Role of CD4⁺ T Cells in Immunobiology of Orthotopic Corneal Transplants in Mice

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PURPOSE. To determine, with the use of mice genetically deficient in expression of CD4 or CD8 molecules, which T cells are responsible for rejection of orthotopic corneal allografts in mice.

METHODS. Corneas were prepared from major histocompatibility complex (MHC)—only incompatible, minor histocompatibility (H)—only incompatible, and MHC-plus-minor H incompatible donors and grafted orthotopically to eyes of CD4 knockout (KO), CD8KO, and wild-type control mice. Graft survival patterns were assessed clinically and compared. Mice that retained healthy corneal allografts beyond 8 weeks were evaluated for evidence of donor-specific tolerance and anterior-chamber-associated immune deviation (ACAID) using local adoptive transfer reactions and challenge with orthotopic skin allografts.

RESULTS. Corneas grafted to CD8KO mice were rejected with an incidence and tempo indistinguishable from that in wild-type control animals. By contrast, MHC-only, and minor-H—only incompatible corneal grafts survived indefinitely in eyes of CD4KO mice. Approximately 50% of corneal grafts that confronted CD4KO recipients with both MHC and minor H alloantigens experienced delayed rejection, whereas similar grafts in wild-type recipients were rejected acutely. CD4KO mice with long-accepted grafts displayed neither donor-specific ACAID nor allograft tolerance.

CONCLUSIONS. CD8⁺ T cells play little or no role in acute rejection of orthotopic corneal allografts. Instead, acute rejection is mediated almost exclusively by CD4⁺ T cells. Moreover, when corneal allografts survive for 8 weeks without acute rejection, CD4⁺ T cells promote donor-specific ACAID thereby insuring long-term graft acceptance. (Invest Ophthalmol Vis Sci. 1999;40:2614–2621)

Allogeneic corneas grafted orthotopically to eyes of humans blinded by corneal disease are the most successful solid organ transplants performed clinically.¹ A similar situation exists in experimental animals in which orthotopic corneal transplants display a high spontaneous rate of acceptance.² The success of corneal transplants is usually explained by the facts that corneal tissue is immune privileged,³ capable of resisting alloimmune-mediated destruction; and the cornea graft forms the anterior wall of the anterior chamber (AC), an immune privileged site.⁴,⁵ However, immune privilege is a dynamic, rather than a passive state, and not all orthotopic corneal allografts succeed, in humans or in experimental animals. In the latter, major and minor histoincompatible corneal grafts placed in normal eyes of BALB/c mice are rejected approximately 50% of the time within 8 weeks;² similar grafts placed in normal eyes of C57BL/6 eyes are rejected more than 80% of the time within 8 weeks.⁶ Analysis of the alloimmune response to grafts of this type has revealed that minor histocompatibility (H)—encoded, rather than major histocompatibility complex (MHC)—encoded antigens, offer the greater barrier to graft acceptance; and rejection correlates better with donor-specific CD4⁺ T cell-dependent delayed-type hypersensitivity (DTH) than with donor-directed CD8⁺ cytotoxic T-cell activity.⁸

When allogeneic corneas are placed in mouse eyes with neovascularized corneas, a situation that resembles high-risk eyes in clinical ophthalmology, the incidence and vigor of graft rejection is enhanced, indicating that immune privilege has been compromised.⁹ However, even in this circumstance, in which rejection of the grafts occurs very rapidly (within 2 weeks), minor H, rather than MHC, antigens are more important, and DTH, rather than cytotoxic T cells, correlates better with graft rejection. Several laboratories have demonstrated that rejection of orthotopic corneal grafts can be more easily prevented by treating recipients with anti CD4⁺, rather than anti-CD8⁺, antibodies.¹⁰ In the aggregate, these various studies suggest that class II MHC-specific or MHC-restricted CD4⁺ T cells play a dominant role in acute rejection of orthotopic corneal allografts.

Because of this situation, we have recently completed a series of experiments in which β2-microglobulin knockout (β2mKO) mice, which are deficient in MHC class I expression and in CD8⁺ T cells, were used as recipients of allogeneic cornea grafts. Irrespective of the degree of immunogenetic disparity between graft donor and recipient (MHC alone, minor H alone, MHC plus minor H), the rate and incidence of rejection was identical in normal mice and in β2mKO mice.¹¹ These findings not only emphasize the importance of CD4⁺ T cells in cornea graft rejection, but they imply that CD8⁺ T cells are

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Supported in part by grant EY19765 from the National Institutes of Health.

