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Immune modulation in corneal transplantation☆

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Abstract

Allograft rejection is the most common reason for corneal transplant failure, despite the immunologic privilege of both the graft and the anterior chamber. To prevent corneal allograft rejection, various immunomodulatory strategies have been used in experimental corneal transplantation. These include (1) anti–T-cell receptor and T-cell depletion therapy; (2) manipulation of costimulatory molecule function, including both down-regulation of positive stimulatory molecules and/or up-regulation of inhibitory molecules and overproduction of tumor necrosis factor–related, apoptosis-induced ligand; (3) modulation of cytokine production by reducing proinflammatory cytokines (tumor necrosis factor α, interleukin [IL]–12, and IL-1) and/or increasing immunoregulatory cytokines (IL-10 and IL-4); (4) macrophage depletion; and (5) overexpression of the immunomodulatory molecule indoleamine 2,3-dioxygenase. Although these approaches appear promising in animal corneal transplantation models, there has been very little translation of these immunomodulatory approaches in human corneal transplantation.

1. Introduction: corneal structure, endothelial function, and immune privilege

Corneal transplantation (keratoplasty) is the only form of therapy for many corneal diseases leading to blindness [1]. It is also the most commonly performed graft, more than all heart, liver, and kidney transplants [2]. Most corneal transplants are performed by excision of a 7–8-mm-diameter circle from the 11-mm diameter recipient cornea and replacement by excised donor cornea of similar diameter. The anatomy of cornea is relatively simple, consisting of 3 major layers (Fig. 1). The anterior surface of the cornea is a 6–8-cell-deep epithelial layer. The main thickness of the cornea is formed by the stroma, consisting of precisely aligned collagen fibers supported by scattered keratocytes. The posterior surface is composed of an endothelial monolayer, critical for maintenance of corneal transparency. In an energy-consuming process, endothelial cells pump water from the stroma to the anterior chamber. The endothelial cell monolayer is nonreplicative in humans. In consequence, corneal disorders or intraocular surgery, which results in loss of a significant number of endothelial cells, lead to decompensation of pump function, stromal swelling, and loss of transparency and vision [3].

The cornea is an immune privileged tissue because of several factors, including (1) the absence of lymphatic and blood vessels in the corneal graft bed, (2) the expression of Fas ligand on corneal cells [4,5], (3) low-level expression of major histocompatibility complex class I and II molecules on corneal cells [6-9], (4) the paucity of indigenous professional antigen-presenting macrophages or Langerhans cells [10,11], (5) the phenomenon of anterior chamber-associated immune deviation (ACAID) in which down-regulation of systemic delayed-type hypersensetivity results from introduction of alloantigens to the anterior chamber [12,13], and (6) the presence in normal aqueous humor in the anterior chamber of immunomodulatory cytokines such as α-melanocyte–stimulating hormone and transforming growth factor [14]. Despite this relative immune privilege, the success rate of corneal transplantation is less than what is generally appreciated, with approximately 25% of corneas being lost by 4–5 years [15]. Loss of components of immune privilege results in high risk of rejection and very much lower survival rates [16-18].

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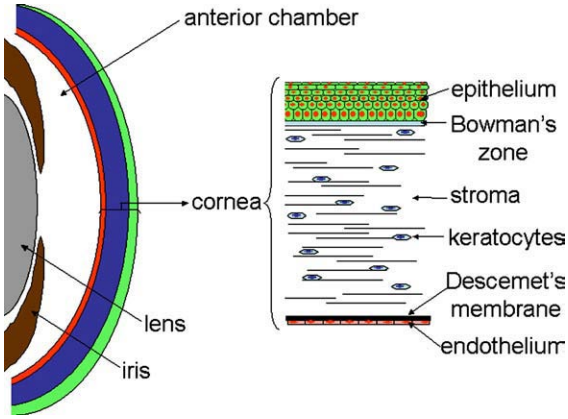

Fig. 1. Anatomy of the cornea. The cornea is a clear, dome-shaped structure at the front of the eye. It is 0.5–0.7mm in thickness and contains 3 layers. The epithelium is on the exterior of the cornea and comprises a 6–8 cell thickness layer resting on Bowman's zone. The stroma forms most of corneal thickness and is composed of a highly organized arrangement of collagen fibrils and kerotocytes aligned parallel to the cornea surface. The endothelium forms a continuous hexagonal monolayer supported by the Descemet's membrane. Reprinted with permission from [3] by Wiley-Blackwell Publishing Ltd.

