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IL-17 Promotes Immune Privilege of Corneal Allografts

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Corneal allograft rejection has been described as a Th1-mediated process involving IFN-γ production. However, it has been reported that corneal allograft rejection soars in IFN-γ−/− mice or mice treated with anti–IFN-γ mAb. Th17 is a recently described IL-17A–producing Th cell population that has been linked to renal and cardiac graft rejection, which was originally thought to be Th1-mediated. We tested the hypothesis that Th17 cells mediate corneal allograft rejection in an IL-17A–dependent fashion and unexpectedly found that depletion of IL-17A increased the incidence of rejection to 90%. We demonstrate that the exacerbated rejection following depletion of IL-17A did not result from a loss of cross-regulation of Th1 cells or exaggerated delayed-type hypersensitivity responses. Instead, inhibition of the Th1 or Th17 cell lineages promoted the emergence of a Th2 cell subset that independently mediated allograft rejection. These findings demonstrate that IL-17A is not required for corneal allograft rejection and may instead contribute to the immune privilege of corneal allografts. The Journal of Immunology, 2010, 185: 4651–4658.

C orneal allografts enjoy an immune privilege that is un-rivaled in the field of transplantation. Every year in the United States alone, >30,000 corneal transplants are performed (http://www.restoresight.org/donation/statistics). The surgical procedure is routinely performed without histocompatibility matching and without use of systemic immunosuppressive drugs, yet corneal transplants experience a 90% success rate under the cover of topical steroids (1, 2). Nonetheless, 10% of corneal transplants undergo immune rejection, and use of corticosteroids carries the added risk of developing cataracts and glaucoma.

The precise immune mechanism underlying graft failure is incompletely understood. It is thought to involve an initial sensitization phase during which host APCs migrate into the graft and internalize soluble Ag derived from the donor’s corneal endothelium. Subsequently, the APCs can induce an oligoclonal T cell response via either the direct or indirect pathway of allore cognition. In the direct pathway, recipient T cells recognize MHC class II molecules expressed on donor APCs. In contrast, indirect allore cognition requires presentation of processed MHC or minor Ags by host APCs (3). Allograft rejection is believed to be mediated by allospecific CD4+ Th1 lymphocytes and is closely associated with the development of delayed-type hypersensitivity (DTH) to donor allo antigens and production of IFN-γ (4). However, recent studies have implicated additional CD4+ Th cell subsets in the process. For example, the incidence and tempo of corneal allograft rejection rises steeply in hosts with either allergic conjunctivitis or allergic asthma (5). In the allergic host, rejection is associated with Th2 allo antigen-specific immune responses involving the production of IL-4, IL-5, and IL-13 (5, 6).

The Th17 cell subset is a recently described IL-17A–producing CD4+ T cell population that has been implicated in the resistance to certain bacterial pathogens (7). IL-17A has also been shown to have several regulatory and protective effects. In mouse models of colitis and graft-versus-host disease, IL-17A has been shown to mitigate the severity of the disease by limiting Th1–associated inflammation (8, 9). Additionally, IL-17A has been shown to negatively regulate asthma via inhibition of dendritic cells and chemokine synthesis in sensitized hosts (10). Nonetheless, dysfunctional Th17 responses have been linked to the pathogenesis of several autoimmune diseases conventionally thought to be Th1 mediated (11–13). In the transplantation setting, Th17 cells have been associated with lung and renal allograft rejection and have been shown to accelerate cardiac allograft rejection in T-bet–deficient hosts (14, 15). Based on the observation that Th1 cell responses are not necessary for corneal allograft rejection, we hypothesized that Th17 cells can also mediate corneal allograft rejection.

We report that the Th17 cell subset contributes to the ocular immune privilege and that the interplay between Th1, Th2, and Th17 cells determines the course of corneal allograft rejection.

