Cellular and Molecular Life Sciences (2018) 75:1509–1520
https://doi.org/10.1007/s00018-017-2739-y

REVIEW

Therapeutic approaches for induction of tolerance and immune quiescence in corneal allotransplantation

Maryam Tahvildari[1,2] · Afsaneh Amouzegar[1] · William Foulsham[1] · Reza Dana[1]

Received: 3 August 2017 / Revised: 12 December 2017 / Accepted: 27 December 2017 / Published online: 6 January 2018

Abstract

The cornea is the most commonly transplanted tissue in the body. Corneal grafts in low-risk recipients enjoy high success rates, yet over 50% of high-risk grafts (with inflamed and vascularized host beds) are rejected. As our understanding of the cellular and molecular pathways that mediate rejection has deepened, a number of novel therapeutic strategies have been unveiled. This manuscript reviews therapeutic approaches to promote corneal transplant survival through targeting (1) corneal lymphangiogenesis and hemangiogenesis, (2) antigen presenting cells, (3) effector and regulatory T cells, and (4) mesenchymal stem cells.

Keywords Corneal transplantation · Graft rejection · Allotolerance · Regulatory T cells · Antigen presenting cells · Corneal hemangiogenesis · Corneal lymphangiogenesis

Introduction

Corneal transplantation is the most common form of tissue transplantation performed worldwide. The cornea, due to its lack of blood and lymphatic vessels and the scarcity of resident immune cells, is regarded an immune privileged tissue, and thereby offers a highly favorable environment for allograft acceptance [1, 2]. Resident antigen presenting cells (APCs) in the cornea normally stay in an inactive state; these immature cells are integral to induction of tolerance against alloantigens [3]. The immune privileged status of cornea is reflected in high rates of graft survival in uninflamed and avascular host beds [4]. Indeed, success rates of corneal grafts in these low-risk graft recipients are estimated to be 90% at 1 year and 55% at 15 years [5, 6]. However, the tolerogenic milieu of cornea is abrogated in inflamed and vascularized host beds, in which failure rates exceed 50% despite maximal immunosuppressive therapy [7].

Immunosuppressive medications, in particular corticosteroids, remain the primary therapeutic strategy for prevention of allograft rejection. However, their use is associated with numerous side effects. The prolonged use of steroids is associated with serious side effects including cataract, glaucoma and opportunistic infections [8]. Over the past three decades, multiple studies have explored the cellular mechanisms underlying immune-mediated corneal graft rejection, with the aim of developing targeted therapies that could dampen the immune response towards the allograft without compromising the integrity of the immune system. The purpose of this review is to summarize the results of these studies, and to propose strategies with potential to prevent alloantigen-specific immune rejection in high-risk human corneal transplantation.

Hemangiogenesis and lymphangiogenesis

The blood and lymphatic vascular systems play critical roles in both delivering oxygen and nutrients to tissues, as well as by draining redundant fluid and enabling the immune system to respond to foreign antigens. Hemangiogenesis describes the growth of new blood vessels, and can be either physiological (as in the case of wound healing)

Maryam Tahvildari, Afsaneh Amouzegar and William Foulsham contributed equally to this work.

✉ Reza Dana
Reza_Dana@meei.harvard.edu

[1] Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, MA, USA

[2] Kresge Eye Institute, Wayne State University, Detroit, MI, USA

or pathological (as with neoplastic or chronic inflammatory diseases). Lymphangiogenesis is the formation of new lymphatic vessels, and can similarly be either physiological or pathological. The correlation between host bed vascularity and corneal allograft rejection has been recognized for several decades [9]. In contrast, the critical role of the lymphatic system in mediating allosensitization to ocular antigens was established more recently, when in 2001, Yamagami et al. demonstrated that the removal of ipsilateral draining cervical lymph nodes prevented the rejection of murine orthotopic high-risk corneal allografts [10]. This observation supported the paradigm that the alloimmune response driving corneal allograft rejection consists of two phases—a sensitization phase and an effector phase. During allosensitization, lymphatic vessels form the conduit by which antigen presenting cells (APCs) are trafficked from the graft site to the regional draining lymphoid tissues, where they present donor antigen to naïve host T cells [4]. During the effector phase, blood vessels permit the transport of alloreactive T cells across the chemotactic gradients from the draining lymphoid tissues to the graft site [11]. Accordingly, the suppression of either the sensitization arm of the response (via lymphatic vessels), or the effector arm (via blood vessels), offer feasible therapeutic approaches to promote allograft survival.

Different therapeutic methods for direct or indirect occlusion of corneal vessels have been evaluated, including the use of laser treatment and fine needle diathermy prior to corneal grafting [12, 13]. Recently, studies have focused on the use of agents that inhibit Vascular Endothelial Growth Factor (VEGF). The family of VEGF ligands and receptors are crucial regulators of both angiogenesis and lymphangiogenesis. The most important molecule that orchestrates blood vessel morphogenesis is VEGF-A, which binds to the receptors VEGFR-1 and VEGFR-2 [14]. Specifically, ligation of VEGFR-2 is the principal mechanism that stimulates endothelial cell differentiation, proliferation and sprouting [14]. Echoing the critical function of VEGF-A in the growth of blood vessels, VEGF-C has been shown to be essential for developmental lymphangiogenesis [15]. Both VEGF-C and VEGF-D are activating ligands for the receptor VEGFR-3 [16]. Despite the discrete functions of the VEGF family that have been described here, there is in fact considerable promiscuity. For example, in both physiological and pathological settings, proteolytically processed VEGF-C and VEGF-D can promote angiogenesis through stimulation of VEGFR-2 [17, 18]. Likewise, VEGF-A has been demonstrated to induce lymphatic vessel formation [19, 20]. In addition to direct effects, there are notable indirect consequences of VEGF interactions on the formation of blood and lymphatic vessels. For example, VEGF-A has been shown to stimulate both lymphangiogenesis and angiogenesis in inflammatory neovascularization via the recruitment of macrophages [21].

Strategies to block the sensitization arm of the alloimmune response with antilymphangiogenic interventions have successfully promoted corneal graft survival [22, 23]. Dietrich et al. selectively inhibited lymphangiogenesis using anti-VEGFR-3 antibodies or anti-integrin α5 small molecules, and found a substantial reduction in murine corneal allograft rejection in the treatment groups despite the presence of pre-existing blood vessels in the host bed [22]. Administration of soluble VEGFR-2 has been shown to inhibit lymphangiogenesis, but not angiogenesis in response to corneal suture injury, with a concomitant decrease in allograft rejection in a high-risk murine model of corneal transplantation [23]. Notably, Albuquerque et al. identified the existence of an endogenous spliced variant of soluble VEGFR-2 that is secreted by corneal epithelial and stromal cells, and demonstrated the essential role it plays in maintaining corneal alymphaticity [23].

Strategies to block the action of VEGF-A have been shown to inhibit the ingress of both blood and lymphatic vessels following keratoplasty [24–27]. Cursiefen et al. conducted normal risk allogeneic and syngeneic corneal allografts in a murine model, and demonstrated that early postoperative neutralization of VEGF-A with VEGF-trap significantly reduced both angiogenesis and lymphangiogenesis, and promoted long-term graft survival [24]. Bachmann et al. corroborated this finding in a high-risk murine model of corneal transplantation [25]. Bevacizumab is a monoclonal antibody that blocks angiogenesis by inhibiting VEGF-A. In a study of high-risk murine corneal transplantation, Dastjerdi et al. demonstrated that subconjunctival bevacizumab both inhibited post-operative neovascularization and promoted graft survival [26]. Noting that strategies targeting both angiogenesis and lymphangiogenesis had been successful in promoting graft survival, Dohlman et al. conducted a study comparing adjunctive therapy of VEGF-trap, anti-VEGF-C and sVEGFR-3 in a murine model of high-risk transplantation [28]. In this study, the authors reported that although all strategies improved graft survival, VEGF-trap was significantly more effective in promoting graft survival compared to anti-VEGF-C and sVEGFR-3. The investigators emphasized that although each of the three agents exerted an effect on both angiogenesis and lymphangiogenesis, VEGF-trap was relatively more effective at limiting angiogenesis, and anti-VEGF-C and sVEGFR-3 were more effective at limiting lymphangiogenesis [28]. Notably, VEGF-trap was the most effective therapy at reducing $CD3^+$ T cell infiltration of the corneal graft, the principal cellular mediators of allograft rejection. This observation is intriguing, since alloprimed T cells themselves have been shown to release VEGF-A and to promote vascular endothelial cell proliferation in corneal transplantation, suggesting a positive feedback mechanism between VEGF-A expression and T-cell recruitment [29, 30].