Submitted for publication December 28, 1998; revised May 19, 1999; accepted June 23, 1999.

Commercial relationships policy: N.

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either unnecessary or are even incapable of rejecting orthotopic corneal grafts. This implication must be formally tested, however, because β2mKo mice have cellular immune deficits beyond those of CD8+ T cells.12 For example, these mice also have no CD1-recognizing NK T cells,13 and cells of this type may have unsuspected roles to play in alloimmunity and rejection.14

To resolve the relative importance of class II-specific CD4+ and class I-specific CD8+ T cells in corneal allograft immunity, we have explored immune responses evoked by orthotopic corneal allografts in two strains of genetically manipulated mice, one of which is deficient in CD4 expression, the other in CD8 expression. We report that CD4-deficient mice were severely impaired in their ability to reject orthotopic corneal allografts. By contrast, corneal allograft rejection proceeded unimpaired in mice deficient in CD8-bearing T cells. Moreover, unlike normal mice, CD4-deficient mice with long-accepted corneal allografts did not acquire donor-specific immune deviation and therefore never accommodated to the cornea grafts by acquiring donor-specific tolerance. On the contrary, in some CD4-deficient mice, MHC-plus-minor H incompatible grafts that had been in residence for many months underwent delayed rejection. We suspect that, given sufficient time, donor-specific CD8+ T cells can eventually emerge as effectors of rejection of orthotopic corneal allografts.

**Materials and Methods**

**Animals**

Adult male BALB/c (H-2b) and C57BL/6 and C57BL/10 (H-2b) mice were purchased from Taconic Farms (Germantown, NY). Adult male BALB.B (C.B10-H2b/LilMcdJ, H-2b), B10.D2 (H-2b), CD4KO (C57BL/10-Cd4 bm1, H-2b), and CD8KO (C57BL/6-Cd8bm1Mak, H-2b) mice were purchased from Jackson Laboratory (Bar Harbor, ME) and used as experimental subjects between 8 and 12 weeks of age. All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Orthotopic Corneal Transplantation**

As described previously,15 each recipient was deeply anesthetized with an intraperitoneal injection of 3 mg ketamine and 0.0075 mg xylazine before all surgical procedures. The central 2 mm of the donor cornea was excised and secured in recipient graft beds with eight interrupted 11-0 nylon sutures (Sharppoint; Vanguard, Houston, TX). Antibiotic ointment was applied to the corneal surface, and the lids were closed for 72 hours with an 8-0 nylon tarsorrhaphy. All grafted eyes were examined after 72 hours; no grafts were excluded from analysis. Grafts were moved in all cases on day 7.

**Assessment of Graft Survival**

Grafts were evaluated by slit lamp biomicroscopy twice a week. At each time point grafts were scored for opacification. A previously described scoring system2 was used to measure the degree of opacification from 0 to 5+: 0, clear and compact graft; 1+, minimal superficial opacity; 2+, mild deep (stromal) opacity with pupil margin and iris vessels (iris structure) visible; 3+, moderate stromal opacity with only pupil margin visible; 4+, intense stromal opacity with the AC visible; 5+, maximal corneal opacity with total obscuration of the AC. Grafts with an opacity score of 2+ or greater after 3 weeks were considered rejected (immunologic failure); grafts with an opacity score of 3+ or greater at 2 weeks that never cleared by 8 weeks were also regarded as rejected.2

**Skin Grafts and Assessment of Skin Graft Survival**

CD4-KO and C57BL/10 mice received an orthotopic skin graft from BALB/c (8 × 8 mm) were placed on graft beds prepared on the thoracic wall of anesthetized mice (halothane). Petrolatum gauze was placed over the graft site followed by an application of mastisol. Antibacterial powder (nitrofurazone) was then applied and the wound covered with dry gauze followed by a plaster cast. One week later, the cast was removed, and the grafts were examined every day. Grafts were regarded as rejected on the day that the surface was judged by clinical inspection to be completely demudded of epidermis.