Materials and Methods

Animals

Eight-to-ten-week-old female mice were used in all experiments. BALB/c (H-2b) and C57BL/6 (H-2b) mice were purchased from Taconic Farms (Germantown, NY), and BALB/c nude mice were obtained from the National Cancer Institute (Frederick, MD). STAT6 knockout (KO) mice were purchased from The Jackson Laboratories (Bar Harbor, ME). All experimental animals were treated in accordance with the Association for Research in Vision and Ophthalmology ARVO Statement for the Use of Animals in Ophthalmic and Visual Research (ARVO Animal Policy).

Orthotopic corneal allograft and clinical evaluation of grafted corneas

Naïve C57BL/6 corneas were grafted onto the right eye of BALB/c mice as described previously (16). Corneal grafts were scored for their graft opacity, neovascularization, and edema twice per week (16). Briefly, degree of opacification ranged between 0 and 4+: with 0, clear; 1+, minimal superficial opacity; 2+, mild deep stromal opacity with pupil margin and iris visible; 3+, moderate stromal opacity with pupil margin visible, but iris structure obscured; and 4+, complete opacity, with pupil and iris totally obscured. Corneal grafts were considered rejected upon two successive scores of 3+. The Journal of Immunology, 2010, 185: 4651–4658.

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Abbreviations used in this paper: DTH, delayed-type hypersensitivity; KO, knockout; MRT, mean rejection time; MST, median survival time; s.c., subcutaneous(lly); Treg, regulatory T cell; WT, wild-type.

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Cytokine depletion protocol
Anti–IFN-γ hybridoma (R4-6A2; catalog number HB-170) was purchased from American Type Culture Collection (Rockville, MD). Anti–IL-17A monoclonal and polyclonal Abs were produced by the University of Texas Southwestern Hybridoma Facility as described previously (17). mAbs were isolated from hybridoma cultures and affinity purified. Rat IgG3 was purchased from Sigma-Aldrich (St. Louis, MO). BALB/c mice were injected intraperitoneally with 500 μg Ab daily from day −4 to day −2, and corneal transplants were grafted on day 0. Biweekly injections of the Abs were continued up to day 60.

DTH assays
An ear swelling assay was used to measure DTH responses to C57BL/6 allografts as described previously (5). A cell suspension of 4 × 10⁵ mitomycin C-treated C57BL/6 spleenocytes in 20 μl HBSS was injected into the right-ear pinna of BALB/c mice. The left-ear pinna received 20 μl HBSS without cells and served as a negative control. Results were expressed according to the following: specific ear swelling = (24 h measurement − 0 h measurement) for experimental ear − (24 h measurement − 0 h measurement) for negative control ear.

Preparation of APCs
APCs were isolated from spleen cells of naive C57BL/6 mice. Briefly, cells were incubated with NLCL erythrocyte lysis solution, washed, and resuspended at 2 × 10⁶ cells/ml HBSS with 400 μg/ml mitomycin C. The cell suspension was incubated at 37°C for 1 h and washed three times with HBSS and was used as a source of APCs in direct MLRs. For the indirect MLR, C57BL/6 cell lysate was initially generated by resuspending C57BL/6 spleenocytes at 3 × 10⁶ cells/ml HBSS and sonicating the suspension with ten 1-s pulsations. Lysates were frozen at −80°C for 10 min and thawed at 37°C for 5 min for two cycles. BALB/c APCs were isolated by incubating the cell suspension of splenocytes onto two 100-mm Petri plates (Franklin Lakes, NJ) (5 ml each plate) at 37°C for 1 h. Nonadherent cells were removed by vigorous washing. Adherent APCs were cultured in a 100-mm Primaria plate containing 4 ml complete RPMI 1640 medium supplemented with 10% FBS and pulsed with the C57BL/6 cell lysate (1 ml). Cell cultures were incubated at 37°C overnight.