It is important to emphasize that the processes of angiogenesis and lymphangiogenesis are usually deeply entwined, and modulating the function of a particular ligand commonly affects both phenomena. As further evidence of this, Chung et al. have demonstrated in a murine model that the implantation of pellets containing VEGFR-3-specific ligands results in not only lymphangiogenesis, but also robust hemangiogenesis with blood vessels that express VEGFR-3 [31]. Moreover, treatment with VEGFR-3-specific ligands prompted the recruitment of VEGF-A-secreting macrophages. Clinical studies of anti-VEGF therapy in corneal transplantation are limited. In a case–control series of 122 patients undergoing high-risk corneal transplantation, Bhatti et al. reported that bevacizumab administered either subconjunctivally or topically resulted in significantly lower corneal neovascularisation post-operatively; however, they did not investigate graft survival [32]. In a prospective, consecutive, interventional case series of 50 eyes of 50 patients, Dekaris et al. treated high-risk penetrating keratoplasty cases with subconjunctival bevacizumab post-operatively combined with topical bevacizumab [33]. The investigators reported a decrease in corneal neovascularization in 42 treated eyes (84%), with 35 (70%) of the high-risk grafts remaining clear over 3 years of follow-up. Fasciani et al. considered the potential role of bevacizumab as a preconditioning therapy, and conducted a prospective interventional case–control series of 27 eyes of 27 patients undergoing high-risk corneal transplantation [34]. The case group of 14 eyes received a cycle of three subconjunctival and/or intrastromal injections of bevacizumab prior to transplantation. The investigators described no corneal graft rejection in the case group over two years of follow-up, contrasting with rejection in 6 of 13 eyes in the control group. In an interventional case series of 14 eyes of 14 patients undergoing high-risk corneal transplantation, Vassileva and Hergeldzhieva treated patients with subconjunctival, perilimbal, and/or intrastromal bevacizumab at the end of surgery and/or at follow-up visits [35]. The authors reported decreased corneal neovascularization in eleven patients (79%) in response to treatment, with twelve grafts (86%) remaining transparent for the observation period (on average 7 months) despite their high-risk status. These preliminary reports are encouraging, yet there is a need for more substantive clinical evidence supporting the use of anti-VEGF therapeutics in corneal transplantation.

Insulin receptor substrate (IRS)-1 proteins have been demonstrated to play an important role in angiogenesis, with human endothelial cells expressing higher levels of IRS-1 proteins in proangiogenic relative to quiescent conditions [36]. In a rat model, Al-Mahmood et al. have demonstrated the dose-dependent inhibition of corneal angiogenesis by aganirsen, an antisense oligonucleotide that inhibits IRS-1 mRNA expression [36]. In the first phase III clinical study of a topical antiangiogenic agent for use at the ocular surface and cornea, Cursiefen et al. conducted a multicenter, double-masked, randomized, placebo-controlled study of 69 patients with keratitis-related progressive corneal neovascularization [37]. The investigators reported a significant reduction in corneal neovascularization in those patients treated with aganirsen. However, the capacity of aganirsen to reduce corneal allograft rejection has not yet been evaluated.

Gene therapy may offer a further therapeutic modality to inhibit angiogenesis and prevent graft failure. Using a rabbit model, Murthy et al. transduced corneas with a lentivirus vector expressing endostatin [38]. The investigators confirmed the presence of the unique fusion gene sequence by RT-PCR in the transduced corneas, and demonstrated reduced neovascularization and improved graft survival in the hosts that received these buttons. Parker et al. transduced rabbit corneas with lentivirus vectors expressing endostatin and angiostatin, and demonstrated significant suppression of neovascularization in the recipient animals relative to control [39]. Furthermore, the treatment group exhibited decreased corneal opacity, edema and inflammatory infiltrates.

Angiogenesis and lymphangiogenesis are complex processes, which are contingent on a plethora of interactions between proangiogenic and antiangiogenic signals. The association between corneal neovascularization and graft failure in penetrating keratoplasty has been confirmed in a meta-analysis of 19 studies including a total of 24,944 grafts [40]. Accordingly, the requirement for a topical inhibitor of corneal angiogenesis has been declared by a panel of experts as an important, yet unmet medical need [41]. As our understanding of the molecular mediators of angiogenesis and lymphangiogenesis deepens, the spectrum of feasible therapeutics broadens. Anti-VEGF antibodies, trap proteins and receptor antagonists have all been shown to successfully limit allograft rejection in murine models of corneal transplantation [22, 25, 26, 28]. Novel antiangiogenic molecules, such as those targeting insulin receptor substrate-1, may prove to be useful therapeutic tools in this setting [37]. Furthermore, ex vivo gene therapy treatment has potential as an effective means of decreasing neovascularization and promoting graft survival [38, 39].

Antigen presenting cells

Antigen-presenting cells (APCs) including macrophages and dendritic cells (DCs) are the principal mediators of the adaptive immune response and play a sentinel role in development, maintenance and regulation of immune memory. It was previously believed that corneal stroma was devoid of immune cells, and that the immune privileged status of the cornea resulted from its lack of passenger leukocytes [42]. However, using mouse models, Liu et al. demonstrated that in the cornea there are distinct populations of resident

APCs that are negative for major histocompatibility complex (MHC) II but capable of expressing class II antigen after transplantation, and of migrating to the draining lymph nodes of grafted hosts [43]. Subsequently, several studies in mouse models established the fact that the corneal stroma harbors heterogeneous populations of bone marrow-derived APCs, including epithelial Langerhans cells (LCs) and dendritic cells (DCs) in the anterior stroma, and macrophages in the posterior stroma [44–47]. In a study on human subjects undergoing corneal transplantation, Flynn et al. characterized the allo-reactive cells and cytokines in the aqueous humor during rejection and reported a significant increase in the population of $CD45^{+}CD14^{+}$ macrophages in aqueous humor of grafts undergoing rejection [48]. Strategies to extinguish immune cells from corneal buttons to enhance transplant survival have had equivocal results. Slegers et al. showed that depletion of macrophages in corneal allografts using administration of clodronate liposomes early after grafting improves allograft survival in a rat model [49]. On the contrary, Zhang et al. used anti-CD45 monoclonal antibodies to deplete APCs in the murine corneal buttons [50] and showed that depletion of these cells in the graft does not significantly promote allograft survival even in the high-risk setting, suggesting that while some donor APCs participate in host sensitization others could be involved in tolerance induction.

In vivo experiments in mice have increased our understanding of the development and functions of DCs and macrophage subsets [51, 52]. Below we summarize important findings regarding APC biology in the context of corneal transplantation. These studies are mainly carried out in rodent models. Relevant human studies in each category are also included in each section.

APC maturation and migration

While the periphery of the cornea contains both mature and immature immune cells, the central cornea is populated exclusively with highly immature and precursor-type APCs. Some of these cells are immunoregulatory, and serve to maintain a quiescent environment in homeostatic conditions. However, the microenvironment of an inflamed host bed (i.e., in high-risk transplantation), induces maturation of these APCs, and overturns the natural tendency of the eye to preserve immune privilege [53]. After corneal allograft transplantation in inflamed host beds, the majority of resident APCs undergo maturation by acquiring high expression levels of MHC class II antigens and co-stimulatory molecules (CD80/CD86 and CD40) [54]. These donor-derived APCs migrate to host cervical lymph nodes and activate host T cells via the direct and indirect pathways of allosensitization [44, 53, 55, 56].