**Assay for DTH Reaction Elicited by Local Adoptive Transfer in Mice Bearing Healthy Corneal Allografts**

On day 14 after subcutaneous injection of 10 × 106 BALB.B spleen cells into naive C57BL/10 mice, spleen cells were harvested as “responder” cells. Spleen cells (1 × 106), obtained from CD4-KO or C57BL/10 mice with long-accepted BALB.C corneal allografts, were used as “regulator” cells. As a positive control, naive CD4-KO spleen cells were used as regulators. Regulators and responders were mixed with 1 × 106 irradiated (200 rad) BALB.B spleen cells as “stimulator” cells. Thus, 3 × 106 cells in 10 μl were injected in 10 μl of Hanks’ balanced salt solution into the right ear pinnae of naive C57BL/10 mice, as described previously.17 After 24 hours and 48 hours, ear thickness was measured with a low-pressure engineer’s micrometer (Mitutoyo–MTI, Paramus, NJ). Ear swelling was expressed as follows: specific ear swelling = (24-hour measurement of left ear − 0-hour measurement of right ear) − (24-hour measurement of left ear − 0-hour measurement of left ear) × 10−3 mm. Ear swelling responses at 24 hours after injection are presented as group mean ± SEM. All experiments were repeated at least twice with similar results.

**Statistical Methods**

Statistical analyses were performed by using Student’s t-test for comparison of DTH responses. We also constructed Kaplan–Meier survival curves and used the Breslow–Gehan Wilcoxon test to compare the probability of corneal graft survival. P < 0.05 was deemed significant.

**Results**

**Survival of Orthotopic Corneal Allografts in Mice Deficient in CD4 or CD8 Expression**

Corneal grafts were prepared from eyes of normal BALB/c mice. These grafts were then placed orthotopically in normal-appearing eyes of CD4KO mice (C57BL/10 background) and CD8KO mice (C57BL/6 background). In control experiments, normal C57BL/10 and C57BL/6 mice received BALB/c corneal grafts. The fate of these MHC-plus-minor H incompatible grafts was assessed clinically at weekly intervals thereafter through
16 weeks or until irreversible graft rejection, whichever occurred first. A summary of the results of these experiments is presented in Figure 1. As anticipated, normal C57BL/10 and C57BL/6 mice rejected a high proportion of BALB/c cornea grafts within 6 weeks. In each recipient strain, only approximately 20% of corneal allografts were clear at 8 weeks, a result similar to that which we have reported previously. According to our previous experience, corneal allografts that are clear at 8 weeks after grafting remain so indefinitely. However, the reason for observing corneal grafts beyond 8 weeks became apparent when we examined the results of experiments conducted in the CD4KO mice. As can be discerned in Figure 1A, CD4KO mice rejected only a minority of the orthotopic corneal allografts (20%) within 8 weeks, and the tempo of these few rejections was markedly less than the speed with which wild-type control animals rejected the grafts. Moreover, because grafts in CD4KO recipients were observed subsequently, the slow rate of rejection continued, so that by 16 weeks, approximately 45% of grafts had been destroyed. By contrast, CD8KO mice rejected the BALB/c cornea grafts with a tempo and an incidence identical with wild-type C57BL/6 mice (see Fig. 1B). These results enable us to make several important conclusions. First, orthotopic corneal allografts can be rejected with apparently normal vigor in the absence of CD8\(^+\) T cells. Thus, CD8\(^+\) cytotoxic T cells are not essential for acute rejection of corneal allografts placed orthotopically in normal eyes of mice. Second, and in distinct contrast, CD4\(^+\) T cells are required for acute corneal allograft rejection that occurs within 8 weeks of grafting in normal mouse eyes. Third, delayed rejection of corneal grafts (beyond 8 weeks after grafting) is observed in CD4KO, but not wild-type, recipients.

**Survival of Orthotopic Skin Allografts in Mice Deficient in CD4 Expression**

To reach the second conclusion, it was necessary to determine whether CD4KO mice are capable of rejecting orthotopic skin allografts, and if so, with what incidence and tempo. Accordingly, full-thickness skin grafts were prepared from BALB/c donors and placed on the thoracic wall of CD4KO mice. In positive control animals, BALB/c skin was grafted orthotopically to wild-type C57Bl/10 mice. The survival of these grafts was observed clinically. The median survival time of skin allografts on CD4KO mice was found to be 32.2 ± 4.1 days, whereas the median survival time of skin grafts on C57BL/10 recipients was significantly lower (9.3 ± 0.4 days). These results indicate that a deficit of CD4\(^+\) T cells attenuated the ability of mice to reject skin allografts. Because all skin allografts placed on CD4KO recipients were rejected by 40 days, alternative immune effectors (presumably CD8\(^+\) T cells) were still able to execute complete graft rejection. The finding that more than 90% of orthotopic corneal allografts on CD4KO mice were still healthy at 40 days strongly suggests that CD8\(^+\) T cells are largely unable to substitute as immune effectors in the eye when CD4\(^+\) T cells are missing.

