraum (H) served as a positive control for the ELISA. Wells were coated with C3H alloantigens. $^* P < 0.05$ by Student’s $t$-test.](image)

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![Figure 2: Corneal allograft rejection in B-cell-knockout mice. BALB/c corneas were grafted orthotopically onto naïve B-cell-knockout mice (on a C57BL/6 background; $n = 7$) or onto wild-type control mice (naïve C57BL/6; $n = 8$). The MST and rejection rate in B-cell-deficient hosts were compared with those of the wild-type control group by Mann-Whitney test and $\chi^2$ test, respectively. $P < 0.05$ by Mann-Whitney; $P > 0.05$ by $\chi^2$.](image)

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Fate of Corneal Allografts in B-Cell–Deficient Hosts

A definitive test for determining whether corneal graft rejection can occur in the absence of alloantibody is to ascertain the fate of corneal allografts in mice that are incapable of producing antibodies. Accordingly, BALB/c corneal grafts were transplanted orthotopically onto B-cell–knockout mouse (C57BL/6 background), a donor–host combination mismatched at multiple minor H loci at the entire MHC as well as multiple minor H loci. We and others have previously shown that wild-type C57BL/6J hosts reject 80% to 100% of orthotopic BALB/c corneal allografts.

The present results indicate that although the incidence of graft rejection in the B-cell–deficient mice was not significantly different from that in immunocompetent control mice, the MST for fully allogeneic corneal grafts in B-cell–deficient hosts was significantly longer than in the control group (Fig. 2). However, the histopathologic features of the rejected corneal allografts in B-cell–knockout mice were virtually identical with those of grafts rejected by normal C57BL/6J hosts (Fig. 3). Thus, in a donor–host combination that is allodisparate at the entire MHC as well as multiple minor H loci, the absence of alloantibody resulted in a significant delay in corneal graft rejection. However, the ultimate fate of the allografts was not influenced by the absence of alloantibody.

Targeting of Fully Allogeneic Corneal Allografts

The results to this point indicate that alloantibody was not needed for corneal allograft rejection in the presence of an
intact T-cell repertoire. However, the possibility remained that antibody-mediated injury to corneal allografts was masked by robust T-cell–mediated effector mechanisms. To test this possibility, alloantiserum was passively transferred to T-cell–deficient nude mice that also received corneal grafts. One donor-equivalent (0.5 mL) of anti-C57BL/6J antiserum was passively transferred IV to naïve BALB/c nude mice 1 to 2 hours before challenge with orthotopic C57BL/6J corneal allografts. Graft recipients received a second IV injection of hyperimmune serum on day 14 after transplantation. The fate of the grafts was observed, and the grafts were scored clinically. The passive transfer of hyperimmune antiserum had a profound effect on the clarity of fully allogeneic corneal grafts, resulting in significantly increased opacity of the grafts (Fig. 4). By contrast, all the nude mice treated with normal BALB/c serum maintained clear C57BL/6J corneal allografts (Fig. 4).

Histopathologic examination of the corneal grafts in the antiserum-treated hosts revealed a conspicuous infiltration of polymorphonuclear neutrophils (Fig. 3). Comparison of the inflammatory infiltrates in T-cell–competent hosts with those in antiserum-treated hosts revealed that corneal graft rejection in the former was characterized by a mononuclear infiltrate, whereas neutrophils were the dominant inflammatory cells in the antiserum-treated group (Table 1).

This study was repeated with a donor–host combination mismatched at multiple minor H loci only. BALB/c corneas were transplanted to DBA/2 hosts treated with serum from DBA/2 mice immunized with BALB/c alloantigens. Passive transfer of putative donor-specific hyperimmune serum failed to affect either the speed or the incidence of corneal graft rejection in this donor–host combination (33% rejection rate in antiserum-treated compared with 36% rejection in control group). We were never able to detect donor-specific IgG or complement-fixing antibodies in this putative hyperimmune serum; however, we were able to verify the efficacy of this immunization protocol in this particular donor–host combination. Adoptive transfer of immune splenocytes from the same donors of the putative hyperimmune serum resulted in 100% rejection of the BALB/c corneal grafts (n = 9) compared with a 36% rejection rate in untreated DBA/2 control mice (data not shown).