The trafficking of corneal APCs to draining lymph nodes is critical in triggering immune responses. The lymphatic system serves as the sensitization arm of the immune response by enabling efficient trafficking of APCs to regional lymph nodes. Several studies on murine models have investigated the role of APC trafficking to draining lymph nodes in corneal transplantation. Jin et al. demonstrated that during an inflammatory response, APCs express chemokine receptor 7 (CCR7) on their cell surface, which interact with CCL21 and facilitate their migration from the cornea to draining lymph nodes via the lymphatics [57]. Expression of chemokines is central to the recruitment of inflammatory cells to the graft site, and modulation of chemokine action has been shown to prevent graft rejection. In 2007, Hamrah et al. demonstrated that targeting specific chemokine pathways significantly promotes the survival of corneal allografts, and have proposed that the selective deletion or suppression of CCR1 may be a useful therapeutic strategy in promoting corneal graft survival [58]. Pillai et al. have also examined the expression of 11 chemokines following corneal allotransplantation [59]. The authors demonstrate that gene delivery of viral macrophage inflammatory protein II (vMIP II), a broad-acting chemokine antagonist, via a non-viral vector promotes corneal allograft survival. In another study, Hajrasouliha et al. showed that APC maturation is associated with upregulation of cell surface receptors of VEGF-C (i.e., VEGFR-2 and R-3), which render APCs more responsive to the VEGF-C gradient induced by inflammation. Further, they demonstrated that APC trafficking could be successfully blocked by anti-VEGF-C therapy [60]. In a recent study by Hua et al., authors showed that CCR7 ligands, CCL19 and CCL21 are expressed at significantly higher levels in the draining lymph nodes of high-risk allograft recipients with inflamed graft beds [61]. This is correlated with an increased migration of mature APCs, which is abolished by neutralizing CCL19 or CCL21. These data suggest that graft site inflammation increases the expression of CCR7 ligands in the draining lymph nodes, which promote homing of mature APCs and thus allorejection. The authors concluded that the graft site microenvironment plays a critical role in alloimmunity by determining APC trafficking through the CCR7–CCL19/21 axis [61]. Endothelial cell-expressed selectins mediate leukocyte tethering and rolling, a prerequisite for subsequent firm adhesion and migration of effector cells into tissue. In a recent study by Dohlman et al., the authors show that E-selectin mediates APC trafficking to lymphoid tissue, and that blockade of E-selectin improves long-term graft survival [62].

Among the family of chemokine receptors are ‘decoy receptors’ that serve as scavenging receptors. Originally defined by their ability to ligate chemokines in a non-signaling fashion, these decoy receptors include chemokine receptor 6 (D6), which is capable of scavenging more

than 12 chemokines (mostly agonists of inflammatory CC chemokine receptors from CCR1 to CCR5) [63]. Thus, in contrast to conventional chemokine receptors (in which chemokine ligation induces leukocyte recruitment in inflammation) ligation via D6 leads to targeted chemokine degradation and consequent reduction of their bioavailability. Consistent with this, the absence of D6 expression has been associated with uncontrolled and sustained inflammation, leading to the theory that expression of D6 by lymphatic endothelial cells plays a crucial role in mediating resolution of inflammation [64]. Hajrasouliha et al. have demonstrated that D6 chemokine receptor expression by APCs has a critical function in mediating allograft rejection through its regulation of APC biology and consequently alloreactive T-cell responses [64]. Using human corneal grafts, Lapp et al. designed an endothelial blood–eye barrier model, in which they targeted monocyte chemotaxis and showed that using inhibitors of chemokine receptors, such as CCR2 and CCR5 significantly attenuated recruitment of monocytes in vitro [65], proposing the potential application of this approach to promote corneal allograft survival. These studies altogether highlight the important role of APC maturation and migration to the draining lymph nodes in T-cell sensitization and graft rejection, and provide evidence for potential therapeutic strategies in clinical studies of human corneal allografting.

Antigen presentation and induction of adaptive immune response

Antigen presentation and T-cell priming not only require binding of MHC molecules to T-cell receptors, but also are dependent on co-stimulatory pathways, including the interaction between CD28 on T cells with B7 molecules (CD80, CD86) on APCs and ligation of CD40 on APCs with CD154 (CD40L) on the T cells. The interaction of CD40–CD154 activates both B7 and interleukin-12 (IL-12) expression by APCs, leading to differentiation of naïve T cells to Th1 cells. Blockade of the CD40–CD154 costimulatory pathway in murine corneal transplantation has been shown to inhibit Th1-mediated responses and suppress ocular chemokine gene expression and leukocytic infiltration into allografts [66, 67]. In another study, treatment of graft recipient mice with recombinant cytotoxic T Lymphocyte antigen-4 (CTLA-4), which is a competitive inhibitor of CD28 and blocks the CD28/B7 interaction and inhibits T-cell activation, has been shown to significantly prolong the survival of corneal allografts [68]. Comer et al. were able to reproduce similar results showing that both protein- or gene-based administration of CTLA4-Ig prolongs allograft survival when treating either the recipient or the donor tissue ex vivo before grafting [69]. In another study, Kagaya et al. showed that treatment of corneal graft recipients with anti-CD80 and anti-CD86 antibodies decreased rejection rates in corneal allografts in a murine model of corneal transplantation [70]. Using a similar animal model, Watson et al. demonstrated that augmented ligation of the PD-1 negative costimulatory molecule with a dimeric PD-L1 Ig fusion protein inhibits in vitro activation of T cells and significantly prolongs corneal allograft survival [71]. Members of the T-cell immunoglobulin domain and mucin domain (TIM) protein family are expressed at the cell surface of APCs as well as T cells, and have emerged recently as important regulators of immune responses [72]. Tan et al. have demonstrated that the anti-Tim-1 monoclonal antibody RMT-10 is effective in promoting corneal allograft survival in a high-risk murine model of corneal transplantation [73].

The expansion of our knowledge on the molecular pathways leading to T-cell sensitization and graft rejection has yielded other novel treatment strategies that target APCs and their function. In a study by Yamada et al., the investigators demonstrated that the local application of *N*,*N*′-diacetyl-L-cystine dimethylester (NM2) to mice receiving corneal allografts improves the survival of MHC-disparate allografts [74]. NM2 has been shown to reduce the intracellular glutathione content in APCs, which in turn downregulates Th1 responses [74]. In a more recent study by our group, Hua et al. showed that treating hosts with a resolvin D1 (RvD1) analogue significantly reduces allosensitization as seen through decreased Th1-cell activation and IFNγ production and reduced T-cell infiltration into the grafts [75]. Resolvins are lipid mediators produced by leukocytes, endothelial and epithelial cells, macrophages, and lymphoid tissues. Among them, resolvin D1 is known for its potent anti-inflammatory actions. It reduces inflammatory and allergic immune responses, as well as APC maturation, migration and IL-12 production. Using the murine model, the investigators demonstrated that graft survival was significantly enhanced in RvD1a-treated hosts compared to vehicle-treated graft recipients, and that enhanced survival was accompanied by suppression of angiogenesis at the graft site [75]. Thrombospondin (TSP)-1 is a matricellular glycoprotein with immunoregulatory properties, such as inhibition of APC function through downregulation of TNF-α and IL-12 expression, with concomitant upregulation of IL-10 expression, as well as a reduced capacity of APCs to sensitize and mount a T cell (Th1) immune response. Using a murine model of corneal transplantation, Saban et al. demonstrated that APC-derived TSP-1 inhibits T-cell allosensitization, and consequently suppresses immune rejection [76]. The investigators showed that TSP-1-null APCs have enhanced expression of MHC class II and B7 maturation markers relative to wild-type APCs in an inflammatory microenvironment, thereby implicating TSP-1 in the regulation of APC maturation. Future strategies aimed at upregulating TSP-1

expression by APCs may, therefore, be effective in promoting transplant survival.

Tolerogenic APCs

Tolerogenic APCs (tolAPCs) have been characterized by their ability to induce T-cell tolerance through various mechanisms, including diminished antigen presentation, production of anti-inflammatory cytokines, and generation and expansion of regulatory T cells [77, 78]. These maturation-resistant APCs are, therefore, thought to be potentially powerful tools for promoting transplant survival [79]. A variety of pharmacological inhibitors have been developed to generate tolAPCs from their undifferentiated precursors to achieve transplant tolerance, such as immunomodulatory cytokines, rapamycin, dexamethasone, and vitamin D [77]. Hattori et al. have shown that ex vivo manipulation of donor-type bone marrow-derived dendritic cells (BMDCs) with immunomodulatory cytokines (IL-10, TGFβ1) renders them tolerogenic [56]. The investigators demonstrated that when systemically transferred to corneal transplant recipients, these tolAPCs significantly improve allograft survival. Khan et al. have demonstrated that intravenous administration of DCs transduced with a lentiviral vector expressing CTLA4-KDEL (a fusion protein that prevents surface CD80/86 expression by retaining the co-stimulatory molecules within the endoplasmic reticulum) promotes corneal allograft survival [80]. In a recent study on mice, we demonstrated that the donor cornea *itself* can be manipulated to generate tolAPCs [3]. We showed that treatment of donor corneal buttons with IL-10 and TGF-β1 induces phenotypic and functional changes in tissue-resident APCs, rendering them tolerogenic and capable of suppressing allosensitization in high-risk allograft recipients that swiftly reject their corneal transplants [3]. This strategy is important, as it is translatable to human corneal allografts and can potentially induce long-term graft acceptance without exposing the recipients to immunosuppressive therapies.