Recipient T-cell recognition of donor major histocompatibility complex (MHC) alloantigens plays a central role in the rejection of organ allografts. This occurs by 2 distinct mechanisms, called the direct and indirect pathways. In the direct pathway intact donor major and minor histocompatibility complex molecules on the surface of donor antigen-presenting cells (APCs) are recognized directly by recipient T cells. It is believed that the direct pathway plays an important role in acute graft rejection. In the indirect pathway, donor MHC molecules are taken up, processed, and presented as peptides in the context of recipient MHC molecules to recipient T cells. This pathway has been associated with chronic graft rejection. Although the direct pathway weakens with time as the donor APCs migrate out of graft, the indirect pathway is likely to be permanently active because of traffic of recipient APCs through the graft [19,20]. Because of the reduced level of MHC expression on corneal tissue and the paucity of APCs in the cornea, allorecognition of donor cornea occurs via the indirect pathway [21-24].

2. Current strategies for prevention of rejection in clinical corneal transplantation

Topical glucocorticosteroid treatment is the mainstay of immunosuppression in corneal transplantation in patients other than those at recognized high rejection risk [25]. Although topical steroid treatment can prevent or reverse rejection episodes [26], it is much less effective in preventing graft failure in cases at high rejection risk [27,28]. This is seen in clinical contexts such as transplantation into a recipient corneal bed, which is vascularized, actively inflamed or bears a rejected previous graft. Graft survival is so much shorter in these settings that most patients with good vision in the contralateral eye are not offered a transplant. This is the impetus for developing immunomodulation approaches, which, by their specificity, would prevent rejection without the adverse effects of systemic immunosuppression [27,29,30]. The evidence base to support the use of systemic immunosuppressive agents in corneal transplantation is limited. Long-term immunosuppression with oral steroid or calcineurin inhibitors is associated with such significant adverse effects that this approach is difficult to justify in patients in whom vision is good in the contralateral eye [31,32]. In contrast also with vascularized organ transplantation, histocompatibility matching in corneal transplantation has not found international consensus among clinicians [33], largely because the findings of many clinical human leukocyte antigen (HLA)–matching studies during the last 30 years have not been consistent. The American Collaborative Corneal Transplant Study reported no benefit from HLA class I and class II antigen matching [30]. In contrast, the UK Collaborative Corneal Transplantation Follow-up Study reported a small increased risk of rejection with HLA-A and HLA-B mismatching and a converse detrimental effect of HLA-DR matching [34]. Development of experimental approaches for local allospecific immunomodulation has clear appeal.

3. Anti–T-cell receptor antibody in corneal transplantation

Anti–T-cell receptor monoclonal antibody (mAb) has been reported to be a potent immunosuppressant and used in various transplant models [35-38]. In 1999, Yamagami et al [39] reported the effect of R73, an anti-$\alpha\beta$ T-cell receptor mAb, on allograft rejection in a rat model of corneal transplantation. R73 was administered intraperitoneally after the kerotoplasty and resulted in indefinite survival rate of R73-treated rats. It was also demonstrated that delayed-type hypersensitivity responses were suppressed in the R73-treated group. Therefore, anti-$\alpha\beta$ T-cell receptor mAb appears to be one modality of immunomodulation, which is effective in preventing corneal allograft rejection [39-42].

4. T-cell depletion therapy

T cells, especially CD4 T cells, have been shown to be important in graft rejection [16-18]. There have been several attempts to prolong corneal graft survival by depletion of T cells using anti-CD4 and/or anti-CD8 monoclonal antibodies. In 1992, Ayliffe et al [43] demonstrated that although CD4 T cells were not eliminated completely by anti-CD4 antibodies, there was a profound delay in the rejection times of orthotopic rat corneal allografts. A third of the CD4-depleted rats failed to reject corneal allografts by 100 days post grafting. In contrast, they found the anti-CD8 antibody treatment did not prolong the graft survival. Pleyer et al [44]

