Published in final edited form as:

Am J Transplant. 2010 March ; 10(3): 527–534. doi:10.1111/j.1600-6143.2009.02961.x.

Decay accelerating factor is essential for successful corneal engraftment

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Abstract

In contrast to immune restrictions that pertain for solid organ transplants, the tolerogenic milieu of the eye permits successful corneal transplantation without systemic immunosuppression, even across a fully MHC disparate barrier. Here we show that recipient and donor expression of decay accelerating factor (DAF or CD55), a cell surface C3/C5 convertase regulator recently shown to modulate T cell responses, is essential to sustain successful corneal engraftment. Whereas wild type (WT) corneas transplanted into multiple minor histocompatibility antigen (mH), or HY disparate WT recipients were accepted, DAF's absence on either the donor cornea or in the recipient bed induced rapid rejection. Donor or recipient DAF deficiency led to expansion of donor-reactive IFN- γ producing CD4⁺ and CD8⁺ T cells, as well as inhibition of antigen induced IL-10 and TGF- β , together demonstrating that DAF deficiency precludes immune tolerance. In addition to demonstrating a requisite role for DAF in conferring ocular immune privilege, these results raise the possibility that augmenting DAF levels on corneal endothelium and/or the recipient bed could have therapeutic value for transplants that clinically are at high risk for rejection.

Introduction

Unlike vascularized organ transplantation, corneal transplantation does not require immunosuppression irrespective of HLA differences between donor and recipient (1). Overall, greater than 85% permanent engraftment with complete corneal retention of clarity is achieved following treatment only with topical steroids (2). This is due to suppression of recipient T and B cell responses to donor tissue in the anterior chamber (a.c.), a process that is essential for the eye to prevent immune/inflammatory processes that could compromise vision.

A number of mechanisms have been implicated in this tolerogenic state of the a.c.: 1) TGF- β , IL-10, and other T cell inhibitory cytokines are locally produced (3), 2) neuropeptides (4, 5), and other immunosuppressive factors also are present (3, 5, 6) 3) costimulatory molecule expression on resident dendritic cells/macrophages in the cornea is downregulated (7), 4) CD4⁺CD25⁺ T regulatory (Treg) cells rather than Th1 cells are generated in response to antigens (8, 9), 5) invariant NK T cells impact responses (10) and 6) Fas ligand, capable of

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inducing apoptosis of T effector cells, is constitutively expressed on corneal endothelium (11).

In recent work (12, 13), we found that decay accelerating factor (DAF), originally characterized as a *intrinsic* complement inhibitor that prevents C3b/C5b deposition on self cell surfaces (14), modulates T cell responses. What initially unmasked this insight were studies with immune cells from mice targeted in the murine homolog (*Daf1*) of the human *DAF* gene. These studies showed that whether DAF is absent on antigen presenting cells (APCs) or on T cells, T cell proliferative and IFN- γ responses are 5–22 fold more robust than when DAF is present (12).

Studies with WT cells showed that this phenomenon is physiologically relevant in that a heretofore unrecognized early event in APC•T cell interactions is that concomitant with locally synthesizing alternative pathway components C3, factor B (fB), and factor D (fD) as well as C5, C5a and C3a receptor (C5aR and C3aR) (13), DAF downregulates on both partners (12, 13). As a consequence of the lifted restraint on junctional activation, C3a and C5a anaphylatoxins locally generate and ligate upregulated C5aR and C3aR on the interacting APCs and T cells. G protein coupled receptor (GPCR) signals resulting from the bidirectional C5a/C3a•C5aR/C3aR interactions play a requisite role in IL-2 production by T cells needed for their expansion as well as in innate cytokine (e.g. IL-12, IL-23) production by APC partners needed for lineage commitment during T cell expansion (13, 15).

These results in conjunction with previous work by ourselves (16–18) and others (19) showing that DAF is highly expressed on human and murine corneas led us to examine whether, in addition to the aforementioned established immuno-modulatory mechanisms associated with ocular tolerance, DAF also is required for allowing successful corneal engraftment. While previous studies by our group (12, 20) and others (21) have shown that dampening of APC and T cell produced complement by DAF suppresses recipient allo responses to major MHC mismatched donor tissue (hearts, skin, and kidney), no study has addressed whether DAF participates in conferring ocular immune privilege or other tolerogenic states.