At 16 weeks after grafting, orthotopic corneal allografts remained healthy and clear in six CD4KO mice, and two such grafts remained clear in wild-type C57BL/10 mice. To determine whether these recipients bearing long-standing healthy corneal allografts had become tolerant of donor alloantigens, each mouse received an orthotopic BALB/c skin allograft at this time. The fate of the skin grafts and the original corneal grafts was assessed clinically. C57BL/10 mice bearing healthy BALB/c skin grafts rejected the orthotopic skin grafts, but the corneal grafts remained clear and healthy. By contrast, CD4KO mice bearing healthy corneal allografts not only rejected orthotopic BALB/c skin grafts, but they also rejected long-standing corneal grafts (see Fig. 2). These findings indicate that a form of tolerance develops in normal (wild-type) mice bearing long-standing orthotopic corneal allografts that protects the graft from systemic, donor-specific alloimmunity. By contrast, cornea graft–bearing CD4KO mice display no such tolerance, because exposure to donor alloantigens on orthotopic skin grafts causes them to reject the corneal grafts. Thus, CD4\(^+\) T cells appear to be important, not only in generating corneal allograft immunity, but in generating tolerance in mice that accept orthotopic corneal allografts for prolonged time intervals.

**Survival of Orthotopic Minor H Corneal Allografts in Mice Deficient in CD4 or CD8 Expression**

We have previously demonstrated that minor H, rather than MHC, alloantigens are more important determinants of ortho-
This conclusion reveals that T cells that recognize graft-derived alloantigens by the indirect pathway of allorecognition are more important than T cells that recognize graft-derived MHC alloantigens directly. Because graft-derived alloantigens that are presented to recipient T cells through the indirect pathway of allore cognition are loaded predominantly on class II MHC molecules, graft rejection is dependent primarily on CD4+ T cells. Circumstantial evidence already suggests that T cells expressing CD4 molecules are the primary mediators of corneal allograft rejection. To explore this issue more definitively, panels of CD4KO and CD8KO mice (and wild-type C57BL/10 and C57BL/6 control animals, respectively) received orthotopic corneal allografts from BALB.B donors. These grafts confront recipients with minor H–only incompatibilities. The fate of these grafts is presented in Figure 3. CD4KO mice were particularly deficient in ability to reject BALB.B grafts. Compared with wild-type C57BL/10 mice that rejected 93% BALB.B corneal grafts within 6 weeks, only 1 of 15 CD4KO mice rejected the BALB.B corneal graft, and this graft was rejected at 8 weeks after grafting (Fig. 3A). By contrast, CD8-deficient mice rejected minor H incompatible BALB.B corneal grafts at least as vigorously as did C57BL/6 mice (Fig. 3B). This indicates that cytotoxic T cells expressing CD8 are not required for acute rejection of minor H–only disparate corneal grafts. These results directly confirm the primary, if not exclusive, role of self class II–restricted CD4+ T cells in the rejection of minor H-incompatible orthotopic corneal grafts.

**Assessment of Donor-Specific Unresponsiveness in CD4KO Mice that Accept Minor H–only Incompatible Orthotopic Corneal Allografts**

The high rate of minor H disparate corneal graft acceptance in CD4KO mice that was noted at 8 weeks after grafting proved to be long lasting. The accepted grafts were observed for an additional 8 weeks (16 weeks total), and no additional rejections were observed. Because of the sustained acceptance of these grafts, the recipients were tested for evidence of donor-specific unresponsiveness in two different assays. In the first, eight corneal allograft-bearing mice received an orthotopic skin graft from BALB/c donors. Selection of this donor strain, rather than BALB.B, was necessitated by our desire to avoid presentation of graft-derived minor H antigens from the test skin allograft by the direct allorecognition pathway, that is, in the context of H-2b. CD4KO mice bearing healthy orthotopic BALB.B corneal allografts rejected orthotopic BALB/c
skin grafts acutely. As revealed in Figure 4, within 2 weeks of skin graft rejection, the previously healthy corneal grafts began to show evidence of opacity and rejection. Within 28 days, all the grafts were rejected. Thus, CD4KO mice bearing minor H–only disparate corneal allografts appeared to resemble CD4KO mice bearing MHC-plus-minor H disparate cornea grafts in the absence of tolerance of donor alloantigens.

