Chapter 13

Corneal Immunosuppressive Mechanisms, Anterior Chamber-Associated Immune Deviation (ACAID) and Their Role in Allograft Rejection

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Abstract

Corneal transplantation is the most frequently performed transplant procedure in humans. Human leukocyte antigen matching, while imperative for other types of organ transplants, is usually not performed before cornea transplantation. With the use of topical steroid immunosuppressants, which are subsequently tailed off to almost zero, most corneal transplants will not be rejected in recipients with low risk of graft rejection. This phenomenon has been described as immune privilege by Medawar many years ago. However, this immune privilege is relative and can be easily eroded, e.g. by postoperative nonspecific inflammation or other causes of corneal or ocular inflammation. Interestingly, corneas that are at high risk of rejection have a higher failure rate than other organs. Considerable progress has been made in recent years to provide a better understanding of corneal immune privilege. This chapter will review current knowledge on ocular immunosuppressive mechanisms including anterior chamber-associated immune deviation and discuss their role(s) in corneal allograft rejection. Ultimately, this evolving information will be of benefit in developing therapeutic strategies to prevent corneal transplant rejection.

Key words Cornea, Transplantation, ACAID, Immunosuppression, Tregs

1 Introduction

The cornea is one of only a select few tissues in the body that enjoy immune-privileged status by maintaining immunological ignorance (others include the brain, testes, the pregnant uterus, and the anterior chamber (AC) of the eye). Any corneal inflammatory events, if allowed to proceed unabated, may break down immunological barriers. The most serious consequence for the cornea is immunological destruction of corneal endothelial cells, which have no capacity to regenerate. The result of this rejection process is corneal edema, corneal opacification, and blindness. The concept of relative immune privilege, as applied to the cornea, is supported by observations of high survival rates of corneal allografts without routine human leukocyte antigen (HLA)

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matching between donor and host tissues and the use of only locally applied topical corticosteroids, as opposed to using systemic immunosuppressants which would be standard for other organ transplants. Corneal allograft-associated immune privilege, however, cannot be ascribed to one single mediator or mechanism and is likely to be the result of multiple anatomical, physiological, and immunomodulatory properties inherent to the allograft itself and also the host graft bed [1].

2 Physiological and Anatomical Properties of the Immune-Privileged Cornea

Physiologically, the normal, healthy cornea is both avascular and devoid of lymphatic vessels, thereby shielding itself from immune-mediated attack by denying potentially harmful infiltrating immune cells getting access to the graft and preventing transport of antigens and antigen-presenting cells (APCs) to secondary lymphoid tissues, such as the draining lymph nodes (DLN) [2]. It has been shown that maintenance of corneal avascularity is due to constitutive expression of soluble vascular endothelial growth factor receptor-1 (VEGFR-1) by epithelial cells [3]; with another study demonstrating that administration of VEGF receptor-3 (VEGFR-3) can also suppress hemangiogenesis [4]. Another naturally occurring angiogenesis inhibitor is endostatin. Endostatin can inhibit endothelial cell functions by several means, including attenuation of VEGF receptor signaling and its subsequent binding to $\alpha_5\beta_1$ integrins [5]. Furthermore, Tan and colleagues could show that both syngeneic and allogeneic corneal grafts produce endostatin and while levels remained high in syngeneic grafts, they began to decrease in allografts 10 days post-transplantation. This correlated with early recruitment of allo-specific T cells into grafts, which led to the destruction of endostatin-producing cells and ultimately allograft rejection in 75 % of cases [6]. The authors also found that local administration of exogenous endostatin treatment could attenuate allograft rejection.

Lymphangiogenesis, on the other hand, is suppressed by secretion of soluble VEGFR-2 (which inhibits VEGF-C activity) by keratocytes and corneal epithelial cells but, interestingly, does not affect hemangiogenesis [7]. In this study, the authors showed that soluble VEGFR-2 could inhibit lymphatic vessel infiltration into "high-risk" corneal grafts, that is, graft beds in which intrastromal sutures had been placed 2 weeks prior to transplantation to induce lymphangiogenesis and hemangiogenesis. Their results also showed that a single intracorneal injection of soluble VEGFR-2 was sufficient to significantly prolong corneal allograft survival compared to untreated recipients [7].