Complement-Dependent, Antibody-Mediated Attack of the Corneal Endothelium

Complement-mediated lysis through the membrane attack complex is an antibody-mediated effector mechanism that is potentially relevant to immune attack of the cornea during corneal allograft rejection. The histopathologic data from the antiserum-treated group (Fig. 3) demonstrated a heavy infiltrate.

FIGURE 3. Histology of rejected donor corneas. Wild-type C57BL/6J (A) and B-cell–knockout (B) hosts rejected BALB/c corneas with a predominantly mononuclear infiltrate typical of cell-mediated rejection, whereas nude mice passively infused with donor-specific hyperimmune serum (C) rejected C57BL/6J corneas with a predominantly neutrophilic infiltrate. H&E staining was performed on all sections. Arrows: lymphocytes (A, B); arrowheads: neutrophils (C). Bar, 24 μm.

FIGURE 4. Effect of passively transferring hyperimmune donor-specific antibodies on the clarity of corneal allografts transplanted onto fully allogeneic hosts. Naïve BALB/c nude mice received a 0.5-mL IV injection of C57BL/6J-specific hyperimmune serum (HIS; n = 10) or normal BALB/c serum (NS; n = 5). 1 to 2 hours before receiving an orthotopic C57BL/6J corneal graft. A second 0.5-mL IV injection of serum was given on day 14 after transplantation. The mean opacity score of the group receiving HIS was compared at multiple time points with that of the group receiving NS. *P < 0.05 by Mann-Whitney test.
tion of neutrophils. This suggested the involvement of complement in antibody-mediated immune attack of the cornea, in that C5a, one of several molecules produced during the complement cascade, acts as a chemotractant to neutrophils. We tested the hypothesis that antibody-mediated immune attack of the cornea was complement-dependent in a passive transfer experiment using T-cell–deficient nude mice that had been depleted of complement. In vivo complement depletion of corneal allograft recipients was achieved with IP injections of 0.4 mg/kg CVF immediately before the application of an orthotopic corneal graft, and every 4 days thereafter until day 28 after transplantation. The complement-depleted animals received one donor equivalent (0.5 mL) IV anti-C57BL/6J antisera on days 4, 5, and 6 after challenge with orthotopic C57BL/6J corneal allografts. A more aggressive protocol was used to introduce hyperimmune donor-specific serum than in earlier passive transfer experiments, because previous findings demonstrated that corneal MHC molecules are upregulated within the first week after corneal transplantation. The fate of the grafts was observed, and the grafts were scored clinically. The results indicated that when complement-depleted, T-cell–deficient nude mice were injected with donor-specific hyperimmune serum during the first week after transplantation, the corneal allografts remained clear for a significantly longer period than those in non–complement-depleted hosts (Fig. 5).

It is widely believed that the corneal endothelium is the most important target for immunologic attack in corneal allograft rejection. Accordingly, in vitro microcytotoxicity assays were performed to determine whether hyperimmune serum would damage either C57BL/6J corneal epithelial or corneal endothelial cells through a complement-dependent pathway. C57BL/6J corneal endothelial and epithelial cells were incubated with anti-C57BL/6J hyperimmune serum in the presence of infant rabbit complement, and specific lysis was determined as described in the Materials and Methods section. The results demonstrated that corneal endothelial cells were highly susceptible to lysis by complement-fixing antibody (Fig. 6). That the corneal endothelial cells were unaffected by the alloantiserum is interesting. The cytotoxic effect was donor-antigen-specific, because third-party corneal cells (C3H/Hej) were unaffected by the alloantiserum and complement.

**Discussion**

Corneal transplantation is one of the most successful transplantation procedures in humans, with immunologic rejection representing one of the last remaining barriers to successful transplantation. Many studies have attempted to elucidate the mechanisms of corneal allograft rejection, but the identification of the precise mediators and their mode of action has been elusive. A preponderance of experimental evidence indicates that cell-mediated immune processes promote orthotopic corneal allograft rejection, but to date there has been very little direct experimental investigation to delineate the role of antibodies in corneal allograft rejection. Accordingly, a series of prospective studies were initiated to evaluate the capacity of donor-specific antibodies to promote corneal allograft rejection.