MLRs and cytokine ELISA
Spleen cells were harvested from BALB/c mice 4–7 d after rejection of the C57BL/6 corneal allografts or at 3 wk posttransplantation in acceptors. CD4⁺ T cells were enriched by positive selection using rat anti-mouse CD4-conjugated magnetic microbeads (Miltenyi Biotec, Auburn, CA). Purified CD4⁺ T cells were incubated at 1 × 10⁶ per well with respective APCs at a 1:1 ratio for 5 d at 37°C in 2 ml complete RPMI 1640. ELISAs for IL-4, IL-5, IL-13, IL-17A, IFN-γ, and TNF-α were performed on culture supernatants according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

Immunohistochemistry
Eyes from corneal allograft rejector mice were enucleated after two successive scores of 3+ and fixed in 10% formalin and were processed for histology. Sections (4 μm) of paraffin-embedded tissue were labeled with mAb against T1/ST2 (D9J; MD Biosciences) to detect Th2 cells using the Vectastain Elite ABC system (Vector Laboratories, Burlingame, CA). Ab specificity was validated using a rat IgG1 isotype control (BD Pharmingen, St. Paul, MN/El Bioscience, San Diego, CA).

Adoptive transfer of Th2 CD4⁺ T cells to nude mice
Spleen cell suspensions were obtained from anti–IL-17A–treated BALB/c recipients 4–7 d after rejection of C57BL/6 corneal allografts. CD4⁺ T cell enrichment was carried out using the magnetic microbead system as described earlier. Each nude mouse received an adoptive transfer of one donor equivalent of the CD4⁺ T spleen-cell population intravenously (10 × 10⁶ to 15 × 10⁶ cellsrecipient). Nude mice were grafted with C57BL/6 corneal allografts within 24 h of the adoptive transfer of CD4⁺ T cells.

Statistical analysis
The log-rank test was used for statistical analysis of the differences in the tempo of corneal graft rejection using Kaplan-Meier survival curves (18). Comparisons yielding p < 0.05 were considered significantly different.

Results

Inhibition of the Th1 and/or Th17 cytokines abrogates immune privilege of corneal allografts
To test the hypothesis that elimination of the Th1 pathway promotes graft survival, we treated BALB/c mice systemically with either anti–IFN-γ mAb or an IgG isotype control Ab given intraperitoneally twice per week before and after the application of orthotopic C57BL/6 corneal allografts. The isotype control-treated BALB/c hosts rejected 50% of their C57BL/6 corneal allografts with a mean rejection time (MRT) of 35.2 ± 8.0 d (Fig. 1). By contrast, depletion of IFN-γ in BALB/c hosts resulted in a 90% incidence of rejection and MRT of 22.2 ± 7.3 d (Fig. 1). The median survival time (MST) for the anti–IFN-γ–treated group was significantly reduced (p = 0.014) compared with that of the IgG isotype control (MST = 23.5 d and 52 d, respectively). This suggests that conventional Th1 cells are not necessary for corneal allograft rejection and implies that either Th2 or Th17 cells are capable of mediating corneal allograft rejection. Accordingly, the role of Th17 cells was evaluated. BALB/c mice were treated with either monoclonal or polyclonal Abs specific for IL-17A. In multiple experiments, we observed an increased tempo and incidence of corneal allograft rejection in BALB/c mice treated with anti–IL-17A (Fig. 2A). Mice treated with either monoclonal or polyclonal anti–IL-17A Abs rejected 90% of their corneal allografts, with MRTs of 26 ± 7.9 d and 24.7 ± 12.8 d and MSTs of 24 and 22.5 d, respectively. The rates of corneal allograft rejection between the rat IgG isotype control and anti–IL-17A–treated groups were significantly different (p < 0.05).

As a cross-regulation between Th17 and Th1 cell subsets has been suggested (9, 19), we tested the hypothesis that elimination of both Th1 and Th17 cytokines would enhance graft survival. BALB/c mice were simultaneously treated with anti–IL-17A and anti–IFN-γ mAbs prior to and after the application of corneal allografts. Treatment with both Abs did not prevent allograft rejection but instead resulted in a 90% incidence of rejection with an MRT of 30 ± 11 d and an MST of 26 d (Fig. 2B). The tempo of rejection was significantly different compared with that of the rat IgG isotype Ab-treated group (p = 0.04). Importantly, neither
anti–IFN-γ nor anti–IL-17A Ab treatment affected the survival of syngeneic BALB/c corneal grafts (data not shown). To our knowledge, these results demonstrate that elimination of the signature cytokines for Th1 and Th17 T cell subsets abolishes ocular immune privilege and exacerbates corneal allograft rejection.