To most sensitively investigate this issue, we employed conditions of minor MHC mismatch *i.e.* between C57BL/6 and 129 both H-2b and between C57BL/6 ♀ vs ♂. We employed minor rather than major mismatched conditions based on our past studies of systemic complement regulation (14, 22) which showed that even though DAF is a critical regulator that distinguishes self vs. nonself for systemic complement activation physiologically, under conditions of massive immune responses, its regulation can be overcome and its importance consequently masked (14, 22).

We transplanted minor antigen disparate or gender disparate congenic corneas from *Daf1*^{−/−} mice into *Daf1*^{+/+} mice and *vice versa*. We 1) measured the duration of viable engraftment, 2) analyzed eyes pathologically and immunohistochemically, and 3) characterized recipient anti-donor T and B cell responses. Our results indicate that DAF indeed plays an essential role in corneal graft acceptance through modulating the recipient immune response directed against the graft.

Materials and Methods

Animals

Daf1^{−/−} and *Daf1*^{+/+} littermates of minor MHC mixed 129/C57BL/6 mice (both H-2b) backcrossed 5 generation or congenic 12 generations in the H-2b C57BL/6 background were used. Differently from humans, mice have two *Daf* (*Daf1* and *Daf2*) (23) genes. The *Daf1*

gene product is predominantly GPI-anchored and ubiquitously expressed whereas the *Daf2* gene product is constitutively expressed primarily in testes (24, 25). For all experiments 8 – 12 wk old mice were used. Animals were housed in the CWRU Animal Resource Center and all experiments performed with an approved Institutional Animal Care and Use Committee protocol.

Corneal harvest/graft bed preparation

Animals were anesthetized by i.m. injection of 0.60–0.80 μ l of 3 ml (50 mg/ml ketamine hydrochloride); 1.5 ml (20 mg/ml xylazine hydrochloride); 0.5 ml (10 mg/ml acepromazine). Transplantations were performed as described in Sonoda and Streilein (1). Corneas were excised with a 2 mm trephine (Katena Products, Inc, Denville, NJ) from both the right and left eyes, and placed in 20°C sterile saline. Recipient corneal beds were prepared by marking corneas with a 1.5 mm trephine, penetrating the a.c. with a Weck blade, and excising endogenous corneas using Vannas scissors while maintaining the a.c. throughout the procedure with intraocular viscoelastic injection. The donor graft was sutured into the recipient bed using 8 to 12 interrupted sutures (11-0 Nylon, Alcon, Inc). Immediately following transplantation, bacitracin ophthalmic ointment was applied on the eye. Eyelids were closed for 72 hr by tarsorrhaphy with 8-0 nylon sutures. On day 7, all sutures were removed.

Clinical Grading of Graft Rejection

Eyes were graded at post-op day 7 and then weekly as follows: 0 = Clear Graft, 1 = minimal superficial non-stromal opacity, 2 = minimal deep stromal opacity with pupil margin and iris vessels visible, 3 = moderately deep stromal opacity with only the pupil margin visible, 4 = intensely deep stromal opacity with the a.c. visible, 5 = maximum stromal opacity with total obscuration of the a.c. Grafts were deemed failures with opacity scores of 3+ or greater for 2 wks starting from day 7 post-op. Post-op exclusion criteria were infection or iris herniation. All grafts with detectable opacity a day 3 were excluded.

Histology

Corneas were fixed in 10% paraformaldehyde, paraffin embedded, and sections stained with H and E. Stained sections were examined in an Olympus microscope and photographs taken with an Olympus capture card.

Antibody Measurements

Levels of anti-HY antibody isotypes in sera were measured using the mouse mono Ab ID/SP kit from Zymed (San Francisco, CA). Following coating of 96 well plates with 10 mM of HY *Dby* or 10 mM of HY *Uty*, 50 μ l of a 1:20 dilution of 21 day post transplant sera was added. After incubation, bound Ig was quantitated by addition of biotin-labeled isotype specific mAb followed by stepwise addition of streptavidin-peroxidase and TNB.