Another important factor with regard to corneal immune privilege is the weak or absence of expression of MHC class I

and II antigens, respectively, by corneal epithelial, stromal, and endothelial cells. This has the effect of limiting immunogenicity to foreign antigens as the capacity for antigen uptake is attenuated [8].

3 Soluble and Cell Membrane-Bound Mediators of Corneal Immunosuppression

In addition to the physiological and anatomical properties outlined above, the cornea also expresses numerous cell membrane-bound immunomodulatory molecules and molecules capable of inducing apoptosis of effector immune cells. These include Fas ligand (FasL, CD95L), complement regulatory proteins (CRPs), tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), programmed death-ligand 1 (PD-L1), and MHC-Ib. FasL is an apoptosis-inducing molecule expressed by a number of ocular cells, including corneal epithelial and endothelial cells and can induce apoptosis of infiltrating Fas receptor (CD95)-expressing effector T cells and neutrophils [9, 10], thereby protecting the corneal graft from immune-mediated rejection. The importance of FasL was highlighted by studies in which murine corneal allografts with defective FasL expression, when transplanted, displayed a much higher incidence of immune-mediated rejection when compared to wild-type controls [11, 12]. Similar to FasL, corneal cells also express PD-L1 which, following engagement with its receptor programmed death (PD)-1 on T cells, leads to inhibition of T-cell proliferation and induction of apoptosis as well as reduced interferon (IFN)-γ secretion, thereby promoting corneal allograft survival [1, 13–15]. Further evidence supporting the key role of PD-L1 in determining the fate of corneal allografts comes by way of a study by Shen and co-workers where they reported that murine C57BL/6 PD-L1$^{-/-}$ allografts were more susceptible to rejection than wild-type allografts when placed into Balb/c hosts [14]. Furthermore, we have shown recently that lentivirus-mediated overexpression of PD-L1 on donor corneas leads to >80 % allograft survival compared to just 20 % in untreated allogeneic control grafts underlining the importance of this molecule in protecting corneal tissues from immune response [16]. TRAIL is another pro-apoptotic molecule that, similar to FasL and PD-L1, can induce apoptosis of inflammatory cells. However, although corneal cells do express TRAIL [17, 18] and evidence suggests adenovirus-mediated overexpression of TRAIL can prolong murine corneal allograft survival [19], no reports have yet been published establishing a link between TRAIL expression and preservation of corneal immune privilege specifically. CRPs play a vital role in protecting cells from complement-mediated damage and are expressed predominantly by the corneal epithelium [20]. Soluble CRPs are also present in physiologically relevant quantities in the aqueous humor (AH) that bathes the corneal endothelium and function to protect

this pivotal nonregenerative cell layer from complement-mediated lysis [20, 21]. Additionally, some CRPs, such as decay accelerating factor (DAF, also known as CD55), can disrupt APC:T cell interactions and contribute to corneal allograft immune privilege in this way [22] . Indeed, Esposito and colleagues [22] demonstrated in a murine corneal transplant model that when either the donor cornea or the recipient graft bed is deficient in DAF, rapid rejection ensues.

The AH contains a large amount of immunosuppressive molecules that contribute significantly to maintaining immunological ignorance. These include anti-inflammatory cytokines (e.g. tissue growth factor (TGF)-β2), complement inhibitors [21], neuropeptides, alpha-melanocyte stimulating hormone, vasoactive intestinal peptide, and calcitonin gene-related peptide [23–26]. A soluble version of FasL is also found in the AH and is an important endogenous immunosuppressant as it can suppress neutrophil recruitment and activation [27, 28]. As mentioned earlier, corneal cells only weakly express MHC class I molecules, if at all and this is an important characteristic in maintaining immune privilege. However, the corneal cells may become a target for natural killer (NK) cells, as these cells are programmed to target and kill any cell that does not express MHC class I molecules [29]. The cells at highest risk of attack are those comprising the corneal endothelium and it has been shown that rats undergoing corneal allograft rejection have NK cells present in their AH, which bathes the endothelium [30]. To counteract this possible NK cell-mediated cytolysis, however, the AH contains physiologically relevant levels of TGF-β and macrophage migration inhibitory factor, both of which are capable of neutralizing the effects of NK cells [31–34].