Depletion of Th17 cytokine does not exaggerate DTH responses

Corneal allograft rejection is closely correlated with the development of DTH responses to donor alloantigens (20–24). Based on the earlier observation that depletion of IL-17A exacerbated the incidence and tempo of corneal allograft rejection, we performed additional experiments to address the possibility that mice treated with anti–IL-17A might develop exaggerated DTH responses to donor alloantigens. In these experiments, mice were treated with either a rat IgG isotype control or anti–IL-17A on days 24, 22, and twice per week over 2 wk after s.c. immunization with C57BL/6 splenocytes on day 0. On day 14, mice were ear challenged with mitomycin C-treated C57BL/6 splenocytes. Negative control animals received an ear challenge only, and positive control animals were immunized s.c. and received an ear challenge. Each group consisted of five animals. This experiment was performed twice with similar results. Results in all three groups differed significantly from those in the negative control group (p < 0.05). Results in the anti–IL-17A Ab-treated group were not significantly different from those in the positive control (p > 0.05).

Depletion of IL-17A and IFN-γ promotes emergence of Th2 alloimmune responses

In nonmanipulated hosts, corneal allograft rejection was characterized by the production of the Th1 cytokine IFN-γ (Fig. 4A). CD4+ T cell production of IL-4 and IL-5 was barely detectable.

FIGURE 2. C57BL/6 corneal allograft survival in BALB/c mice treated with anti–IL-17A, anti–IFN-γ, or a rat IgG isotype control Ab. A, C57BL/6 corneal allografts underwent rejection in 50% of hosts treated with the isotype control IgG (n = 10) and had an MST of 52 d. C57BL/6 corneal allografts transplanted to BALB/c recipients treated with either monoclonal anti–IL-17A or polyclonal anti–IL-17A were rejected in 90% of hosts with an MST of 24 and 22.5 d, respectively (n = 10 in each group). B, C57BL/6 corneal allografts underwent rejection in 50% of the untreated BALB/c mice (n = 10) and in 90% of the BALB/c mice treated with anti–IL-17A and anti–IFN-γ and had an MST of 26 d (n = 10). p < 0.05 by Kaplan-Meier survival analysis between cytokine-depleted groups and rat IgG isotype control-treated allograft recipients. These experiments were performed three times with similar results giving a total of 30 mice in each group. Syngeneic recipients treated with anti–IL-17A or anti–IFN-γ did not reject their corneal syngrafts (data not shown).

FIGURE 3. Anti–IL-17A treatment does not exacerbate DTH. A, BALB/c animals were treated with either anti–IL-17A or rat IgG isotype control on days −4, −2, and twice per week over 2 wk after s.c. immunization with C57BL/6 splenocytes on day 0. On day 14, mice were ear challenged with mitomycin C-treated C57BL/6 splenocytes. Negative control animals received an ear challenge only, and positive control animals were immunized s.c. and received an ear challenge. Each group consisted of five animals. This experiment was performed twice with similar results. Results in all three groups differed significantly from those in the negative control group (p < 0.05). Results in the anti–IL-17A Ab-treated group were not significantly different from those in the positive control (p > 0.05).

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Depletion of Th17 cytokine does not exaggerate DTH responses

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Depletion of IL-17A and IFN-γ promotes emergence of Th2 alloimmune responses