reported that topical application of anti-CD4 mAb prolonged corneal allograft survival in a rat model. Most recently, another group used a corneal rat-to-mouse xenotransplantation model to show that treatment of xenograft recipients with anti-CD4 antibody resulted in a significant prolongation of corneal xenograft survival [45]. It is clear from these reports that treatment with anti-CD4 antibodies may have a useful clinical application. Although there are no reports of a beneficial effect of anti-CD8 treatment on corneal allograft survival, the role of CD8 T cells in mediating delayed rejection of corneal xenografts was reported by Higuchi et al [46]. They reconstituted severely combined immunodeficiency mice, which do not normally reject guinea pig grafts, with purified CD8 T cells and found that guinea pig cornea xenografts that avoided acute rejection in CD4 T cell–depleted mice were vulnerable to rejection by CD8 T cells. These CD8 T cells can destroy xenografts through release of proinflammatory mediators such as interferon (IFN)–γ rather than cytotoxity. Further studies are needed to clarify the effect of anti-CD8 treatment on graft survival. It is noteworthy that use of anti-CD3 mAb in treatment, rather than prevention, of corneal allograft rejection in humans was reported some years before the above studies [40]. In addition, Newman et al [41] reported in 1 patient that corneal graft rejection was inhibited by depletion of lymphocytes with the anti-lymphocyte mAb, CAMPATH-1H. This is a fully humanized mAb which recognizes the pan-lymphocyte antigen CD52.

5. Modulation of costimulatory molecules to inhibit T-cell activation

5.1. Inhibition of CD28-B7 pathway by cytotoxic T-lymphocyte antigen 4 (CTLA4)–immunoglobulin (Ig) and anti-CD28 antibody

The specific recognition of peptide-MHCs by T-cell receptor is not sufficient to fully activate the T cells. Costimulation is required, in the absence of which the T cell will become unresponsive [47]. The most potent costimulatory molecules are the B7 family, including CD80 and CD86 [48] (Table 1). CD28 is the main costimulatory ligand expressed on naive T cells [49]. It has been shown that CD28/B7 ligation synergizes with the T-cell receptor engagement to lower T-cell activation threshold, enhance proliferation, and increase cell survival by augmenting secretion of multiple cytokines [48,50]. Cytotoxic T-lymphocyte antigen 4 (CD152), the alternative T cell ligand for B7, is an inhibitory receptor limiting T-cell activation [50,51]. The importance of CTLA4 as a negative regulatory costimulatory molecules for T cells is highlighted by the finding that CTLA4-deficient mice develop a fatal lymphoproliferative disorder with multiple autoimmune diseases [52,53]. CTLA4 binds B7 with a higher affinity than does CD28. Ligation of CD28 by B7 can be blocked by CTLA4-Ig (a fusion protein comprising CTLA4 and the Fc part of an antibody molecule).

Table 1
Expression and function of costimulatory molecules

	T cell	APC	Function
"Positive" costimulators	CD28	B7-1 or B7-2 (CD80 or CD86)	Increase T-cell proliferation and cytokine production, such as IL-2
	ICOS	B7RP-1	ICOS is induced after TCR engagement. Signaling through ICOS is important for T-cell activation and proliferation.
	CD154 (CD40L)	CD40	Binding of CD154 to CD40 induces up-regulation of B7 to enhance T-cell activation.
"Negative" costimulators	CTLA4	B7-1 or B7-2 (CD80 or CD86)	Decrease T-cell proliferation and cytokine production; promote energy and tolerance
	PD-1	PD-L1 or PD-L2	Engagement of PD-2 by its ligands decreases T-cell proliferation and cytokine production.

Blockage of costimulatory molecules can be used to induce anergy in alloreactive T cells [54].

Several studies have been carried out to investigate the role of CTLA4-Ig in preventing corneal allograft rejection. In 1997, Hoffmann et al [55] have found that intraperitoneal injection of 300 μg of CTLA4-Ig prolonged the survival of murine corneal allografts, whereas topical and systemic application of 130 μg of CTLA4-Ig had no influence on graft survival. In 1999, Gebhardt et al [56] incubated donor corneas from Dutch belted rabbits in culture medium containing different concentrations of soluble CTLA4-Ig and then transplanted the cornea into the prevascularized and normal avascular corneas of New Zealand White rabbit recipients. They found that grafts placed in avascular corneas showed no difference in survival times. Among the grafts placed in vascularized corneas, those incubated with CTLA4-Ig at a concentration of 250 μg/mL rejected at normal tempo, whereas grafts incubated with CTLA4-Ig at concentrations of 1 and 10 mg/mL had extended survival times. These data suggest that CTLA4 protein is needed in excess to effectively block costimulation via CD28. Investigation of this pathway was taken further in the rat model by Comer et al [57]. They incubated the excised Brown Norway rat donor cornea with CTLA4-Ig protein or a recombinant adenovirus vector bearing CTLA4-Ig complementary DNA (cDNA) before grafting into Lewis strain recipients. Both protein and gene-based administration of CTLA4-Ig prolonged allograft survival. The same fusion protein and viral vector were administered by intraperitoneal injection post grafting, resulting in significant extension of graft survival in the cDNA-treated graft recipients only.