An initial attempt to demonstrate a role of alloantibody in a multiple minor H only mismatched donor–host combination was unsuccessful, because we were never able to detect elevated levels of donor-specific alloantibody in the serum of hosts that had rejected the corneal grafts. Furthermore, it was not possible to detect any donor-specific IgG or complement-fixing antibodies in hyperimmune serum raised in DBA/2 mice against BALB/c allografts. These results were consistent with earlier findings from several groups regarding the difficulty of raising antibodies against minor H antigens. Thus, it appears that alloantibody does not play a role in corneal allograft rejection.
allograft rejection in a donor–host combination mismatched at multiple minor H loci only.

The role of antibodies in the rejection of combined MHC and minor H mismatched corneal grafts was more intriguing. The ability of B-cell-deficient hosts to reject donor corneas with the same incidence as wild-type control mice indicated that antibodies are not essential for corneal allograft rejection, in agreement with Goslings et al.48 However, the MST of corneal allografts in B-cell–deficient mice was significantly longer that in wild-type control animals, suggesting that alloantibody may play a minor role in the initiation of corneal allograft rejection. In this regard, it has been proposed that alloantibody directed against MHC class I determinants may exacerbate T-cell-mediated rejection of kidney allografts.37 In the present study, histologic examination of rejected corneas indicated no significant differences in pathologic appearances between corneas rejected by B-cell–deficient mice and wild-type control animals, further supporting the notion that antibodies do not play a major role in corneal allograft rejection. It may be possible to definitively resolve this question by performing passive alloantibody transfer experiments in BALB/c hosts that typically reject approximately 50% of C57BL/6 corneal allografts. If anti-C57BL/6 alloantibody exacerbates T-cell-mediated rejection of C57BL/6 corneal allografts, we would expect to observe a higher incidence or a swifter tempo of corneal allograft rejection in BALB/c hosts infused with anti-C57BL/6 antisera in uninfused control mice.

In the absence of conventional T-cell–mediated immunity, passive transfer of donor-specific antibodies resulted in a significant increase in the opacity of corneal allografts. Histopathologic examination of the corneal grafts revealed a predominantly neutrophilic infiltration, in direct contrast to the characteristic mononuclear infiltrate observed in the corneas rejected by cell-mediated immune mechanisms. The presence of neutrophils in the corneal transplants of antisum recipients may be attributed to the presence of neutrophil chemotacticants, such as C5a, that are generated by the complement cascade and are powerful chemotacticants for granulocytes. The role of neutrophils in antibody-dependent graft rejection is unclear. In some studies alloantibody-mediated rejection of rat cardiac allografts occurred in the absence of neutrophils,38 whereas other studies indicate that neutrophil depletion inhibits the rejection of skin xenografts in mice.19 In the present study, alloantibody-mediated injury was most likely due to the generation of the complement membrane attack complex. In vitro cytolysis assays indicated that donor-specific hyperimmune serum mediated complement-dependent lysis of the corneal endothelium, not the corneal epithelium. The resistance of the corneal epithelium to complement-mediated damage may be related to the corneal epithelium’s expression of at least three complement regulatory proteins, which counteract the cytolytic action of complement.10,41 This is in sharp contrast to the corneal endothelium, which does not express complement-regulating proteins.40 These findings suggest a minor, albeit real, role for alloantibody in corneal allograft failure. This effect, however, is significantly affected by the histocompatibility disparity between the donor and the host. Donor–host mismatches involving differences only at multiple minor H loci do not appear to be capable of provoking an antibody response. By contrast, mismatches involving differences at the MHC and multiple minor H loci, can result in the generation of donor-specific antibodies capable of initiating an immune attack of corneal allografts. The present data suggest that antibodies could inflict injury on corneal grafts by both direct and indirect mechanisms. Antibodies could damage the corneal endothelium directly by complement-mediated lysis and indirectly by recruiting inflammatory cells, such as neutrophils. Neutrophils could be recruited through chemotactic factors, such as C5a, that are generated during the complement cascade. It is also possible that alloantibodies damage the cornea through antibody-dependent cellular cytotoxicity (ADCC) or by inducing apoptosis. However, in the presence of an intact T-cell repertoire, antibody-induced injury to the corneal allograft is overshadowed by robust T-cell-mediated effector mechanisms. A similar mechanism for antibody has been proposed in the rejection of cardiac allografts in mice42 and skin and kidney allografts in rats.37 The results presented in this article add to a growing body of evidence underscoring the redundancy of immune mechanisms that are capable of jeopardizing the success of organ allografts.
References


Acknowledgment

The authors thank Elizabeth Mayhew for expert assistance with the histology.