4 Anterior Chamber-Associated Immune Deviation and Corneal Allograft Fate

As described previously, key mechanisms which characterize ocular immune privilege are the unique anatomical and cellular barriers of the eye and the expression of key immunomodulatory molecules including but not limited to interleukin (IL)-10, TGF-β, FasL, and PD-L1 [9, 12, 14, 16, 20, 35–37]. In addition to maintaining a local immunosuppressive environment, the eye is also capable of orchestrating systemic immunoregulatory responses against intraocular antigens. This mechanism of ocular immunosuppression has been given the term anterior chamber-associated immune deviation (ACAID) [38–40]. In corneal transplantation, the donor allografts are in direct contact with the AC and it is this location that correlates closely with the allograft's capacity to survive and induce ACAID to donor alloantigens [41–43].

ACAID, an atypical systemic response to alloantigens is not, as once believed, due to immunological ignorance but rather an active process that induces unique cellular mechanisms which can suppress destructive cellular responses such as delayed type

hypersensitivity (DTH) and cytotoxic T lymphocytes (CTLs) [44]. Studies have illustrated AC injection of donor cells prior to orthotopic corneal transplantation results in a significant increase in the acceptance of corneal allografts in rats and mice [41, 45]. However, it is the introduction of alloantigens during and after routine keratoplasty that is believed to contribute to the ACAID role in corneal transplantation survival [41].

The AC contains AH consisting of biologically relevant concentrations of various immunomodulatory factors as previously described [20, 35–37]. Compelling evidence demonstrates that the F4/80-positive APCs in the eye, maintained in an immature state due to the presence of constitutively expressed TGF-β2, capture intraocular antigens [46]. These antigen-bearing APCs subsequently migrate through the blood stream to the marginal zone of the spleen where they induce the formation of ACAID-regulatory T cells (Tregs) [39, 41, 43, 47]. Both cell-associated and soluble antigen injected into the eye has been detected in the DLN at 6 h and in the spleen after 16–24 h post injection [48, 49]. Once ocular-derived APCs enter the spleen, a series of complex cellular interactions which are not yet fully understood involving CD4 + T cells, natural killer (NK) T cells, B cells, and γδT cells culminate in the generation of Tregs that suppress DTH responses in an antigen-specific manner [42, 50, 51].

Eventually, antigen-specific Tregs that mediate ACAID emerge from these cell clusters in the spleen [39, 44]. It is these antigen-specific CD4+ and CD8+ Tregs that contribute to ocular immune privilege by down-regulating immune responses and protecting a graft from immune rejection after transplantation [52]. The first "afferent" set of cells made up of $CD4^{+}$Tregs prevent the activation and differentiation of antigen-specific effector T helper cells (Th1). Following this, a second set of "efferent" cells consisting of the $CD8^{+}$Tregs are associated with the inhibition of DTH [40]. Interestingly, it is these different forms of immune tolerance which have been demonstrated to be involved in the induction of ACAID and play a role in the promotion of corneal allograft survival [53, 54]. These findings suggest that ACAID may be required for long-term survival of corneal allografts and indicates that immune privilege in the eye is sustained through the cooperation of various cells from organs other than the eye itself.

The concept of ACAID, in which antigen-bearing APCs migrate from the eye, is not yet proven in humans and animal studies have demonstrated that cells do not need to leave the ocular microenvironment for antigen to induce a reduced DTH [55, 56]. The nature of the APC which promotes ACAID-induced tolerance is unclear, but mice deficient in cells expressing the macrophage surface marker F4/80 fail to generate tolerance after injection following donor antigen challenge [57]. Others have suggested that ocular fluids containing material such as soluble proteins from incoming inflammatory cells enter the blood circulation and arrive

at the spleen where further amplification of the NKT cell/F4/80 spleen cell-mediated process of T-cell apoptosis occurs [58]. Winton et al. also suggest antigen may travel from the eye to the spleen, lymph nodes of the head and neck, and mesenteric lymph nodes in a soluble form through blood and lymph [56].