We have previously shown that normal mice bearing long-standing orthotopic allogeneic corneal grafts acquire donorn-specific, AC-associated immune deviation (ACAID). It is impossible to immunize these mice with donor alloantigens in a manner that generates donor-specific DTH. Moreover, spleen cells from these mice contain regulatory cells that, in adoptive transfer assays, can prevent the induction of donor-specific DTH in naive syngeneic mice. To examine this point in CD4KO recipients of minor H–only incompatible corneal grafts, spleen cells were obtained at 16 weeks after keratoplasty. These cells were then used as “regulators” (10⁶) in local adoptive transfer reactions in which responder C57BL/10 anti-BALB.B spleen cells (10⁶) were mixed with x-irradiated (2000R) BALB.B spleen cells (10⁶) and then injected into the ear pinnae of normal C57BL/10 mice. Control regulator cells were generated by using spleen cells from C57BL/10 mice bearing healthy BALB.B corneal allografts for more than 8 weeks. One of these donors bore a healthy BALB.B cornea graft for 16 weeks. Positive control animals received spleen cells from ungrafted CD4KO mice as “regulators” in local adoptive transfer injections. Negative control animals received intrapinnae injections containing CD4KO spleen cells, normal C57BL/10 spleen cells, and irradiated BALB.B spleen cells. When ear-swelling responses were assayed 24 and 48 hours later, positive control animals showed ear-swelling responses significantly greater than negative control animals (see Fig. 5).

Adoptive transfers containing regulators from C57BL/10 mice in which ACAID had been induced by long-standing healthy BALB.B corneal grafts displayed ear-swelling responses not significantly higher than negative control animals. Spleen cells from CD4KO mice bearing long-standing BALB.B corneal allografts did not suppress local adoptive transfer reactions. Ear-swelling responses induced by injections containing CD4KO spleen cells as regulators were similar in thickness to those in the positive control animals. Thus, CD4KO mice that accept BALB.B corneas for 16 weeks displayed no evidence of donorn-specific ACAID.

**Survival of Orthotopic MHC-only Disparate Corneal Allografts in Mice Deficient in CD4 Expression**

Having discerned the patterns of rejection of minor H–only disparate grafts and of MHC-plus-minor H disparate grafts in CD4KO mice, it remained to examine the fate of corneal grafts confronting recipient mice with only MHC-encoded alloantigens. In these experiments, CD4KO and C57BL/10 mice received orthotopic corneal allografts prepared from B10.D2 donors. Except for the H-2 region of chromosome 17, C57BL/10 mice and B10.D2 mice are syngeneic. Thus, B10.D2 grafts confront recipients only with alloantigens encoded by genes within H-2. The results of this experiment are summar-
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FIGURE 6. Fate of orthotopic B10.D2 corneal allografts in eyes of CD4KO and C57BL/10 mice.

discussed in Figure 6. None of the grafts placed in eyes of CD4KO mice was rejected, either acutely, or in a delayed fashion. As reported previously, a small but significant number of B10.D2 cornea grafts were rejected by wild-type C57BL/10 mice. Because of the small number of CD4KO recipients used in this experiment, the difference between the survival patterns of B10.D2 grafts in B10 (wild-type) and CD4KO mice was not statistically significant. These results support, but do not prove, the hypothesis that CD4+ T cells are also required for rejection of corneas expressing only MHC alloantigenic disparities.