Similar data were also obtained from other studies, which showed corneal allograft survival was significantly prolonged by blockage of the CD28-B7 pathway with CTLA4-Ig [58-60]. Most recently, Gong et al [61] analyzed the effects of ex vivo or in vivo administration of adenovirus-bearing CTLA4-Ig cDNA on prolonging allograft survival using rat corneal transplant model. They showed that ex vivo gene transfer into donor cornea resulted in more modest prolongation of graft survival than systemic gene therapy.

Other approaches have been used to study the effect of blockage of the CD28 pathway on corneal allograft survival. Thiel et al [59] reported significantly prolonged rat corneal allograft survival after systemic short-term administration of anti-CD28 mAb. Han et al [62] investigated the effects of mouse CTLA4-Ig gene-modified dendritic cells (DCs) on the survival of the corneal allografts in rats: the plasmid-expressing CTLA4-Ig was transfected into DCs of F344 rats. Corneal transplantation was performed from F344 rats to Lewis rats. The DCs modified with CTLA4-Ig gene were injected into the Lewis rats on the day 0 and 3 after transplantation, resulting in reduced proliferation of allogeneic T cells and prolonged survival of corneal allografts. In addition to these studies, Konig Merediz et al [63] investigated the role of CTLA4-Ig in corneal allograft rejection by ballistic transfer of CTLA4-Ig expressing construct to the corneal epithelium in mice after corneal allograft transplantation using a gene gun. They demonstrated a beneficial effect of gene transfer of CTLA4-Ig in comparison with the gene gun–treated control group but not to the corticosteroid-treated control group. Although the beneficial effect of CTLA4-Ig gene therapy appeared attenuated by the epithelial destruction associated with the gene gun technique, the beneficial effect of CTLA4-Ig gene transfer to the corneal epithelium was again demonstrated in this study.

There are a few mechanisms by which CTLA4-Ig may down-regulate the immune response. Firstly, CTLA4 ligation may augment the production of inhibitory cytokines, such as transforming growth factor-β [64]. In natural settings, ligation of CD80 and CD86 may occur through CTLA4 that is expressed at the surface of regulatory $CD4^+$ T cells [65]. Regulatory $CD4^+$ T cells have been shown to up-regulate indoleamine 2,3-dioxygenase (IDO) expression in DCs in a CTLA4 dependent manor. Evidence has recently emerged that CTLA4 regulates tryptophan catabolism through induction of IDO. This causes a localized tryptophan deficiency, to which proliferating T cells are particularly sensitive [66-69].

5.2. Inhibition of inducible costimulatory molecule costimulation molecule

Inducible costimulatory molecule (ICOS), a CD28 homologue, is also a T-cell costimulatory molecule. ICOS is induced after TCR engagement and expressed only on activated T cells and resting memory T cells [70]. The expression of the ICOS ligand, B7RP-1, is poorly understood, and little is known about the pathway through which ICOS signals. Despite this, it has been demonstrated that signaling through ICOS is important in T-cell activation and proliferation [71]. Blockage of ICOS-B7RP-1 ligation with ICOS-Ig fusion protein suppresses proliferation of T cells responding to allogeneic DCs [72], and ICOS blockade or ICOS deficiency prevents rejection of vascularized cardiac allografts [73]. However, Watson et al [74] found that survival of fully mismatched corneal allografts was similar in BALB/c recipients treated with anti-ICOS antibody or isotype control, suggesting that signaling through ICOS may be less important in corneal allergenic rejection. Further studies are needed to clarify the significance of the ICOS signaling pathway in corneal transplantation and determine the immunologic features of cornea compared with other transplanted tissues which underlie the divergent effects of ICOS modulation.

5.3. Enhancement of programed death–1 inhibitory signal

One of the most recently characterized members of CD28 family, programed death (PD)–1 (PD-1), shares approximately 20% homology with CTLA4. Programmed death–1 expression is induced on activated CD4 and CD8 T cells, B cells, and macrophages [75]. PD-1 binds to 2 ligands, PD-L1 (B7-H1) and PD-L2, which are expressed by APC [76,77]. Like CTLA4, engagement of PD-1 results in a negative regulatory effect with decreased cellular proliferation and cytokine production by T cells. Nishimura et al [78] showed that PD-1 deficiency leads to autoimmune diseases.