IFN- γ ELISPOT Assays

Wells of 96 well ELISPOT plates (Cellular Technology Ltd., Cleveland, OH) were coated with anti-IFN- γ mAb (Endogen, Woburn, MA; 4 mg/ml) in PBS overnight at 4°C, blocked with 1% bovine serum albumin (BSA, Sigma, St. Louis, MO) in PBS and washed three times with PBS. Spleen cells (6×10^5) were added to wells together with 0.01–100 μ g/ml of HY *Dby* peptide or HY *Uty* peptide. After 24 hr of incubation, spots were counted on a computer assisted Immunospot image analyzer (Cellular Technology Ltd., Shaker Hts, OH).

Cytokine ELISA Assay

Spleen cells from mice obtained on day 21 were cultured with 10 μ M/ml *Dby* or *Uty* at 37°C for 48 hr. Supernatants were assayed for IL-10 and TGF- β 1 by ELISAs (R & D system). For TGF- β 1, the supernatant was first treated with 1 M HCl to activate latent TGF- β 1 to its immunoreactive form and then neutralized with 1.2 M NaOH.

Statistics

Clarity score data were analyzed statistically using an unpaired 2 tailed Student's T-test. Kaplan-Meier statistical analysis was used of survival graft tolerance plots for each animal group. Log-rank test were used to compare animals on survival across the study period. The statistical analysis was performed using SAS software (version 9; Cary, NC). A value of 0.05 was used to establish significance for all experiments.

Results

The absence of DAF either in the recipient or on the corneal graft leads to corneal graft rejection

The *Daf1*^{-/-} mouse was originally produced on a mixed H-2b/129 background and then backcrossed further (F12) to C57BL/6. In initial studies directed at assessing how donor and recipient DAF deficiency impact corneal engraftment under conditions when antigen differences are minor, we transplanted grafts from 129 mice backcrossed 5 times to B6 (F5) into F5 recipients where animals share MHC alleles but are different at multiple minor (mH) loci. First we transplanted F5 σ *Daf1*^{+/+} corneas into multiple mH disparate σ F5 *Daf1*^{+/+} (n=10) or σ *Daf1*^{-/-} (n=9) recipients or *vice versa* (n=6) and followed clinical scores posttransplant. Whereas >70% of *Daf1*^{+/+} grafts transplanted in *Daf1*^{+/+} recipients did not fail for > 21 days (Fig 1A), 70% of the *Daf1*^{+/+} grafts transplanted into *Daf1*^{-/-} recipients ($p < 0.006$ for *Daf1*^{+/+} $\rightarrow$ *Daf1*^{+/+} vs. *Daf1*^{+/+} $\rightarrow$ *Daf1*^{-/-}) and 100% of the *Daf1*^{-/-} grafts transplanted into *Daf1*^{+/+} recipients rejected by day 21 ($p < 0.001$ for *Daf1*^{+/+} $\rightarrow$ *Daf1*^{+/+} vs. *Daf1*^{-/-} $\rightarrow$ *Daf1*^{+/+}). Analyses of corneal nonclarity (using the standardized 0–5 scoring system) at day 14 demonstrated that in the absence of DAF in either the donor or recipient, injury was accelerated.

We euthanized an additional four mice from each transplant combination after day 21 posttransplant and examined corneal sections by H&E (at 100x magnification). Specimens from *Daf1*^{+/+} to *Daf1*^{+/+} transplants exhibited minimal corneal swelling, small numbers of infiltrating leukocytes, and near normal corneal architecture (Fig 1B). In contrast, those from *Daf1*^{+/+} corneas transplanted into *Daf1*^{-/-} recipients (Fig 1C) and from *Daf1*^{-/-} corneas transplanted into *Daf1*^{+/+} recipients exhibited marked cellular infiltration (Fig 1D), providing a histopathological correlate to the elevated non-clarity index. High power examination showed mixtures of lymphocytes, monocytes, and polymorphonuclear cells (PMN) only in transplants where DAF was absent in the recipient or cornea (not shown).