Effector and regulatory t cells

Effector T cells

IFNγ-producing $CD4^+$ Th1 cells are considered the principal mediators of corneal allograft rejection [81, 82]. The mechanism through which Th1 cells mediate allograft rejection is still not yet fully understood; studies have shown that these cells induce corneal endothelial cell apoptosis in vitro [83, 84]. Furthermore, high levels of Th1-type cytokines, IL-2 and IFNγ, are detected in corneas undergoing rejection [85]. Studies performed in mouse and rat models of orthotropic corneal transplantation have shown that depletion of $CD4^+$ T cells using anti-CD4 monoclonal antibodies significantly prolongs corneal allograft survival [86, 87]. However, depletion of neither $CD4^+$ T cells nor IFNγ completely prevents allograft rejection, suggesting the involvement of CD4-independent effector mechanisms in the rejection process [87–89]. Alloprimed cytotoxic $CD8^+$ T cells have been implicated in high-risk corneal allograft rejection as well, though evidence suggests that despite their activation, $CD8^+$ T cells cannot induce rejection in the absence of proper co-stimulatory signals and are, thus, not essential for corneal allograft rejection [90, 91].

The role of Th17 cells in the pathogenesis of corneal allograft rejection has been controversial. It has been suggested that the presence of IL-17 in early timepoints after corneal transplantation is actually essential for allograft survival, which is supported by studies demonstrating that anti-IL-17A treatment or IL-17A depletion can accelerate the tempo of corneal allograft rejection early after transplantation [92–94]. These observations have been attributed to both the emergence of a Th2 type immune response that mediates graft rejection upon IL-17 blockade, and the critical role of IL-17 in Treg-mediated immunosuppression [92–94]. Interestingly, however, IL-17 seems to play a role in graft rejection in later stages after transplantation, and anti-IL-17A treatment has been shown to significantly reduce late-term corneal graft allorejection [93]. Continuous treatment of transplanted mice displaying early signs of graft rejection with anti-IL-17A antibody significantly suppresses graft opacity and vascularization and reverses late-term corneal allograft rejection [93].

Various strategies to improve corneal allograft survival have focused on reducing the migration of effector T cells to draining lymph nodes and to the site of graft. Th1 cells have been found to express PSGL-1 and glyco-CD43 ligands, which interact with P- and E-selectins expressed by vascular endothelial cells, respectively, and mediate the tethering, adhesion and subsequent tissue migration of activated T cells. Treatment of corneal allograft recipients with E-selectin neutralizing antibody results in a significant decrease in frequencies of graft infiltrating Th1 cells and leads to a modest improvement in corneal allograft survival in mice [62]. Therapeutic modalities targeting sphingosine 1-phosphate receptor 1 (S1P1), the cell receptor involved in sequestration of lymphocytes in lymphoid tissues, have also yielded promising results. Topical and systemic treatment with S1P1 receptor both lead to retention of $CD4^+$ T cells in peripheral lymphoid tissues and decrease the frequencies of these cells in the blood [95, 96]. Systemic combination therapy with S1P1 receptor agonist and cyclosporine/rapamycin, and topical combination therapy with S1P receptor agonist and cyclosporine have both been associated with prolonged corneal allograft survival in mice [95, 96]. Thus, strategies that inhibit Th1 and Th17 cells, either through

inducing apoptosis or via inhibiting their migration to graft site, can potentially induce long-term allograft acceptance.

Regulatory T cells

Regulatory T cells (Tregs) play a pivotal role in curbing the effector response to alloantigens, and Treg-based immunotherapies have emerged as promising therapeutic tools in promoting corneal allograft survival [97]. Several mechanisms have been identified in Treg mediated immunosuppression. Tregs can outcompete effector T cells in interacting with APCs [through lymphocyte function associated antigen-1 (LFA-1)]; downregulating CD80/CD86 expression by APCs (via CTLA-4), and destroying or inactivating effector T cells (through granzymes, perforins, IL-10, TGF-β, IL-35) [98, 99]. Tregs derived from corneal graft acceptors have been shown to express comparably higher levels of Foxp3 (the transcription factor implicated in the suppressive function of Tregs), demonstrate greater potency in suppressing naïve T-cell proliferation, and have the ability to grant protection against corneal allograft rejection upon adoptive transfer to transplanted mice [100]. Hori et al. demonstrated that constitutive expression of glucocorticoid-induced tumor necrosis factor receptor family-related protein ligand (GITRL) by corneal cells is critical for recruitment of $GITR^+$ Tregs to the graft bed and subsequent improvement in corneal allograft survival [101].

While adoptive transfer of naïve Tregs does not prevent graft rejection, a recent report by Hildebrand et al. demonstrated that subconjunctival injection of naïve Tregs to grafted baby rats improves corneal allograft survival [102]. Different approaches to control allograft rejection through in vivo expansion of Tregs or transfer of in vitro-expanded Tregs have been studied. Xu et al. demonstrated that subconjunctival treatment of transplanted mice with TGF-β-induced Tregs promotes corneal allograft survival [103]. In vivo expansion of Tregs using a combination of systemic rapamycin and IL-2 has also been associated with reduced graft opacity scores and neovascularization early after transplantation [104]. IL-2 maintains Treg suppressive function by improving Foxp3 and immunoregulatory cytokine expression [105, 106]. In a more recent study from our group, intravenous injection of mice with low dose IL-2 alone proved effective in expanding $CD4^+$ $CD25^+$ Tregs and improving corneal allograft survival in the high-risk setting [107]. Proper homing of Tregs to draining lymph nodes has also been implicated in their ability to suppress alloimmune responses. Adoptive transfer of CCL21-treated Tregs, which express higher levels of CCR7 and CD62L lymph node homing receptors has been associated with significantly enhanced corneal allograft survival in mice [108].

Apart from strategies to expand Treg population, other therapeutic modalities have been proposed, which alter the balance between Tregs and Th1/Th17 cells. Wang et al. demonstrated that systemic treatment of mice with all-trans retinoid acid (ATRA), a metabolite of vitamin A, in conjunction with TGF-β increases the frequencies and suppressive function of Tregs, skews the Th17-Treg balance towards Tregs, and subsequently improves corneal allograft survival [109]. Systemic treatment with anti-CD154 monoclonal antibody has been found to prolong corneal graft survival by preferentially increasing Treg-associated anti-inflammatory cytokines and suppressing Th1 inflammatory immune response in mice [110]. CD154 (or CD40 ligand) binds to its receptor CD40 on antigen-presenting cells (APC), providing a co-stimulatory signal for T cell priming [111]. Anti-CD154 antibody immunosuppression has also been investigated in a pig-to-primate xenocorneal transplantation model, resulting in significantly reduced inflammatory cell infiltration and Th1-associated cytokine expression, and improved corneal allograft survival [112]. In aggregate, these experimental animal studies can serve as a guide for designing novel Treg-based therapeutic strategies in patients at high risk of corneal allograft rejection.

Mesenchymal stem cells

Bone marrow-derived mesenchymal stem cells (MSCs) are multipotent nonhematopoietic stem cells that interact with cells of both innate and adaptive immune systems to modulate the effector response [113]. MSCs have been shown, in vivo, to migrate to injured tissue and limit the release of pro-inflammatory cytokines [113]. Consequently, MSCs have attracted attention as a potential therapeutic tool in preventing corneal allograft rejection [114–117].