Using a murine corneal transplantation model, Watson et al [74] studied the effect of PD-1 modulation on corneal allograft survival. They found PD-L1–Ig fusion protein treatment of BALB/c mice prolonged fully MHC-mismatched C3H donor corneal allograft survival. In another study, Hori et al [79] showed that PD-L1 plays a critical role in corneal allograft survival. In PD-L1–expressing corneal allografts, apoptosis of the infiltrating PD-1(+), CD4(+), or CD8(+) T cells was observed, after which there was allograft acceptance. In contrast, PD-L1 blockade suppressed apoptosis of infiltrating PD-1(+) T cells, which led to allograft rejection. These reports suggest that PD-L1 expressed on corneal endothelial cells maintains long-term acceptance of the corneal allografts by inducing apoptosis of effector T cells within the cornea.

5.4. Modulation of CD154-CD40 pathway

The CD154-CD40 is the prototypic costimulatory pathway. CD154 (CD40L) is expressed after activation of CD4 T cells, some CD8 T cells, natural killer cells and eosinophils. Its ligand CD40 is constitutively expressed on APC such as B cells, macrophages, and DCs. CD40 can also be induced on endothelial cells and fibroblasts. The interaction between CD154 and CD40 provides a bidirectional signal for T cell and B cell activation. Binding of CD154 to CD40 on the B cells delivers additional signals to the B cells, which are essential for the B cells to switch antibody class. Individuals with CD154 gene mutations cannot make functional CD154,

which results in hyper-IgM syndrome [80]. On the other hand, transduction of signals through CD40 induces up-regulation of CD80/86 and therefore helps to provide further costimulatory signals to T cells and so enhance T-cell activation [81-83].

Blockade of CD154-CD40 interaction has been shown to prevent allograft rejection in many transplant models. Two groups have demonstrated that CD40 blockade using anti-CD154 antibody is effective in preventing acute allograft rejection in a mouse cardiac graft model [84,85]. Kirk et al [86,87] showed that treatment of rhesus monkeys with an antibody to CD154 allowed for prolonged survival of renal allografts. Similar data were also obtained in an islet transplant model [88,89]. In the corneal transplant setting, Qian et al [90,91] assessed the effect of either locally (subconjunctival injection) or systemically (intraperitoneal injection at surgery and once weekly after surgery) administered anti-CD154 mAb on survival of murine corneal allografts They found that both local ocular and systemic administration of anti-CD154 was effective in the prevention of corneal allograft rejection. However, termination of anti-CD154 mAb treatment led to some loss in graft survival. These authors also demonstrated the effects of anti-CD154 therapy on T-cell cytokine profiles and ocular chemokine gene expression after corneal transplantation in recipients at high risk for rejection. Frequencies of T_H1 but not T_H2 cytokine-producing T cells were significantly reduced in anti-CD154, mAb-treated hosts, and leukocyte infiltration was profoundly suppressed in grafts in anti-CD154 mAb-treated hosts. These data demonstrated that blockade of the CD40-CD154 costimulatory pathway after corneal transplantation inhibits T_H1-mediated responses and leukocytic infiltration into allografts [92]. In another study, Ardjomand et al [58] examined the effect of modulating the CD154-CD40 pathway in a murine model of corneal allograft rejection. The BALB/c mice received corneal grafts from fully MHC-mismatched C3H donors and were treated with anti-CD154 mAb on days 0, 2, and 4 after transplantation. Corneal allograft survival was significantly prolonged. However, as Rothstein and Sayegh [93] pointed out, this therapy appeared to induce prolonged immunosuppression rather than true tolerance, as recipients in which therapy was withdrawn ultimately rejected their grafts. It is possible the combination of blockage of CD40-CD154 costimulatory pathway with other approaches could achieve a better outcome. Some studies indicated the synergy between B7 and CD154 blockage in murine skin and cardiac allograft survival model [84]. The efficacy of CTLA4-Ig and anti-CD154 antibody in primate islet and renal transplantation model has also been demonstrated [54,86]. Ardjomand et al [58] tested the combination of blockage of CD40-CD154 costimulatory pathway with CTLA4-Ig on murine corneal transplantation model. They found that corneal allograft survival in wild-type BALB/c mice (median survival time [MST], 14 days) was significantly prolonged by blockade of the costimulatory pathways with CTLA4-Ig or anti-CD154 antibody (MST 21 days and 25 days, respectively). Median survival time in recipients treated with CTLA4-Ig and anti-CD154 antibody in combination was 29 days, not significantly longer than graft survival in single-treatment groups.