As a prerequisite to allow testing of DAF's role on recipient immune responses against corneal grafts acceptance, we transplanted F5 σ corneas into F5 σ recipients. Additionally to allow more stringent testing of DAF's importance on corneal acceptance, we performed a second set of studies using *Daf1*^{-/-} mice backcrossed 12 generations to the C57BL/6 strain, a strain in which σ mice reject σ skin but not corneal grafts through a T cell dependent mechanism (26). In the F5 animals (Fig 2A), DAF deficiency in the donor or the recipient resulted in failure of 100% of σ corneas by days 28–35, while >85% of *Daf1*^{+/+} σ corneas transplanted into *Daf1*^{+/+} female recipients did not fail for > 35 days ($p < 0.001$ for *Daf1*^{+/+} $\rightarrow$ *Daf1*^{+/+} vs. *Daf1*^{-/-} $\rightarrow$ *Daf1*^{+/+} and $p < 0.001$ for *Daf1*^{+/+} $\rightarrow$ *Daf1*^{+/+} vs. *Daf1*^{+/+} $\rightarrow$ *Daf1*^{-/-}). As in the previous studies, histopathological examination of the corneas revealed

significantly enhanced mononuclear cell and PMN infiltration in the absence of DAF either in the cornea or recipient (not shown). In the F12 transplants, like the F5 transplants, similar differences in corneal graft acceptance were obtained (Fig 2C). Control isografts of *Daf1*^{-/-} σ corneas to *Daf1*^{-/-} σ recipients and *Daf1*^{-/-} σ corneas into *Daf1*^{+/+} recipients did not fail for 35 days (Fig 2D). Together, the findings indicate that recipient and corneal expression of DAF are required to permit long term corneal transplant survival.

The absence of DAF either in the recipient or donor does not induce systemic C3b deposition

Because DAF's originally characterized function is to circumvent systemic complement activation on self cells, DAF deficiency could lead to increased complement activation at the transplant interface which theoretically could contribute to corneal transplant injury. Consequently, we examined eyes from a subset of the different transplant combinations for complement deposition. Immunohistochemical analyses on day 21 (Fig 3) showed no significant C3 deposition in any cornea, regardless of DAF expression. As a positive control, the detectability of C3 deposition was documented in tissue sections from mice sensitized *in vivo* with tissue specific antibody.

The absence of DAF either in the recipient or on the cornea induces anti-graft cellular and humoral immune responses

In the C57BL/6 mouse strain, T cells from σ animals recognize and respond to several immune dominant peptides derived from the σ antigen HY, including the class II MHC-restricted peptide HY*Dby* (presented by I-Ab) and the class I MHC-restricted peptide HY*Uty* (presented by Db) (26). To test the impact of donor or recipient DAF deficiency on the HY disparate transplant-induced cellular immune response, we assayed recipient spleen cells in subsets of each F5 group at day 21 and each F12 group at day 28 for IFN- γ production in response to APC presented *Uty* and *Dby* using ELISPOT assays. In the F5 mice, the frequency of HY*Uty* specific (class I, CD8) and HY*Dby* specific (class II, CD4) IFN- γ producers in the spleens of all groups was low (Fig 4A). However, 2-fold more HY*Dby*-reactive and HY*Uty* reactive IFN- γ producers were detected in the spleens of *Daf1*^{-/-} σ transplanted with *Daf1*^{+/+} σ grafts and vice versa. Similarly, 2-fold more HY*Dby*-reactive IFN- γ producing cells were detected in the spleens of *Daf1*^{+/+} σ recipients of *Daf1*^{-/-} σ corneas. Analyses at day 35 in the F12 mice (Fig 4B) similarly yielded higher responses to HY*Dby* in the transplants in which DAF was deficient (consistent with the T cell response to the cornea being mediated principally by CD4⁺ T cells; see Discussion).

We also obtained plasmas on day 21 from each transplantation pair and tested them for anti-*Dby/Uty* specific antibody isotypes by ELISA (Fig 4C). Compared to *Daf1*^{+/+} to *Daf1*^{+/+} transplants, anti-*Dby* and anti-*Uty* IgG2a and IgG2b Ab isotypes were increased in transplant recipients in which either donor or recipient DAF was deficient.