Jia et al. have evaluated the immunomodulatory effects of MSCs in a rat model of corneal allograft rejection [114]. In their study, MSCs (isolated and cultured from Wistar rats) were delivered intravenously following transplantation of a donor corneal button from Wistar rats to Lewis rat recipients. The investigators reported that treatment with MSCs prolonged graft survival, modulated the effector T-cell response, and upregulated Tregs [114]. Oh et al. shed light on the mechanism of action of human MSCs in promoting corneal allograft survival, when in a murine model, they demonstrated that the anti-inflammatory protein tumor necrosis factor-α-stimulated gene/protein 6 (TSG-6) was essential for the suppression of inflammation [115]. This study corroborated the prolongation of allograft survival by MSC therapy. The authors noted that most of the intravenously delivered human MSCs became trapped in the lungs, where they increased the expression of the TSG-6 gene. Importantly, the therapeutic effect of MSCs was repealed when human MSCs with a knockdown of TSG-6 were used, and IV infusion of recombinant TSG-6 emulated the effects

of MSC therapy [115]. Omoto et al. further investigated the homing of systemically MSCs injected following corneal transplantation, using MSCs derived from either wild-type BALB/c or GFP C57/BL/6 mice [116]. The investigators reported that numerous GFP^+ MSCs were found in the transplanted cornea, ipsilateral conjunctiva and ipsilateral lymph nodes, but not in the contralateral tissues. Moreover, the study revealed a significant decrease in the frequencies of mature antigen presenting cells (MHC II^+CD11c^+) in the corneas and draining lymph nodes of MSC-injected allograft recipients. The apparent conflict in MSC homing between the two previously referenced studies [115, 116] may be due to the xeno-species barrier inhibiting MSC migration in the work by Oh et al., where human MSCs were used. Using an allogeneic rat model of corneal transplantation with Dark Agouti donors and Lewis recipients, O'Treacy et al. have evaluated the capacity of MSCs from three distinct sources (syngeneic Lewis, allogeneic Dark Agouti and third-party Wistar Furth rats) to prolong rat corneal allograft survival [118]. The investigators showed that corneal allograft survival was significantly prolonged in those mice treated with allogeneic or third party MSCs, but not in untreated mice or those treated with syngeneic MSCs. In aggregate, these studies suggest that MSCs and specifically TSG-6 may be viable tools for promoting corneal allograft survival.

Future directions

Animal keratoplasty models (particularly mouse and rat systems) have been critical for gaining insight into the cellular and molecular pathways that mediate allograft rejection. The onset of rejection in these models is determined based upon clinical scoring systems that assess graft opacity and corneal neovascularization. This subjective evaluation is inherently vulnerable to issues of reproducibility and interobserver variability. Recently, new tools (such as anterior segment spectral domain optic coherence tomography) have been introduced for defining graft rejection [119, 120]. In the future, these tools may help researchers to improve readout reproducibility and reduce interobserver variability.

Despite the considerable progress made with murine models of corneal transplantation in identifying potential therapeutics, the goal of improving clinical outcomes by promoting allotolerance in humans remains elusive. This translational discrepancy between bench-side discoveries and clinical solutions is troubling, particularly given the failure rates seen in high-risk grafts and the manifest clinical need for effective therapies [7]. One of the issues that animal keratoplasty models face concerns the genetic background of the system—we know that murine immunology is contingent on the strain used [121]. An illustrative example of this phenomenon in corneal transplantation is the increased incidence and tempo of allograft rejection in C57BL/6 mice relative to BALB/c mice [122]. Certainly, there are substantial challenges in moving from mouse models to clinical trials [123]. Nevertheless, there is an exigent need for human in vitro and translational clinical studies to permit progress in this field. While to date no randomized controlled clinical studies have been conducted to ascertain the efficacy of tolerance induction in corneal transplantation, it is clear that this is the next frontier in the field given the immerse progress that has been made toward a better understanding of the cellular and molecular bases of allotolerance and immune quiescence in the recent past.

Conclusion

Multiple cellular and molecular pathways have been identified in the immunopathology of corneal graft rejection, including corneal lymphangiogenesis and hemangiogenesis, antigen presentation, and induction of both antigen-specific effector and regulatory T cells. Despite the immune privileged status of the healthy cornea, inflammation and subsequent neovascularization compromise the cornea's ability to maintain its clarity upon allografting. Several immunomodulatory approaches have been developed based on our current knowledge of immune mechanisms underlying corneal allograft rejection. Targeting heme/lymphangiogenesis, inhibiting maturation and migration of APCs and inducing tolerogenic APCs, suppressing effector T cells and expanding regulatory T cells are successful examples of such approaches that have been investigated in various experimental models. Preliminary clinical studies using anti-VEGF therapies have shown promising results in reducing neovascularization in inflamed corneas and have improved short-term outcomes in high-risk grafts. Induction of tolerogenic APCs in the donor cornea is another potential therapeutic approach that can be achieved by pre-treatment of human donor corneas with immunomodulatory cytokines prior to transplantation without exposing graft recipients to toxic side effects of systemic immunosuppression. Finally, expanding regulatory T cells using IL-2 therapy and the use of MSCs are strategies that hold promise in promoting graft survival with the potential to be translated into human corneal allografting.

Acknowledgements This work was supported by National Institutes of Health/National Eye Institute Grant EY012963.

Compliance with ethical standards

Conflict of interest The authors have no financial conflicts of interest.