In nonmanipulated hosts, corneal allograft rejection was characterized by the production of the Th1 cytokine IFN-γ (Fig. 4A). CD4+ T cell production of IL-4 and IL-5 was barely detectable,
whereas moderate levels of IL-13 and small quantities of IL-17A and TNF-α were found. To confirm that disabling the Th1 subset with anti–IFN-γ did not exacerbate rejection via the Th17 lineage, we evaluated the cytokine profile of CD4+ T cells that were isolated from BALB/c mice 4–7 d after their rejection of C57BL/6 corneal allografts. CD4+ spleen cells were stimulated with C57BL/6 alloantigens for 5 d in either direct or indirect MLR cultures. A, CD4+ cell supernatants from BALB/c mice treated with rat IgG isotype control Ab. B, CD4+ cell supernatants from BALB/c mice treated with anti–IFN-γ Ab. C, CD4+ cell supernatants from BALB/c mice treated with anti–IL-17A. Six mice were in each group. This experiment was performed 6 times with similar results. D, Th2 (T1/ST2+) cells in the corneal stroma and adhering to the corneal endothelium during allograft rejection in anti–IL-17A–treated hosts. E, Isotype control Ab tested on same tissue sample shown in D reveals no Ab staining. F, Th2 (T1/ST2+) cells in anterior chamber of rejecting corneal allograft in an anti–IL-17A–treated BALB/c mouse. G, Isotype control Ab tested on same tissue sample shown in F. All specimens were counterstained with methylene green. Original magnification ×600.

**FIGURE 4.** Th1, Th2, and Th17 cytokine production by CD4+ spleen cells from corneal allograft rejector mice. CD4+ spleen cells were isolated from BALB/c mice 4–7 d after their rejection of C57BL/6 corneal allografts. CD4+ spleen cells were stimulated with C57BL/6 alloantigens for 5 d in either direct or indirect MLR cultures. A, CD4+ cell supernatants from BALB/c mice treated with rat IgG isotype control Ab. B, CD4+ cell supernatants from BALB/c mice treated with anti–IFN-γ Ab. C, CD4+ cell supernatants from BALB/c mice treated with anti–IL-17A. Six mice were in each group. This experiment was performed 6 times with similar results. D, Th2 (T1/ST2+) cells in the corneal stroma and adhering to the corneal endothelium during allograft rejection in anti–IL-17A–treated hosts. E, Isotype control Ab tested on same tissue sample shown in D reveals no Ab staining. F, Th2 (T1/ST2+) cells in anterior chamber of rejecting corneal allograft in an anti–IL-17A–treated BALB/c mouse. G, Isotype control Ab tested on same tissue sample shown in F. All specimens were counterstained with methylene green. Original magnification ×600.

with anti–IL-17A showed a similar cytokine profile, implicating Th2 cells in corneal allograft rejection in hosts lacking IL-17 (Fig. 4C). Although IL-17A is known to cross-regulate IFN-γ, in vivo treatment with anti–IL-17A Abs did not result in an increased production of IFN-γ (Fig. 4C). Thus, disabling the Th1 and Th17 alloimmune responses favors the expression of Th2-mediated corneal allograft rejection. Collectively, these results support the notion that IL-17A and IFN-γ can independently cross-regulate the activity and cytokine secretion of Th2 cells.
Based on the Th2 cytokine profiles observed from the MLRs, we hypothesized that the rejected corneas of anti–IFN-γ–treated and/or anti–IL-17A–treated BALB/c corneal allograft rejector mice would display a predominant infiltration of Th2 cells and eosinophils into the graft rejection site. To assess infiltration of Th2 cells within the rejection site, we used Abs specific for T1/ST2, which is expressed on Th2 cells but not Th1 cells (25). Mononuclear cells infiltrating the corneas of anti–IL-17A–treated animals stained positively for the T1/ST2 Ag (Fig. 4D–G), and no significant eosinophilic infiltrate was detected at the rejection site (data not shown).