In the absence of DAF on donor corneas or in recipients, immunosuppressive cytokines are not produced by spleen cells

Published work by others has documented that prolonged corneal engraftment is associated with the absence of pathogenic type 1 (IFN- γ producing) anti-donor T cell immunity in conjunction with the upregulation of immunodulatory cytokines TGF- β and IL-10 (3). To determine the effect of DAF deficiency on elaboration of these immunosuppressive cytokines, we assayed supernatants of spleen cells from the above transplant recipients for IL-10 and TGF- β (normalized in each case to *Daf1*^{+/+} σ recipients of *Daf1*^{+/+} σ corneas). Strikingly, while both *Uty* and *Dby* stimulation induced IL-10 and TGF- β production by spleen cells from *Daf1*^{+/+} σ to *Daf1*^{+/+} σ transplants (Fig 5), following identical

stimulation of spleen cells from transplant recipients deficient in DAF, no increase in either cytokine occurred.

Discussion

In this study, we investigated ocular immune responses to minor mismatched grafts. We transplanted *Daf1*^{+/+} corneas into *Daf1*^{+/+} and *Daf1*^{-/-} recipients and *vice versa*. Clinical evaluations showed that in contrast to acceptance in *Daf1*^{+/+} recipients of *Daf1*^{+/+} grafts, when DAF was absent in either donor or recipient tissue, transplants rapidly failed. Histopathological analyses showed markedly increased cellular infiltration. Analyses of spleen cells from ♂ to ♀ transplants showed that CD4⁺ and CD8⁺ T cell responses to both ♂ antigens were up to augmented in the absence of DAF, again whether recipient or donor tissue was DAF deficient. ELISAs showed that, in contrast to the characteristic elaboration by spleen cells of immunosuppressive IL-10 and TGF-β in *Daf1*^{+/+} ♂ to *Daf1*^{+/+} ♀ transplants, little change or decreases in these cytokines occurred when DAF was absent in recipients. Analyses of sera showed that in contrast to *Daf1*^{+/+} ♂ to *Daf1*^{+/+} ♀ transplants where no anti-HY antibodies were made, complement fixing IgG2a and IgG2b were produced when DAF was deficient on the donor cornea. Taken together, the findings demonstrate that across mH or HY disparities DAF is essential to suppress recipient T and B cell responses against donor corneas. DAF regulation thus plays a requisite role in establishing the immunological milieu that allows successful corneal engraftment in the absence of systemic immunosuppression.

Experiments in this study with fully congenic (F12) mice gave comparable results to those with (F5) mixed C57BL/6/129 mice establishing the importance of DAF's contribution to the conditions which allow for successful corneal engraftment even when the smallest differences are present. While rejection rates overall were fast due to studies on the Th1-skewed C57BL/6 rather than the generally used Th-2 skewed Balb/c background, control experiments with syngeneic *Daf1*^{+/+} ♀ to *Daf1*^{+/+} ♀ transplants showed rejection rates consistent with published reports (27–29). Our findings that the 1) elaboration of donor specific T and B cell responses, and 2) reductions in immunosuppressive cytokines by spleen cells, did not occur in the absence of DAF, argues that donor and recipient DAF activity are needed to suppress recipient adaptive responses against corneas irrespective of how low the threshold is of antigen mismatch. Because we wanted to examine the ocular tolerogenic immune response under conditions of limited antigen disparity where DAF's functional importance could be most sensitively tested, we focused on minor mismatch.

A large body of evidence (30), both in “high risk” transplants in humans and in allogeneic transplants in mice, has established that CD4⁺ T cells are the primary effectors that mediate corneal graft rejection (29). According to current concepts (2, 30, 31) while perforin and granzyme release by antigen specific CD8⁺ effector cells can contribute, the main CD4⁺ cell responses that cause corneal rejection are antigen specific generation of IFN-γ and TNF-α (21, 27).