5.5. Modulation of tumor necrosis factor–related apoptosis-induced ligand in corneal transplantation

Tumor necrosis factor (TNF)-related apoptosis-induced ligand (TRAIL) is a newly identified member of the TNF superfamily [94]. Other members of the TNF family, such as Fas ligand, have been shown to contribute to the immune privilege of ocular tissues and protects allografts from rejection [4,5]. The TRAIL has been shown to inhibit autoimmune diseases such as arthritis [95]. Xie et al [96] found that donor mouse corneas transduced by adenovirus vector expressing TRAIL survived significantly longer than the control grafts. Furthermore, blocking the endogenous TRAIL accelerated allograft rejection. More apoptotic cells were found in the graft of TRAIL-treated group than in other groups, which suggests that TRAIL-induced apoptosis of inflammatory cells in the corneal allograft. The TRAIL appears to play an important role in corneal allograft rejection although the exact mechanism by which TRAIL inhibits corneal allograft rejection is not clear.

6. Modulation of cytokine production

Cytokines secreted by T cells, especially CD4 T cells, are critical to allogeneic responses. The immune response resulting in graft rejection is characterized by the strong expression of T_H1-type cytokines, such as IFN-γ and interleukin (IL)–2 and, in general, lower expression of T_H2-type cytokines, such as IL-10 and IL-4 [97-99]. Sagoo et al [100] reported that inflammatory cytokines, such as IL-1, TNF-α, and IFN-γ, induced apoptosis of corneal endothelium through a nitric oxide dependent pathway. By contrast, T_H2 cytokines have been shown to have immunosuppressive properties and, thus, can down-regulate the cell-mediated response and production of T_H1 cytokines [101]. Thus, a shift from a proinflammatory T_H1 response to the IL-4 and IL-10 producing T_H2 response has been associated with allograft survival [102,103]. One reported example is that prolonged allograft survival in CTLA4-Ig–treated animals is associated with the increased production of IL-4 and IL-10 [104]. Therefore, inhibition of proinflammatory cytokines, such as TNF-α and IL-12, or up-regulation of the T_H2 subtype cytokines, is an attractive strategy for prevention of corneal graft rejection.

6.1. Inhibition of TNF

Corneal allograft rejection is a T-cell–mediated process with CD4 T cells as a pivotal contributor [16-18]. $CD4^+$ T cells secrete a variety of cytokines, such as TNF, that may result in corneal allograft rejection. There is a considerable

body of evidence that TNF has a role in corneal allograft rejection. In 1997, Larkin et al [105] reported staining of graft stroma–infiltrating cells for TNF after rejection onset. In 1999, Zhu et al [106] also reported that high levels (by enzyme-linked immunosorbent assay) of TNF were present in murine corneal allografts. In agreement with these data, Rayner et al [107,108] demonstrated spikes of bioactive TNF in aqueous humor in the anterior chamber before and at observed onset of endothelial graft rejection. They went on to study the effect on rabbit corneal allograft survival of prior ex vivo graft transfection with an adenovirus vector with cDNA encoding soluble TNF receptor fusion protein. Transplanted gene-modified corneas showed marginally increased survival time over mock-transfected control corneas but a significantly increased survival time over vector control adenovirus-transfected control corneas. It was concluded that this immunogenic response produced by adenovirus infection might partially counteract the beneficial effect on graft survival of anti-TNF therapy [108].

6.2. Inhibition of IL-12

IL-12 is the most important cytokine in determining T_H1 development. It binds to IL-12 receptor on $CD4^+$ T cells and signals differentiation toward the T_H1 pathway. It can also stimulate the production of IFN-γ [109]. IL-12 consists of disulfide-linked 35-and 40-kd subunits. Both subunits are required to make functional IL-12 [110,111]. The IL-12 p40 subunits act as a natural antagonist for IL-12 in vivo [112,113]. Antagonist activity of I-12 p40 for IL-12 as an immunosuppressant has been examined in many transplantation models. Klebe et al [114] studied the effect of local production of IL-12 p40 in sheep donor corneas on prolongation of allograft survival using an adenovirus-mediated gene transfer, finding that IL-12 p40 gene modified corneas survived significantly longer than control corneas.