The Th2 pathway is sufficient to mediate corneal graft rejection

The results up to this point suggested that the exacerbation of corneal allograft rejection associated with depletion of either IL-17A or IFN-γ was the result of a Th2 alloimmune response. To explore whether Th2 cells could independently cause graft rejection, we collected CD4+ T cells from anti–IL-17A–treated mice that had rejected their corneal allografts (MRT = 16.7 ± 4.7 d; MST = 13 d) and transferred them into naive BALB/c nude mice. The transferred CD4+ T cells were predominantly of the Th2 phenotype as confirmed by their preferential production of Th2 cytokines when confronted with C57BL/6 alloantigens in both indirect and direct MLRs (Fig. 5A). Nude mice that received adoptively transferred CD4+ Th2 cells rejected 100% of their C57BL/6 corneal allografts with an MRT of 23.6 ± 9.2 d and an MST of 17 d (Fig. 5B). Immunohistochemical analysis of the rejected corneas revealed a significant infiltration of T1/ST2+ mononuclear cells (Fig. 5C, 5D). These results suggest that anti–IL-17A treatment elicits an immune deviation that favors the emergence of allospecific Th2 cells that are sufficient to mediate corneal allograft rejection. With this in mind, we examined the fate of corneal allografts transplanted into STAT6 KO mice, which do not generate IL-4–mediated functions including Th2 cell differentiation (26, 27). Accordingly, STAT6 KO mice on a BALB/c background were treated with a combination of anti–IFN-γ and anti–IL-17A mAbs or were untreated and then challenged with C57BL/6 corneal allografts. For comparison, wild-type (WT) BALB/c mice were treated with either anti–IFN-γ or anti–IL-17A mAbs and then challenged with C57BL/6 corneal allografts. As expected, WT mice treated with either anti–IFN-γ or anti–IL-17A mAbs rejected 90% of their corneal allografts. By contrast, only one of the eight STAT6 KO mice treated with both anti–IFN-γ and anti–IL-17A rejected their C57BL/6 corneal allograft (Fig. 6). STAT6

![Figure 5](http://www.jimmunol.org/)

**FIGURE 5.** Corneal allograft survival in anti–IL-17A–treated nude BALB/c mice after adoptive transfer of CD4+ T splenocytes from corneal allograft rejector BALB/c mice treated with anti–IL-17A. A, Cell supernatant cytokine profiles of CD4+ cells that were isolated from anti–IL-17A–treated corneal allograft rejector donor mice and adoptively transferred to nude BALB/c mice. This experiment was performed 4 times with similar results. B, Corneal allograft rejection in anti–IL-17–treated BALB/c mice and in BALB/c nude mice that received adoptively transferred CD4+ T cells from either anti–IL-17–treated or untreated BALB/c corneal allograft rejector donors. C57BL/6 corneal allografts underwent rejection in 100% of the nude BALB/c recipient hosts that received CD4+ T cells from either anti–IL-17 donors (n = 9) or untreated donors (n = 5) and had an MST of 17 and 7 d, respectively. WT BALB/c CD4+ T cell donors rejected their corneas with an MST of 13 d (n = 9), p > 0.05 by Kaplan–Meier survival analysis. C, T1/ST2 expression in cell infiltrates of rejected corneal allografts from nude mice that received adoptively transferred CD4+ T cells. D, Isotype control staining of same corneas. All specimens were counterstained with methylene green. Original magnification ×600.
relates with corneal allograft survival, CD4+ T cells were isolated from corneal allograft acceptors and were stimulated in vitro with C57BL/6 corneal allografts. WT mice treated with either anti–IL-17A or anti–IFN-γ mAbs and transplanted with C57BL/6 corneal allografts. WT mice treated with either anti–IL-17A (n = 10) or anti–IFN-γ (n = 10) Abs rejected 90% of their corneal allografts with an MST of 25.5 and 19 d, respectively. By contrast, only one of eight STAT6 KO mice treated with anti–IFN-γ and anti–IL-17A Abs rejected its corneal allograft (MST = 60 d).

KO mice grafted with C57BL/6 corneal allografts rejected 50% of their corneal allografts. These results support the hypothesis that blockade of Th17 and Th1 pathways favors the emergence of a Th2-mediated form of immune rejection of corneal allografts. Moreover, simultaneous blockade of Th1, Th2, and Th17 pathways inhibits the CD4+ T cell-mediated rejection of corneal allografts. Our initial hypothesis proposed that Th17 T cells mediated corneal allograft rejection. However, the weight of evidence shown here indicates the opposite and suggests that IL-17 may contribute to the immune privilege of corneal allografts.