References

1. Niederkorn JY (2013) Corneal transplantation and immune privilege. Int Rev Immunol 32(1):57–67
2. Niederkorn JY (2015) Immunology of corneal allografts: insights from animal models. J Clin Exp Ophthalmol 6(3):429
3. Tahvildari M, Emami-Naeini P, Omoto M, Mashaghi A, Chauhan SK, Dana R (2017) Treatment of donor corneal tissue with immunomodulatory cytokines: a novel strategy to promote graft survival in high-risk corneal transplantation. Sci Rep 7(1):971
4. Amouzegar A, Chauhan SK, Dana R (2016) Alloimmunity and tolerance in corneal transplantation. J Immunol 196(10):3983–3991
5. Williams KA, Coster DJ (2007) The immunobiology of corneal transplantation. Transplantation 84(7):806–813
6. Sangwan VS, Ramamurthy B, Shah U, Garg P, Sridhar MS, Rao GN (2005) Outcome of corneal transplant rejection: a 10-year study. Clin Exp Ophthalmol 33(6):623–627
7. Price FW Jr, Whitson WE, Collins KS, Marks RG (1993) Five-year corneal graft survival. A large, single-center patient cohort. Arch Ophthalmol 111(6):799–805
8. Renfro L, Snow JS (1992) Ocular effects of topical and systemic steroids. Dermatol Clin 10(3):505–512
9. Khodadoust AA (2008) The allograft rejection reaction: the leading cause of late failure of clinical corneal grafts. Wiley, New York, pp 151–167
10. Yamagami S, Dana MR (2001) The critical role of lymph nodes in corneal alloimmunization and graft rejection. Investig Ophthalmol Vis Sci 42(6):1293–1298
11. Tan Y, Abdulreda MH, Cruz-Guilloty F, Cutrufello N, Shishido A, Martinez RE, Duffort S, Xia X, Echegaray-Mendez J, Levy RB, Berggren PO, Perez VL (2013) Role of T cell recruitment and chemokine-regulated intra-graft T cell motility patterns in corneal allograft rejection. Am J Transplant 13(6):1461–1473
12. Baer JC, Foster CS (1992) Corneal laser photocoagulation for treatment of neovascularization. Efficacy of 577 nm yellow dye laser. Ophthalmology 99(2):173–179
13. Pillai CT, Dua HS, Hossain P (2000) Fine needle diathermy occlusion of corneal vessels. Investig Ophthalmol Vis Sci 41(8):2148–2153
14. Adams RH, Alitalo K (2007) Molecular regulation of angiogenesis and lymphangiogenesis. Nat Rev Mol Cell Biol 8(6):464–478
15. Karkkainen MJ, Haiko P, Sainio K, Partanen J, Taipale J, Petrova TV, Jeltsch M, Jackson DG, Talikka M, Rauvala H, Betsholtz C, Alitalo K (2004) Vascular endothelial growth factor C is required for sprouting of the first lymphatic vessels from embryonic veins. Nat Immunol 5(1):74–80
16. Alitalo K, Tammela T, Petrova TV (2005) Lymphangiogenesis in development and human disease. Nature 438(7070):946–953
17. Rissanen TT, Markkanen JE, Gruchala M, Heikura T, Puranen A, Kettunen MI, Kholova I, Kauppinen RA, Achen MG, Stacker SA, Alitalo K, Yla-Herttuala S (2003) VEGF-D is the strongest angiogenic and lymphangiogenic effector among VEGFs delivered into skeletal muscle via adenoviruses. Circ Res 92(10):1098–1106
18. Cao Y, Linden P, Farnebo J, Cao R, Eriksson A, Kumar V, Qi JH, Claesson-Welsh L, Alitalo K (1998) Vascular endothelial growth factor C induces angiogenesis in vivo. Proc Natl Acad Sci USA 95(24):14389–14394
19. Hong YK, Lange-Asschenfeldt B, Velasco P, Hirakawa S, Kunstfeld R, Brown LF, Bohlen P, Senger DR, Detmar M (2004) VEGF-A promotes tissue repair-associated lymphatic vessel formation via VEGFR-2 and the alpha1beta1 and alpha2beta1 integrins. FASEB J 18(10):1111–1113
20. Hirakawa S, Kodama S, Kunstfeld R, Kajiya K, Brown LF, Detmar M (2005) VEGF-A induces tumor and sentinel lymph node lymphangiogenesis and promotes lymphatic metastasis. J Exp Med 201(7):1089–1099
21. Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, D'Amore PA, Dana MR, Wiegand SJ, Streilein JW (2004) VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. J Clin Investig 113(7):1040–1050
22. Dietrich T, Bock F, Yuen D, Hos D, Bachmann BO, Zahn G, Wiegand S, Chen L, Cursiefen C (2010) Cutting edge: lymphatic vessels, not blood vessels, primarily mediate immune rejections after transplantation. J Immunol 184(2):535–539
23. Albuquerque RJ, Hayashi T, Cho WG, Kleinman ME, Dridi S, Takeda A, Baffi JZ, Yamada K, Kaneko H, Green MG, Chappell J, Wilting J, Weich HA, Yamagami S, Amano S, Mizuki N, Alexander JS, Peterson ML, Brekken RA, Hirashima M, Capoor S, Usui T, Ambati BK, Ambati J (2009) Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. Nat Med 15(9):1023–1030
24. Cursiefen C, Cao J, Chen L, Liu Y, Maruyama K, Jackson D, Kruse FE, Wiegand SJ, Dana MR, Streilein JW (2004) Inhibition of hemangiogenesis and lymphangiogenesis after normal-risk corneal transplantation by neutralizing VEGF promotes graft survival. Investig Ophthalmol Vis Sci 45(8):2666–2673
25. Bachmann BO, Bock F, Wiegand SJ, Maruyama K, Dana MR, Kruse FE, Luetjen-Drecoll E, Cursiefen C (2008) Promotion of graft survival by vascular endothelial growth factor a neutralization after high-risk corneal transplantation. Arch Ophthalmol 126(1):71–77
26. Dastjerdi MH, Saban DR, Okanobo A, Nallasamy N, Sadrai Z, Chauhan SK, Hajrasouliha AR, Dana R (2010) Effects of topical and subconjunctival bevacizumab in high-risk corneal transplant survival. Investig Ophthalmol Vis Sci 51(5):2411–2417
27. Rocher N, Behar-Cohen F, Pournaras JA, Naud MC, Jeanny JC, Jonet L, Bourges JL (2011) Effects of rat anti-VEGF antibody in a rat model of corneal graft rejection by topical and subconjunctival routes. Mol Vis 17:104–112
28. Dohlman TH, Omoto M, Hua J, Stevenson W, Lee SM, Chauhan SK, Dana R (2015) VEGF-trap aflibercept significantly improves long-term graft survival in high-risk corneal transplantation. Transplantation 99(4):678–686
29. Di Zazzo A, Tahvildari M, Subbarayal B, Yin J, Dohlman TH, Inomata T, Mashaghi A, Chauhan SK, Dana R (2017) Proangiogenic function of T cells in corneal transplantation. Transplantation 101(4):778–785
30. Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM (2010) VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res 107(3):408–417
31. Chung ES, Chauhan SK, Jin Y, Nakao S, Hafezi-Moghadam A, van Rooijen N, Zhang Q, Chen L, Dana R (2009) Contribution of macrophages to angiogenesis induced by vascular endothelial growth factor receptor-3-specific ligands. Am J Pathol 175(5):1984–1992
32. Bhatti N, Qidwai U, Hussain M, Kazi A (2013) Efficacy of subconjunctival and topical bevacizumab in high-risk corneal transplant survival. J Pak Med Assoc 63(10):1256–1259
33. Dekaris I, Gabric N, Draca N, Pauk-Gulic M, Milicic N (2015) Three-year corneal graft survival rate in high-risk cases treated with subconjunctival and topical bevacizumab. Graefes Arch Clin Exp Ophthalmol 253(2):287–294
34. Fasciani R, Mosca L, Giannico MI, Ambrogio SA, Balestrazzi E (2015) Subconjunctival and/or intrastromal bevacizumab

injections as preconditioning therapy to promote corneal graft survival. Int Ophthalmol 35(2):221–227
35. Vassileva PI, Hergeldzhieva TG (2009) Avastin use in high risk corneal transplantation. Graefes Arch Clin Exp Ophthalmol 247(12):1701–1706
36. Al-Mahmood S, Colin S, Farhat N, Thorin E, Steverlynck C, Chemtob S (2009) Potent in vivo antiangiogenic effects of GS-101 (5′-TATCCGGAGGGCTCGCCATGCTGCT-3′), an antisense oligonucleotide preventing the expression of insulin receptor substrate-1. J Pharmacol Exp Ther 329(2):496–504
37. Cursiefen C, Viaud E, Bock F, Geudelin B, Ferry A, Kadlecova P, Levy M, Al Mahmood S, Colin S, Thorin E, Majo F, Frueh B, Wilhelm F, Meyer-Ter-Vehn T, Geerling G, Bohringer D, Reinhard T, Meller D, Pleyer U, Bachmann B, Seitz B (2014) Aganirsen antisense oligonucleotide eye drops inhibit keratitis-induced corneal neovascularization and reduce need for transplantation: the I-CAN study. Ophthalmology 121(9):1683–1692
38. Murthy RC, McFarland TJ, Yoken J, Chen S, Barone C, Burke D, Zhang Y, Appukuttan B, Stout JT (2003) Corneal transduction to inhibit angiogenesis and graft failure. Investig Ophthalmol Vis Sci 44(5):1837–1842
39. Parker M, Bellec J, McFarland T, Scripps V, Appukuttan B, Hartzell M, Yeager A, Hady T, Mitrophanous KA, Stout T, Ellis S (2014) Suppression of neovascularization of donor corneas by transduction with equine infectious anemia virus-based lentiviral vectors expressing endostatin and angiostatin. Hum Gene Ther 25(5):408–418
40. Bachmann B, Taylor RS, Cursiefen C (2010) Corneal neovascularization as a risk factor for graft failure and rejection after keratoplasty: an evidence-based meta-analysis. Ophthalmology 117(7):1300–1305 e1307
41. Cursiefen C, Colin J, Dana R, Diaz-Llopis M, Faraj LA, Garcia-Delpech S, Geerling G, Price FW, Remeijer L, Rouse BT, Seitz B, Udaondo P, Meller D, Dua H (2012) Consensus statement on indications for anti-angiogenic therapy in the management of corneal diseases associated with neovascularisation: outcome of an expert roundtable. Br J Ophthalmol 96(1):3–9
42. Streilein JW, Toews GB, Bergstresser PR (1979) Corneal allografts fail to express Ia antigens. Nature 282(5736):326–327
43. Liu Y, Hamrah P, Zhang Q, Taylor AW, Dana MR (2002) Draining lymph nodes of corneal transplant hosts exhibit evidence for donor major histocompatibility complex (MHC) class II-positive dendritic cells derived from MHC class II-negative grafts. J Exp Med 195(2):259–268
44. Hamrah P, Dana MR (2007) Corneal antigen-presenting cells. Chem Immunol Allergy 92:58–70
45. Hamrah P, Huq SO, Liu Y, Zhang Q, Dana MR (2003) Corneal immunity is mediated by heterogeneous population of antigen-presenting cells. J Leukoc Biol 74(2):172–178
46. Nakamura T, Ishikawa F, Sonoda KH, Hisatomi T, Qiao H, Yamada J, Fukata M, Ishibashi T, Harada M, Kinoshita S (2005) Characterization and distribution of bone marrow-derived cells in mouse cornea. Investig Ophthalmol Vis Sci 46(2):497–503
47. Hamrah P, Zhang Q, Liu Y, Dana MR (2002) Novel characterization of MHC class II-negative population of resident corneal Langerhans cell-type dendritic cells. Investig Ophthalmol Vis Sci 43(3):639–646
48. Flynn TH, Mitchison NA, Ono SJ, Larkin DF (2008) Aqueous humor alloreactive cell phenotypes, cytokines and chemokines in corneal allograft rejection. Am J Transplant 8(7):1537–1543
49. Slegers TP, Broersma L, van Rooijen N, Hooymans JM, van Rij G, van der Gaag R (2004) Macrophages play a role in the early phase of corneal allograft rejection in rats. Transplantation 77(11):1641–1646
50. Zhang X, Shen L, Jin Y, Saban DR, Chauhan SK, Dana R (2009) Depletion of passenger leukocytes from corneal grafts: an effective means of promoting transplant survival? Investig Ophthalmol Vis Sci 50(7):3137–3144
51. Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K (2010) Development of monocytes, macrophages, and dendritic cells. Science 327(5966):656–661
52. Liu K, Victora GD, Schwickert TA, Guermonprez P, Meredith MM, Yao K, Chu FF, Randolph GJ, Rudensky AY, Nussenzweig M (2009) In vivo analysis of dendritic cell development and homeostasis. Science 324(5925):392–397
53. Hamrah P, Liu Y, Zhang Q, Dana MR (2003) Alterations in corneal stromal dendritic cell phenotype and distribution in inflammation. Arch Ophthalmol 121(8):1132–1140
54. Huq S, Liu Y, Benichou G, Dana MR (2004) Relevance of the direct pathway of sensitization in corneal transplantation is dictated by the graft bed microenvironment. J Immunol 173(7):4464–4469
55. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392(6673):245–252
56. Hattori T, Saban DR, Emami-Naeini P, Chauhan SK, Funaki T, Ueno H, Dana R (2012) Donor-derived, tolerogenic dendritic cells suppress immune rejection in the indirect allosensitization-dominant setting of corneal transplantation. J Leukoc Biol 91(4):621–627
57. Jin Y, Shen L, Chong EM, Hamrah P, Zhang Q, Chen L, Dana MR (2007) The chemokine receptor CCR7 mediates corneal antigen-presenting cell trafficking. Mol Vis 13:626–634
58. Hamrah P, Yamagami S, Liu Y, Zhang Q, Vora SS, Lu B, Gerard CJ, Dana MR (2007) Deletion of the chemokine receptor CCR1 prolongs corneal allograft survival. Investig Ophthalmol Vis Sci 48(3):1228–1236
59. Pillai RG, Beutelspacher SC, Larkin DF, George AJ (2008) Expression of the chemokine antagonist vMIP II using a non-viral vector can prolong corneal allograft survival. Transplantation 85(11):1640–1647
60. Hajrasouliha AR, Funaki T, Sadrai Z, Hattori T, Chauhan SK, Dana R (2012) Vascular endothelial growth factor-C promotes alloimmunity by amplifying antigen-presenting cell maturation and lymphangiogenesis. Investig Ophthalmol Vis Sci 53(3):1244–1250
61. Hua J, Stevenson W, Dohlman TH, Inomata T, Tahvildari M, Calcagno N, Pirmadjid N, Sadrai Z, Chauhan SK, Dana R (2016) Graft site microenvironment determines dendritic cell trafficking through the CCR7–CCL19/21 axis. Investig Ophthalmol Vis Sci 57(3):1457–1467
62. Dohlman TH, Di Zazzo A, Omoto M, Hua J, Ding J, Hamrah P, Chauhan SK, Dana R (2016) E-selectin mediates immune cell trafficking in corneal transplantation. Transplantation 100(4):772–780
63. Nibbs RJ, Kriehuber E, Ponath PD, Parent D, Qin S, Campbell JD, Henderson A, Kerjaschki D, Maurer D, Graham GJ, Rot A (2001) The beta-chemokine receptor D6 is expressed by lymphatic endothelium and a subset of vascular tumors. Am J Pathol 158(3):867–877
64. Hajrasouliha AR, Sadrai Z, Lee HK, Chauhan SK, Dana R (2013) Expression of the chemokine decoy receptor D6 mediates dendritic cell function and promotes corneal allograft rejection. Mol Vis 19:2517–2525
65. Lapp T, Zaher SS, Haas CT, Becker DL, Thrasivoulou C, Chain BM, Larkin DF, Noursadeghi M (2015) Identification of therapeutic targets of inflammatory monocyte recruitment to modulate the allogeneic injury to donor cornea. Investig Ophthalmol Vis Sci 56(12):7250–7259
66. Qian Y, Hamrah P, Boisgerault F, Yamagami S, Vora S, Benichou G, Dana MR (2002) Mechanisms of immunotherapeutic intervention by anti-CD154 (CD40L) antibody in high-risk corneal transplantation. J Interferon Cytokine Res 22(12):1217–1225