6.3. Inhibition of IL-1

Interleukin–1 is a potent proinflammatory cytokine that plays an important role in initiating and maintaining immunogenic inflammation [115,116]. IL-1 activity has been correlated with corneal vascularization [117], and IL-1–mediated Langerhans cell migration can play a critical role in host allosensitization in the setting of corneal transplantation [116,118]. IL-1 receptor antagonist (RA) is a naturally occurring IL-1 isoform with high-affinity to IL-1 receptor but has no agonist activity [119]. Dana et al [120] reported a series experiments to determine whether the topical application of IL-1 RA can prolong mouse corneal transplant survival. Survival rates of transplants increased significantly in IL-1 RA–treated mice compared with the control group. The effect of IL-1 RA on graft survival has also been demonstrated by another group using rat keratoplasty model [121]. Dana et al further demonstrated that IL-1 RA–treated grafts had significantly less inflammation and Langerhans cells infiltration [122,123]. Because topical IL-1 RA can prevent delayed-type hypersensitivity-type sensitization to corneal grafts by down-regulating APC function and local inflammation, the same group went on further to determine whether topical treatment with IL-1 RA can promote the early induction of tolerogenic allospecific ACAID in grafted eyes. However, they found that treatment with IL-1 RA did not alter induction of donor-specific allospecific ACAID after transplantation, suggesting that IL-1 RA promotes graft survival almost exclusively by virtue of suppressing inflammation and not by directly promoting tolerance [124]. In addition, Zhang et al [125] reported that cell apoptosis played an important role in corneal allograft rejection, and IL-1 RA treatment can prolong the survival time of corneal grafts by suppressing cell apoptosis in corneal grafts. It seems that there are multiple mechanisms that can explain the effect of IL-1 RA in suppressing graft rejection.

6.4. Overexpression of IL-10

The immunoregulatory cytokine IL-10 is produced by T_H2-subtype T cells, B cells, monocytes, and macrophages. This cytokine down-regulates MHC class II and costimulatory molecules on DCs, monocytes, and macrophages. It also inhibits the synthesis of proinflammatory cytokines such as TNF [126,127]. The Epstein-Barr virus–encoded IL-10 homologue, vIL-10, shares 84% sequence identity with human IL-10 and shows several of its immunosuppressive activities but not stimulatory effects on cytotoxic T cells and natural killer cells [128,129]. Therefore, vIL-10 is an attractive target to be expressed in allogeneic transplants to decrease graft immunogenicity, reduce effective antigen presentation, and inhibit inflammation. In 1999, Torres et al [130] investigated whether the administration of recombinant murine IL-10 prolonged rat corneal allograft survival. Mice were injected intraperitoneally with murine IL-10 before the surgery; on the day of grafting; and on postoperative days 2, 4, and 6. However, they found that IL-10 treatment did not prolong corneal allograft survival. Using a different approach, Klebe et al [131,131] studied the effect of IL-10 overexpression in donor corneas on corneal allograft survival using a sheep model. In this gene-based approach, donor corneas were transduced ex vivo with an adenoviral vector encoding ovine IL-10 before transplantation. The transfected donor corneas had prolonged survival (median, 55 days) compared with control corneas (median, 21 days). No inflammatory response was noted with recombinant adenovirus in this study. In contrast, Gong et al [132] showed that local expression of vIL-10 in donor rat corneas (transfected with a vIL-10–expressing adenovirus vector or liposome/vIL-10 plasmid DNA mixtures) did not prolong corneal allograft survival. However, in contrast to this local approach, systemic adenovirus-mediated vIL-10 gene therapy (graft recipients treated with intraperitoneally vector 1 day before transplantation) did significantly prolong allograft survival. Although corneal graft survival was prolonged, antiadenovirus immunity was induced after systemic injection of

adenoviral vector encoding IL-10. When high dose of adenovirus (1.0×10^{10}) was injected, no prolongation of corneal graft survival was observed, and no IL-10 protein was detected. [133,134]. High levels of antiadenovirus antibodies were found in the serum samples of high-dose adenovirus treated animals, suggesting that high doses of adenovirus may induce a strong immune response which is able to overcome the beneficial effect of IL-10 [132].