The CD4+ T cells which recognize alloantigen via the indirect pathway are the cells which are believed to be required for induction of corneal allograft rejection [59]. It also has been described that IFN-γ is not necessary for the rejection of MHC-mismatched corneal grafts. However, IFN-γ and Th1 immune mechanisms have been demonstrated to be necessary for the rejection of MHC-matched but minor histocompatibility mismatched corneal allografts [60]. Interestingly, it has been recently demonstrated that IFN-γ is needed for alloantigen-specific ACAID CD8+ Tregs to execute their suppressive function but not required for the establishment of ACAID CD8+ Tregs [51]. Paunicka et al. provide evidence that the Tregs induced by AC injection of alloantigens (i.e. ACAID) are different from the Tregs induced by corneal allografts [51]. For example, in vivo administration of anti-CD8 antibody abolishes ACAID but has no effect on the immune privilege of corneal allografts. The authors suggest that an additional role of IFN-γ in exerting suppression during ACAID may be its ability to enhance the susceptibility of CD4+ effector cells to be suppressed by CD8+ Tregs [51].

Several cell-based therapies have been explored for their capacity to modulate the immune system of the corneal transplant recipient [61–63]. As described, APCs are key players in determining the induction of ACAID or tolerance. APCs are the cells with the capacity to transmit antigen-specific signals and direct adaptive immune responses. In one study by Khan et al. corneal allograft survival was prolonged by intravenously administering CTLA4-KDEL-expressing dendritic cells (DCs), however, this was only when the DCs were capable of indirect presentation of alloantigen [62]. Using donor-derived tolerogenic DCs, Hattori et al. demonstrated that corneal allograft recipients significantly suppress the indirect pathway of allorecognition and that this led to the inhibition of CD4+IFN-γ T cell frequencies. This DC cell therapy was also associated with an increase in Foxp3 expression in the Treg cell compartment [61]. We have recently shown that intravenous injection of donor bone marrow-derived DCs (BMDCs) or donor BMDCs treated with dexamethasone significantly prolongs corneal allograft survival without the need for additional immunosuppression. With both cell therapies, a significant reduction in the level of allograft cellular infiltration and a significant increase in the ratio of intragraft FoxP3 expressing regulatory cells in both the allograft and the DLNs were observed [63].

As well as examining APC-derived cell therapies, much interest has also been focused on the development of mesenchymal stromal cell (MSC)-based therapies to promote corneal allograft survival

[64, 65]. We recently demonstrated that allogeneic MSC treatment prolongs corneal allograft survival by suppressing peripheral immune responses and promoting an intragraft immunoregulatory milieu. This response was associated with a higher proportion of splenic CD4+Foxp3+ Treg cells [64]. Interestingly, Zhang et al. illustrated that systemic administration of human umbilical cord-derived MSCs (hUC-MSC) could potentiate the antigen-specific immune-suppressive responses induced by ACAID. The authors also demonstrated how administration of hUC-MSC was associated with increased cytokine production and Treg cell expansion within the spleen, capable of promoting and maintaining ACAID [66].

Aside from Treg expansion in the spleen, the possibility that Tregs can be induced locally within the eye has been a topic of much debate [55]. However, there is some evidence to suggest that naïve T cells that gain access to the ocular microenvironment may be skewed toward a Treg phenotype in situ [55]. Tregs recruited into the eye from the periphery, therefore, may be critical to tip the balance and together with local conversion help induce an immunosuppressive microenvironment and bring about resolution of potential transplantation rejection [55]. It must be noted that ACAID is not maintained when DTH is present under normal conditions and ACAID may be disturbed/abolished due to surgery-induced trauma, viral infection or chronic inflammation, all of which may contribute to corneal allograft rejection.

5 Conclusions

Relative ocular immune privilege is a fascinating area of immunology research. It is not the result of a single immunosuppressive mechanism, but rather is a combination of both local and systemic immunomodulation involving soluble factors as well as regulatory cells. Although corneal transplants benefit from relative ocular immune privilege, this privilege can be lost with subsequent failure of the transplant and blindness. Transplant immunology and eye research is proving beneficial at identifying factors and processes that protect cells and tissues from immune-mediated destruction or rejection. Future research will further elucidate the mechanisms of ocular immune privilege and open new areas of immunomodulation, particularly with respect to patients at high risk of corneal transplant rejection, that may also benefit other transplant models or immune-mediated diseases.

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