Our recent work (12, 13, 15) has shown that DAF, originally characterized as an *intrinsic* cell surface complement inhibitor which protects self cells from autologous C3/C5 convertase activity on their surfaces (14), plays a central role in regulating T cell responses. The underlying mechanism is that during cognate APC•T cell interactions, both partners downregulate their DAF expression levels in conjunction with locally synthesizing C3, fB, fD, C5, C3aR and C5aR. The lowered junctional C3/C5 convertase inhibitory activity allows the assembly of alternative pathway C3 and C5 convertases at the adjacent APC and T cell surface. These convertases cleave C3a and C5a from the locally synthesized C3 and C5. GPCR signals conferred by the engagement of upregulated C3aR and C5aR on APCs and T

cells by the locally generated C3a and C5a induces phosphatidylinositol 3-kinase p110 γ (PI-3K γ) dependent phosphorylation of AKT in T cells necessary both for T cell costimulation (13) and T cell viability (13, 32). The locally produced anaphylotoxins also bind to their receptors on APCs inducing IL-12 production which elicits Th1 immunity (13, 33).

DAF thus functions as a barrier against the induction of proinflammatory type I cytokine secreting T cell responses known to mediate rejection. This new insight that APC and T cell produced complement is involved in T cell responses explains several past findings concerning effects of complement deficiency/depletion on outcomes of corneal and other transplantations. Notable among these are findings that a) corneal rejection rates of orthotopic guinea pig corneas in murine *C3*^{-/-} recipients are slower than in WT mice (29, 34), b) cobra venom factor (CoVF) depletion of serum complement, (which our studies with bone marrow chimeras (32) have shown) does not impact local complement production by APC and T cell partners, does not affect corneal transplantation outcomes (28), and c) in *Daf1*^{+/+} C3H recipients of renal transplants from allogeneic C57BL/6 *C3*^{-/-} mice, recipient allo responses do not occur and transplants are not rejected (35). Our recent work (13) has shown that while the absence of C3 in and of itself can have variable effects on rejection, the combined absence of C3 and C5 or of C3aR and C5aR and consequent abrogation of APC and T cell C5aR/C3aR signal transduction virtually abolishes costimulation-dependent T cell responses.

While cellular effectors are the primary mediators of corneal rejection, humoral immune responses have been implicated (2). In principle, alloantibody initiation of systemic complement activation could damage corneal endothelium either via terminal pathway mediated membrane injury or C3a/C5a-mediated recruitment of inflammatory cells. While our isotype ELISAs (Fig 4D) showed anti-HY*Dby* and HY*Uty* IgG2a and IgG2b antibodies absent DAF, immunohistological staining of transplants (Fig 3) showed no C3 deposition. That antibodies against HY antigens were elaborated is consistent with findings by others (36, 37) in σ to φ transplants. That systemic complement was not activated is consistent with the fact that HY antigens are intracellular (38, 39). In further support of the corneal rejection being T cell mediated, our studies of the effects of DAF deficiency on the tolerogenic state of the eye using chimeric *Daf1*^{+/+} mice reconstituted with *Daf1*^{-/-} marrow (Liu et al, unpublished) yielded results recapitulating those in unmanipulated *Daf1*^{-/-} animals. Collectively, the data reinforce the proposition that DAF is essential for shielding against recipient T and B cell responses to corneal grafts.

The findings in this study concur with data in other models, e.g. experimental autoimmune encephalitis (EAE) (15) and experimental autoimmune uveitis (EAU) (40), which have documented comparably enhanced (autoantigen) T cell reactivity in the absence of DAF. DAF is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (41). Since purified GPI-anchored proteins, when added to cells *in vitro*, reincorporate into their surface membranes, and once incorporated exert their native biological functions (14, 42), our findings open the possibility that increasing DAF expression by “painting” corneal endothelium or the ocular bed with purified GPI-anchored recombinant DAF prior to surgical implantation could have value in certain settings *e.g.* high risk transplants.

Acknowledgments

Supported by EY11288 (MEM), P30 EY11373 (JHL) and AI071125 (PSH). This work was initially motivated by the late Dr. Milton Singer.

This work was supported by NIH grant EY11288 (MEM), P30 Core EY015476, and AI071125 (PSH)

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FIGURE 1.