6.5. Overexpression of IL-4

Interleukin–4, regarded as one of the key cytokines that support T_H2 response, has been associated with the acquisition of tolerance [102,135,136]. To this end, several groups have studied the role of IL-4 in corneal transplantation. Pleyer et al [137] studied the effect of adenovirus-mediated gene transfer of IL-4 to rat corneas ex vivo before transplantation and found that overexpression of IL-4 did not reduce the rejection rate of corneal allografts. Consistent with this work, Klebe et al [114] also found that local expression of IL-4 by ovine donor corneas after ex vivo infection by adenovirus encoding IL-4 did not prolong the corneal allograft survival in a proportion of sheep recipients. Adenovirus was used by these 2 groups. It is known that adenovirus is a nonintegrative virus and that viral DNA remains episomal. Moreover, as discussed above, adenovirus can induce a immune response which may be able to overcome the beneficial effect of transgene [133,134]. This may possibly explain why Konig Merediz et al [63] observed prolongation of murine corneal allograft survival after IL-4 gene transfer to corneal epithelium using a gene gun technique. However, the gene gun technique itself caused epithelial destruction, which could overcome the favorable effect of IL-4. Therefore, the functional effect of IL-4 modulation on corneal allograft survival requires further studies.

7. Function of IDO in prolongation of allograft survival

Indoleamine 2,3-dioxygenase is important enzyme in the regulation of the immune response. It catabolizes the essential amino acid tryptophan, leading to depletion of tryptophan and to production of metabolites, such as kynurenine [138]. This has the effect of down-regulating T-cell activation [139-142]. The importance of IDO was first recognized in the context of immune privilege of the placenta, where high levels of the enzyme in syncytial trophoblasts in part prevent immunologic rejection of the fetus [143]. Localized depletion of tryptophan in vivo has also been implicated in the immune evasion of certain tumors [144,145].

Manipulation of IDO expression has been shown to delay graft rejection. IDO overexpression in pancreatic islets prolongs graft survival [146]. Beutelspacher et al [147] investigated the effect of IDO overexpression in donor murine cornea on allograft survival. Excised donor C3H corneas were transduced ex vivo with equine infectious anemia virus expressing IDO before transplantation into fully mismatched BALB/c recipients. Significantly prolonged survival of IDO-transduced allografts (MST, 21 days) resulted, compared with green fluorescent protein-transduced or mock-transduced corneas (MST, 11 days). Immunohistochemistry indicated IDO expression in the endothelial layer.

8. Effect of macrophage depletion on corneal allograft survival

As discussed in the Introduction, the current opinion is that the corneal allograft rejection is mediated by T lymphocytes [16-18]. However, using histopathologic studies of corneal allograft rejection, Holland et al [148] found an influx of not only T lymphocytes but also macrophages. In 1994, using clodronate liposomes to selectively deplete macrophages, Van der Veen et al [149] investigated the function of macrophages in corneal allograft rejection. They found that subconjunctival administration of clodronate liposomes to recipients after corneal allograft transplantation resulted in complete graft survival in all treated rats. In 2000, Slegers et al [150] determined the effect of macrophage depletion on immune effector mechanisms during corneal allograft rejection in rats. They concluded that local depletion of macrophages by local treatment with clodronate liposomes down-regulated local and systemic cytotoxic T-lymphocyte responses and prevented the generation of antibodies, which led to a less vigorous attack on the graft tissue and therefore promoted allograft survival.

9. Conclusion

Different strategies to modulate the immune responses have been discussed in corneal allograft transplantation. Although individual approach appears to be promising in prolongation of allograft survival, it may not be sufficient to induce long-term tolerance in transplants [93]. Therefore, there is still space to develop new protocols or improve the old ones to induce true tolerance in transplantation. It may also be necessary to combine different methods to achieve the ultimate goal of prevention of allograft rejection [84,151].

Corneal transplantation represents a useful model for studying the indirect pathway of allorecognition. The indirect pathway has been regarded as the driving force for chronic graft rejection in other solid organ transplantation. Although acute rejection is better understood and treated, late loss of graft due to chronic rejection mediated via the indirect pathway is an ongoing problem in the transplantation field [152]. The cornea is an excellent candidate for investigation of the indirect pathway because of its accessibility, the relative simple corneal anatomy, and the ability to maintain grafts in culture for up to 4 weeks [153]. Progress in prolonging corneal allografts will shed light on long-term transplant survival in other organs.

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