DISCUSSION

Compared with other solid tissues and organs, the cornea is rather simple. It comprises only three types of cells (epithelial cells, keratocytes, and endothelial cells), each of which forms a horizontal layer (surface epithelium, stroma, and endothelium). The cornea possesses neither blood nor lymph vessels,22 and it is devoid of bone marrow–derived cells, except for rare Langerhans' cells in the epithelium near the limbus.23,24 Because corneal cells express moderate to abundant amounts of class I MHC molecules and no class II MHC molecules,4 rejection of orthotopic corneal allografts would be expected to be mediated primarily by CD8+ T cells. However, that was not the case. Mice deficient in expression of class I MHC molecules (by virtue of intentional deletion of the β2-microglobulin gene) reject orthotopic corneal allografts with the same vigor as wild-type mice.11 It has also been reported that treatment of mice with anti-CD8 antibodies does not extend the survival of orthotopic corneal allografts.10 Our current experimental results show that mice genetically deficient in CD8 expression rejected both MHC-plus-minor H and minor H–only disparate corneal allografts with comparable incidence and tempo. We can only conclude that CD8+ T cells are not required for rejection of orthotopic corneal allografts.

Despite the absence of bone marrow–derived cells within the normal cornea, and the absence of class II MHC gene expression on corneal parenchymal cells, class II-restricted, CD4+ T cells play the prime role in mediating rejection of orthotopic corneal allografts. The evidence from the literature in support of this conclusion is considerable. Treatment of mice with anti-CD4 antibodies inhibits rejection of orthotopic corneal allografts.10 Rejection of corneal grafts correlates better with recipient acquisition of donor-specific DTH than cytotoxic T cells.8 Our current experimental results show that CD4KO mice possess virtually no capacity to reject either minor H–only or MHC-only disparate corneal grafts. In fact, the only corneal grafts that CD4KO mice eventually rejected were grafts expressing MHC-plus-minor H incompatibilities. Even so, only 50% of these grafts were destroyed, and rejection was significantly delayed (many between 56 and 112 days after grafting) compared with corneal grafts rejected by wild-type recipients.

We have previously provided evidence to support the view that corneal alloantigens are recognized by recipient T cells largely, albeit not exclusively, through the so-called indirect pathway of allorecognition.7 In this pathway, peptides derived from MHC and/or minor alloantigens are processed and presented by recipient antigen-presenting cells that infiltrate the graft. Conventional wisdom dictates that peptides generated in this manner are loaded predominately on recipient class II MHC molecules for presentation to CD4+ T cells. We presume that this is one reason why CD4+ T cells are so important in corneal allograft immunity. In fact, therapeutic strategies that prevent recipient APC from migrating into the graft not only promote corneal allograft acceptance,25 but they suppress induction of donor-specific DTH, a CD4+ T-cell-mediated response.25 The inability of CD4KO mice to reject minor H–only disparate corneal grafts can be explained by the absence of CD4+ T cells required to recognize donor alloantigens through the indirect pathway.

Approximately 30% of MHC-only disparate (B10.D2) corneal grafts were rejected by normal C57BL/10 mice. The absolute failure of CD4KO mice to reject B10.D2 corneal grafts tells us that CD4+ T cells that recognize donor class II alloantigens are the mediators. However, we cannot be sure whether the effector T cells recognized peptides derived from class II alloantigens presented in the context of self class II molecules (indirect pathway of allorecognition), or whether these CD4+ T cells directly recognize donor class II alloantigens (direct pathway). We favor the former (indirect) pathway because corneal cells do not normally express class II molecules. However, we are aware that Peeler and Niederkorn,26 and Nicholls et al.27 have demonstrated that grafted corneal tissue can upregulate the expression of class II molecules. Experiments to distinguish between the direct and indirect pathways of class II recognition in CD4KO mice grafted orthotopically with allogeneic corneas are under way.

It is more challenging to explain why CD4KO mice, which utterly failed to reject MHC-only or minor H–only disparate grafts, mounted more successful immune reactions against grafts expressing MHC-plus-minor H disparities. At the very least, we suspect that CD8+ T cells must be involved. It is generally believed that activation of naive CD8+ T cells requires help and that CD4+ T cells are usually regarded as the source of helper factors. However, helper cells of this type are missing in CD4KO mice. Therefore, alternative sources must be considered. Activated CD8+ T cells are known to secrete cytokines, including IL-2 and interferon-γ, which can provide help, and therefore should be considered. It may be relevant that C57BL/6 recipients of orthotopic BALB/c corneal allografts acquire activated CD8+ T cells that recognize class I H-2d...
antigens directly. Although these cells were detected in cytotoxicity assays, it is possible that they also secrete helper factors in response to recognition of donor class I antigens. If cells such as this were activated in CD4KO recipients of BALB/c corneal grafts, then they may have been able to provide help for CD8+ T cells that recognize BALB/c-derived minor H alloantigens through the indirect pathway. Therefore, we propose that CD4KO mice reject BALB/c corneal grafts through the actions of minor H-specific cytotoxic T cells, and that these cells are promoted by factors released from CD8+ T cells that recognize H-2k class I antigens directly. It is of interest that BALB/c corneas were rejected by CD4KO mice in a delayed fashion compared with graft rejection in wild-type C57BL/10 mice. Perhaps the tempo of orthotopic corneal allograft rejection is dictated by whether CD4+ T cells participate or not. In their absence, rejection is desultory and incomplete.