67. Qian Y, Dana MR (2002) Effect of locally administered anti-CD154 (CD40 ligand) monoclonal antibody on survival of allogeneic corneal transplants. Cornea 21(6):592–597
68. Hoffmann F, Zhang EP, Pohl T, Kunzendorf U, Wachtlin J, Bulfone-Paus S (1997) Inhibition of corneal allograft reaction by CTLA4-Ig. Graefes Arch Clin Exp Ophthalmol 235(8):535–540
69. Comer RM, King WJ, Ardjomand N, Theoharis S, George AJ, Larkin DF (2002) Effect of administration of CTLA4-Ig as protein or cDNA on corneal allograft survival. Investig Ophthalmol Vis Sci 43(4):1095–1103
70. Kagaya F, Hori J, Kamiya K, Kaji Y, Oshika T, Amano S, Yamagami S, Tsuru T, Tanaka S, Matsuda H, Yagita H, Okumura K (2002) Inhibition of murine corneal allograft rejection by treatment with antibodies to CD80 and CD86. Exp Eye Res 74(1):131–139
71. Watson MP, George AJ, Larkin DF (2006) Differential effects of costimulatory pathway modulation on corneal allograft survival. Investig Ophthalmol Vis Sci 47(8):3417–3422
72. Kuchroo VK, Dardalhon V, Xiao S, Anderson AC (2008) New roles for TIM family members in immune regulation. Nat Rev Immunol 8(8):577–580
73. Tan X, Jie Y, Zhang Y, Qin Y, Xu Q, Pan Z (2014) Tim-1 blockade with RMT1-10 increases T regulatory cells and prolongs the survival of high-risk corneal allografts in mice. Exp Eye Res 122:86–93
74. Yamada J, Hamuro J, Terai K, Kinoshita S (2007) Major histocompatibility complex semi-matching improves murine corneal allograft survival under oxidative macrophage dominancy. Transplantation 84(7):899–907
75. Hua J, Jin Y, Chen Y, Inomata T, Lee H, Chauhan SK, Petasis NA, Serhan CN, Dana R (2014) The resolvin D1 analogue controls maturation of dendritic cells and suppresses alloimmunity in corneal transplantation. Investig Ophthalmol Vis Sci 55(9):5944–5951
76. Saban DR, Bock F, Chauhan SK, Masli S, Dana R (2010) Thrombospondin-1 derived from APCs regulates their capacity for allosensitization. J Immunol 185(8):4691–4697
77. Morelli AE, Thomson AW (2007) Tolerogenic dendritic cells and the quest for transplant tolerance. Nat Rev Immunol 7(8):610–621
78. Natarajan S, Thomson AW (2010) Tolerogenic dendritic cells and myeloid-derived suppressor cells: potential for regulation and therapy of liver auto- and alloimmunity. Immunobiology 215(9–10):698–703
79. Ezzelarab M, Thomson AW (2011) Tolerogenic dendritic cells and their role in transplantation. Semin Immunol 23(4):252–263
80. Khan A, Fu H, Tan LA, Harper JE, Beutelspacher SC, Larkin DF, Lombardi G, McClure MO, George AJ (2013) Dendritic cell modification as a route to inhibiting corneal graft rejection by the indirect pathway of allorecognition. Eur J Immunol 43(3):734–746
81. Boisgerault F, Liu Y, Anosova N, Ehrlich E, Dana MR, Benichou G (2001) Role of $CD4^+$ and $CD8^+$ T cells in allorecognition: lessons from corneal transplantation. J Immunol 167(4):1891–1899
82. Yamada J, Kurimoto I, Streilein JW (1999) Role of $CD4^+$ T cells in immunobiology of orthotopic corneal transplants in mice. Investig Ophthalmol Vis Sci 40(11):2614–2621
83. Chauhan SK, Jurkunas U, Funaki T, Dastjerdi M, Dana R (2015) Quantification of allospecific and nonspecific corneal endothelial cell damage after corneal transplantation. Eye (Lond) 29(1):136–144
84. Hegde S, Beauregard C, Mayhew E, Niederkorn JY (2005) CD4(+) T-cell-mediated mechanisms of corneal allograft rejection: role of Fas-induced apoptosis. Transplantation 79(1):23–31
85. Sano Y, Osawa H, Sotozono C, Kinoshita S (1998) Cytokine expression during orthotopic corneal allograft rejection in mice. Investig Ophthalmol Vis Sci 39(10):1953–1957
86. Ayliffe W, Alam Y, Bell EB, McLeod D, Hutchinson IV (1992) Prolongation of rat corneal graft survival by treatment with anti-CD4 monoclonal antibody. Br J Ophthalmol 76(10):602–606
87. He YG, Ross J, Niederkorn JY (1991) Promotion of murine orthotopic corneal allograft survival by systemic administration of anti-CD4 monoclonal antibody. Investig Ophthalmol Vis Sci 32(10):2723–2728
88. Yamada J, Hamuro J, Fukushima A, Ohteki T, Terai K, Iwakura Y, Yagita H, Kinoshita S (2009) MHC-matched corneal allograft rejection in an IFN-gamma/IL-17-independent manner in C57BL/6 mice. Investig Ophthalmol Vis Sci 50(5):2139–2146
89. Hargrave SL, Hay C, Mellon J, Mayhew E, Niederkorn JY (2004) Fate of MHC-matched corneal allografts in Th1-deficient hosts. Investig Ophthalmol Vis Sci 45(4):1188–1193
90. Niederkorn JY, Stevens C, Mellon J, Mayhew E (2006) Differential roles of $CD8^+$ and $CD8^-$ T lymphocytes in corneal allograft rejection in 'high-risk' hosts. Am J Transplant 6(4):705–713
91. Yamada J, Ksander BR, Streilein JW (2001) Cytotoxic T cells play no essential role in acute rejection of orthotopic corneal allografts in mice. Investig Ophthalmol Vis Sci 42(2):386–392
92. Cunnusamy K, Chen PW, Niederkorn JY (2010) IL-17 promotes immune privilege of corneal allografts. J Immunol 185(8):4651–4658
93. Yin XT, Zobell S, Jarosz JG, Stuart PM (2015) Anti-IL-17 therapy restricts and reverses late-term corneal allorejection. J Immunol 194(8):4029–4038
94. Cunnusamy K, Chen PW, Niederkorn JY (2011) IL-17A-dependent $CD4^+$ $CD25^+$ regulatory T cells promote immune privilege of corneal allografts. J Immunol 186(12):6737–6745
95. Zhu J, Liu Y, Pi Y, Jia L, Wang L, Huang Y (2014) Systemic application of sphingosine 1-phosphate receptor 1 immunomodulator inhibits corneal allograft rejection in mice. Acta Ophthalmol 92(1):e12–21
96. Zhu J, Liu Y, Huang Y (2015) Topical application of sphingosine 1-phosphate receptor 1 prolongs corneal graft survival in mice. Mol Med Rep 11(5):3800–3807
97. Roncarolo MG, Battaglia M (2007) Regulatory T-cell immunotherapy for tolerance to self antigens and alloantigens in humans. Nat Rev Immunol 7(8):585–598
98. Sakaguchi S, Yamaguchi T, Nomura T, Ono M (2008) Regulatory T cells and immune tolerance. Cell 133(5):775–787
99. Ohkura N, Sakaguchi S (2011) Maturation of effector regulatory T cells. Nat Immunol 12(4):283–284
100. Chauhan SK, Saban DR, Lee HK, Dana R (2009) Levels of Foxp3 in regulatory T cells reflect their functional status in transplantation. J Immunol 182(1):148–153
101. Hori J, Taniguchi H, Wang M, Oshima M, Azuma M (2010) GITR ligand-mediated local expansion of regulatory T cells and immune privilege of corneal allografts. Investig Ophthalmol Vis Sci 51(12):6556–6565
102. Hildebrand A, Jarsch C, Kern Y, Bohringer D, Reinhard T, Schwartzkopff J (2014) Subconjunctivally applied naive Tregs support corneal graft survival in baby rats. Mol Vis 20:1749–1757
103. Xu Q, Tan X, Zhang Y, Jie Y, Pan Z (2015) Subconjunctival injection of in vitro transforming growth factor-beta-induced regulatory T cells prolongs allogeneic corneal graft survival in mice. Int J Clin Exp Med 8(11):20271–20278
104. Wang X, Wang W, Xu J, Le Q (2013) Effect of rapamycin and interleukin-2 on regulatory $CD4^+CD25^+Foxp3^+$ T cells in mice after allogenic corneal transplantation. Transplant Proc 45(2):528–537

105. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY (2005) A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol 6(11):1142–1151
106. Barron L, Dooms H, Hoyer KK, Kuswanto W, Hofmann J, O'Gorman WE, Abbas AK (2010) Cutting edge: mechanisms of IL-2-dependent maintenance of functional regulatory T cells. J Immunol 185(11):6426–6430
107. Tahvildari M, Omoto M, Chen Y, Emami-Naeini P, Inomata T, Dohlman TH, Kaye AE, Chauhan SK, Dana R (2016) In vivo expansion of regulatory T cells by low-dose interleukin-2 treatment increases allograft survival in corneal transplantation. Transplantation 100(3):525–532
108. Chauhan SK, Saban DR, Dohlman TH, Dana R (2014) CCL-21 conditioned regulatory T cells induce allotolerance through enhanced homing to lymphoid tissue. J Immunol 192(2):817–823
109. Wang X, Wang W, Xu J, Wu S, Le Q (2015) All-trans retinoid acid promotes allogeneic corneal graft survival in mice by regulating Treg–Th17 balance in the presence of TGF-beta. BMC Immunol 16:17
110. Tan X, Zeng H, Jie Y, Zhang Y, Xu Q, Pan Z (2014) CD154 blockade modulates the ratio of Treg to Th1 cells and prolongs the survival of allogeneic corneal grafts in mice. Exp Ther Med 7(4):827–834
111. Grewal IS, Flavell RA (1998) CD40 and CD154 in cell-mediated immunity. Annu Rev Immunol 16:111–135
112. Choi HJ, Lee JJ, Kim DH, Kim MK, Lee HJ, Ko AY, Kang HJ, Park C, Wee WR (2015) Blockade of CD40–CD154 costimulatory pathway promotes long-term survival of full-thickness porcine corneal grafts in nonhuman primates: clinically applicable xenocorneal transplantation. Am J Transplant 15(3):628–641
113. Uccelli A, Moretta L, Pistoia V (2008) Mesenchymal stem cells in health and disease. Nat Rev Immunol 8(9):726–736
114. Jia Z, Jiao C, Zhao S, Li X, Ren X, Zhang L, Han ZC, Zhang X (2012) Immunomodulatory effects of mesenchymal stem cells in a rat corneal allograft rejection model. Exp Eye Res 102:44–49
115. Oh JY, Lee RH, Yu JM, Ko JH, Lee HJ, Ko AY, Roddy GW, Prockop DJ (2012) Intravenous mesenchymal stem cells prevented rejection of allogeneic corneal transplants by aborting the early inflammatory response. Mol Ther 20(11):2143–2152
116. Omoto M, Katikireddy KR, Rezazadeh A, Dohlman TH, Chauhan SK (2014) Mesenchymal stem cells home to inflamed ocular surface and suppress allosensitization in corneal transplantation. Investig Ophthalmol Vis Sci 55(10):6631–6638
117. Murphy N, Lynch K, Lohan P, Treacy O, Ritter T (2016) Mesenchymal stem cell therapy to promote corneal allograft survival: current status and pathway to clinical translation. Curr Opin Organ Transplant 21(6):559–567
118. Treacy O, O'Flynn L, Ryan AE, Morcos M, Lohan P, Schu S, Wilk M, Fahy G, Griffin MD, Nosov M, Ritter T (2014) Mesenchymal stem cell therapy promotes corneal allograft survival in rats by local and systemic immunomodulation. Am J Transplant 14(9):2023–2036
119. Lapp T, Hildebrand A, Bohringer D, Betancor PK, Schlunck G, Reinhard T (2016) Optimizing rejection readouts in a corneal allograft transplantation model. Mol Vis 22:1248–1255
120. Liu YC, Lwin NC, Chan NS, Mehta JS (2014) Use of anterior segment optical coherence tomography to predict corneal graft rejection in small animal models. Investig Ophthalmol Vis Sci 55(10):6736–6741
121. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM (2000) M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 164(12):6166–6173
122. Yamada J, Streilein JW (1998) Fate of orthotopic corneal allografts in C57BL/6 mice. Transpl Immunol 6(3):161–168
123. Shanks N, Greek R, Greek J (2009) Are animal models predictive for humans? Philos Ethics Humanit Med 4:2