To test the hypothesis that IL-17A and IFN-γ production correlates with corneal allograft survival, CD4+ T cells were isolated from corneal allograft acceptors and were stimulated in vitro with C57BL/6 alloantigens, and cytokine production was measured by ELISA. Unlike CD4+ T cells from anti–IFN-γ–treated or anti–IL-17A–treated mice, which preferentially produce IL-4, IL-5, and IL-13 and reject 90% of their corneal allografts, CD4+ T cells from untreated mice that had accepted their corneal allografts produced large amounts of IL-17A and IFN-γ and moderate levels of IL-13 and TNF-α (Fig. 7).

Discussion

Corneal transplantation is arguably one of most successful forms of solid organ transplantation performed in humans. The high success rate of keratoplasty is attributable to the immune privilege of the cornea and rests on factors intrinsic to the tissue and the ocular environment (2, 28). When the delicate balance required for the maintenance of immune privilege is disrupted, graft rejection ensues by an immune response classically thought to be mediated by Th1 cells. However, in contrast with this prevailing paradigm, our attempt to inhibit the Th1 lineage by depletion of its signature cytokine, IFN-γ, abolished the immune privilege of corneal allografts and exacerbated rejection. The observation that the Th1 subset was not required for graft rejection led us to examine the role of Th17 T cells via its effector cytokine, IL-17A, in corneal immune privilege.
some immune-mediated diseases. In T cell-dependent colitis and graft-versus-host disease, IL-17A appears to limit Th1-associated inflammation and differentiation (8, 9). However, our observations suggest that a completely distinct regulatory mechanism is involved in corneal transplantation. It appears that the increased incidence of rejection is not due to a loss of Th1 cross-regulation or an exaggeration of DTH. The cytokine and the immunohistological analyses suggest that anti–IL-17A treatment lifts the alloimmune response toward a Th2 pathway. Adoptive transfer of CD4+ T cells from anti–IL-17A–treated hosts to nude mice culminates in accelerated corneal allograft rejection and the appearance of a putative Th2 cell infiltrate in the rejected corneal allografts, which is further evidence that anti–IL-17A treatment promotes preferential emergence of Th2 alloimmunity and Th2-mediated corneal allograft rejection.

Interestingly, the Th2-mediated graft rejection observed in IL-17A–depleted hosts appears to be independent of an eosinophilic infiltrate into the rejecting corneal allografts. Although eosinophils are often associated with Th2-based inflammation, recent studies indicate that hosts with Th2-mediated airway hyperreactivity have a similar exacerbation of corneal allograft rejection, which is associated with a Th2 cytokine profile, yet rejection occurs in the absence of infiltrating eosinophils (5).

It appears that the cytokine signature of the CD4+ T cells from grafted mice reflects the mechanism of allograft rejection. In untreated WT mice, the Th1 arm of the alloimmune response predominates and mediates corneal allograft rejection in ~50% of the hosts. Corneal allografts in the remaining 50% of WT hosts enjoy immune privilege, which correlates with concomitant expression of IFN-γ and IL-17A. It should also be noted that although the CD4+ T cells from acceptor mice also produced IFN-γ, the amount of cytokine produced in acceptor mice was higher than that produced by isotype IgG-treated mice that rejected their corneal allografts. Additional experiments are needed to elucidate the role of IL-17A in graft survival. Our report is not the first to suggest that IL-17 might mitigate Th2-based inflammation. Using a murine model of allergic asthma, Schnyder-Candrian et al. (10) found that in vivo neutralization of IL-17 exacerbated allergic asthma, whereas administration of exogenous IL-17 reduced macrophage chemotactic protein-1 and interleukin-Iβ during inflammatory but not basic fibroblast growth factor-dependent neovascularization in the mouse cornea. Lab. Invest. 83: 927–938.

In summary, our study demonstrates that IL-17A is required for corneal allograft survival and is preferentially expressed by CD4+ T cells from mice with surviving allografts. Our data also indicate that neither the Th1 nor the Th17 subset is required for allograft rejection and that inhibition of these lineages promotes emergence of a Th2 subset that can independently mediate allograft rejection.

Disclosures
The authors have no financial conflicts of interest.

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