The idea that allogeneic MHC-specific T cells can act as adjuvants, promoting the immunogenicity of minor H antigens, is suggested by our experimental results. This idea has been advanced previously. In the early 1970s, rejection of skin grafts bearing weak transplantation antigens was promoted in Syrian hamsters by sensitizing the recipients simultaneously with adjuvants, promoting the immunogenicity of minor H antigens, their absence, rejection is desultory and incomplete.

The proposed mechanism advanced to explain the allogeneic effect focused on T cells that recognize class II MHC alloantigens. Our evidence leads us to focus on class I-recognizing CD8+ T cells, but the outcome would be expected to be the same.

Although the evidence from studies of orthotopic corneal allografts emphasizes the key role CD4+ T cells play in immune rejection, evidence from experiments with other types of solid tissue allografts is less clear on this point. Experiments attempting to determine which effector cell (CD4+ or CD8+) is more important in graft rejection were popular in the 1980s. Monoclonal antibodies were used to negatively or positively select CD4+ and CD8+ T cells, which were then tested for relative capacity to cause graft rejection when adoptively transferred into appropriate recipients bearing skin, heart, or kidney grafts. In some studies, CD8+ T cells alone—without the participation of any CD4+ T cells—were sufficient to cause graft rejection. In other studies, CD4+ T cells provoked graft rejection independently of CD8+ T cells. In yet a third type of study, both CD4+ and CD8+ T cells were required to achieve acute, rather than delayed or chronic, graft rejection. More recently, skin allograft survival was assessed in CD4KO mice. Depending on the genetic background of the strain, one group claimed that the grafts were not rejected, whereas another group observed that CD4KO mice can still reject skin allografts. Based on recent studies from other laboratories, as well as our current results, we believe that CD4+ T cells play the central, if not the only, role as effector cells in orthotopic corneal allograft rejection. We suspect that this is the case, because the normal cornea has no “passenger leukocytes,” a deficit that requires recipient antigen-presenting cells to process exogenous donor transplantation antigens for presentation through the class II pathway to T cells. CD4+ T cells are the responding cells in this situation.

In the aggregate, our experimental results confirm that CD4+ T cells determine the tempo and incidence of acute rejection of orthotopic corneal allografts. In addition, they illuminate a role for CD4+ T cells in regulating allograft immunity, thereby promoting the long-term survival of orthotopic corneal allografts. In normal mice that accept orthotopic corneal allografts for prolonged intervals (beyond 8 weeks), donor-specific ACAID emerges and secures the future integrity of the graft by preventing subsequent development of destructive allograft immunity. However, in CD4KO mice bearing corneal allografts for more than 8 weeks, no evidence of ACAID was detected. Instead, cornea graft-bearing CD4KO mice rejected orthotopic skin grafts acutely, and shortly thereafter, the longstanding cornea grafts were destroyed. Thus, CD4+ T cells have two antithetical roles to play in the immunobiology of corneal allografts. On the one hand, CD4+ T cells play a critical role in mediating acute rejection of orthotopic corneal grafts. On the other hand, CD4+ T cells are needed to promote allograft tolerance and ACAID, thereby ensuring the long-term survival of grafts that manage to survive acute graft rejection mechanisms. If this knowledge is to be used eventually to enhance the survival of corneal allografts in humans, it is important to understand the conditions that enable CD4+ T cells to promote tolerance without promoting acute graft rejection.

Acknowledgments

The authors thank Takeshi Kezuka for helpful discussions and Jacqueline Doherty and Marie Ortega for managerial support.

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