A) F5 ♂ $Daf1^{+/+}$ corneas were transplanted into F5 ♂ $Daf1^{+/+}$ (◆, n=10) or ♂ F5 $Daf1^{-/-}$ (▲, n=9) recipients and F5 ♂ $Daf1^{-/-}$ corneas were transplanted into F5 ♂ $Daf1^{+/+}$ (■, n=6) recipients. For the $Daf1^{+/+} \rightarrow Daf1^{+/+}$ group, the non-failure was 100% (1.0) at day 7, 80% at day 21, and >70 % at day 28. P values are given in the text. B–D) Corneas analyzed by H&E staining on day 28 from ♂ $Daf1^{+/+}$ to ♂ $Daf1^{+/+}$ transplants compared to those from ♂ $Daf1^{+/+}$ to $Daf1^{-/-}$ and from ♂ $Daf1^{-/-}$ to ♂ $Daf1^{+/+}$ transplants. *While some sections showed pigmented cells, others did not and there was no correlation with nonclarity.

FIGURE 2.

A) F5 ♂ $Daf1^{+/+}$ corneas were transplanted into F5 ♀ $Daf1^{+/+}$ (◆, n=9) or F5 $Daf1^{-/-}$ (▲, n=6) recipients and F5 ♂ $Daf1^{-/-}$ (■, n=7) corneas were transplanted into F5 ♀ $Daf1^{+/+}$ recipients. P values are given in the text. B) F12 ♂ $Daf1^{-/-}$ corneas were transplanted into F12 ♀ $Daf1^{+/+}$ (solid line, X, n=2) recipients, and F12 $Daf1^{+/+}$ ♂ (dotted line, △, n=2) were transplanted into F12 $Daf1^{-/-}$ ♀ recipients. As controls F12 ♂ $Daf1^{+/+}$ corneas were transplanted into F12 ♂ $Daf1^{+/+}$ (solid line, ◆, n=6) recipients, and F12 ♂ $Daf1^{+/+}$ (dotted line, ▲, n=6) corneas were transplanted into F12 $Daf1^{+/+}$ ♀ recipients. C) F12 ♂ $Daf1^{-/-}$ (◆, n=2) corneas were transplanted into F12 ♀ $Daf1^{-/-}$ recipients. As controls, F12 ♀

Daf1^{-/-} (■, n=5) or F12 ♂ *Daf1*^{-/-} (▲, n=3) corneas were transplanted into F12 ♂ *Daf1*^{-/-} recipients. P values are given in the text.

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FIGURE 3.

C3 deposition in 21 day $\text{♂ } Daf1^{+/+}$ corneas transplanted into $\text{♀ } Daf1^{-/-}$ or $\text{♀ } Daf1^{+/+}$ recipients (100x magnification). Isotype control stained sections are shown for comparison. Lower left insets in top panels are positive staining controls of sections of livers from ova transgenic mice sensitized *in vivo* with anti-ova antibody.

FIGURE 4.

A) CD4⁺ and CD8⁺ T cell IFN- γ responses of spleen cells harvested on day 28 from F5 ♀ *Daf1*^{+/+} and *Daf1*^{-/-} recipients of F5 ♂ *Daf1*^{+/+} corneas and from F5 ♀ *Daf1*^{+/+} recipients of ♂ *Daf1*^{+/+} or ♂ *Daf1*^{-/-} corneas. B) CD4⁺ and CD8⁺ T cell IFN- γ responses of spleen cells harvested on day 35 from F12 ♂ *Daf1*^{+/+} corneas to F12 ♀ *Daf1*^{+/+} or F12 ♀ *Daf1*^{-/-} recipients and F12 ♂ *Daf1*^{-/-} corneas to F12 ♀ *Daf1*^{+/+} recipients. C) Anti-Dby and anti-Uty Ig isotype levels were assayed in 21 day plasma from ♀ *Daf1*^{+/+} recipients of ♂ *Daf1*^{+/+} or *Daf1*^{-/-} corneas.

FIGURE 5.

♂ *Daf1*^{+/+} corneas were transplanted into ♀ *Daf1*^{-/-} or *Daf1*^{+/+} recipients. Day 35 recipient splenocytes were incubated for 3 days with HY *Dby* or HY *Uty* after which IL-10 and TGF-β levels in supernatants were assayed by specific ELISAs.