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Novel insights into the immunoregulatory function and localization of dendritic cells

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Abstract

Dendritic cells (DCs) are antigen-presenting cells that stimulate T cell-dependent immune responses upon antigen presentation. However, tolerogenic DCs (CD11c⁺MHC-II^{low}CD80^{low}/–CD86^{low}/–) induce immune tolerance by stimulating regulatory T cells (Tregs: CD4⁺CD25⁺Foxp3⁺). Although tolerogenic DCs are used to treat autoimmune diseases and to prevent transplantation rejection, the mechanisms involved are poorly understood. Here, we review our previous studies to elucidate the mechanisms involved in immune rejection of corneal allografts using a corneal transplant model. We found that donor-derived tolerogenic DCs significantly prolonged cornea allograft survival by suppressing indirect allosensitization. We also report the precise distribution of specific corneal DCs, termed Langerhans cells (LCs: CD11c⁺Langerin⁺MHC-II^{+/–}) in normal cornea. Using confocal microscopy, we constructed three-dimensional images of corneal LCs, which demonstrated that their cell bodies are present in the basal cell layer of the corneal epithelium. Furthermore, LC dendrites extend toward the ocular surface, but do not connect to epithelial tight junctions, indicating they cannot directly interact with ocular surface antigens. We confirm the potential of DC therapy for corneal graft rejection and report the function of LCs in normal cornea.

Keywords

tolerogenic dendritic cell; Langerhans cell; direct pathway; indirect pathway; tight junction

INTRODUCTION

Dendritic cells (DCs) and Langerhans cells (LCs) are professional antigen-presenting cells (APCs) that stimulate T cell-dependent immune responses. Based on the basic research of these cells, DCs are currently used in the clinic for the treatment of several diseases.¹ This article reviews DC therapy for corneal transplantation survival, and reviews the distribution of LCs in the normal cornea.

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FUNCTIONS OF MATURE, IMMATURE, AND TOLEROGENIC DCS

DCs are APCs that stimulate T cell-dependent immune responses upon antigen presentation.² Tissue DCs are considered immature (CD11c⁺MHC-II^{low}CD80/86^{low}) because they do not have the capacity to sensitize T cells, and induce immune tolerance by inducing apoptosis or anergy of T cells, or by stimulating regulatory T cells (Tregs, CD4⁺CD25⁺forkhead box [Fox] p3⁺) (Fig. 1).^{3, 4} It is thought that DCs act as sentinels in tissues by capturing and processing antigens. After charging their surface MHC class II molecules with immunogenic peptides, they develop into mature DCs (mDCs, CD11c⁺MHC-II^{high}CD80/86^{high}), traffic to the lymphoid organs and sensitize antigen-specific T cells. (Fig. 1).⁴ Immature DCs (iDCs), which maintain their immunosuppressive functions even during inflammation, were recently termed tolerogenic DCs (CD11c⁺MHC-II^{low}CD80^{low/-}CD86^{low/-}) (Fig. 1).⁵ In an effort to generate tolerogenic DCs, *in vitro*-propagated DCs have been manipulated by exposure to various anti-inflammatory and immunosuppressive agents.⁶

USE OF TOLEROGENIC DCS FOR TRANSPLANTATION

Tolerogenic DCs are effective for preventing transplantation rejection in various animal models of organ transplantation.^{5, 7, 8} Rapamycin-conditioned DCs administered to host mice prolonged heart^{9, 10} and skin graft survival.¹¹ Administration of DCs treated with the vitamin D₃ metabolite, 1 α ,25-dihydroxyvitamin D₃ (1 α ,25(OH)₂D₃), promoted tolerance to mouse pancreatic islet allografts.¹² Administering donor-derived DCs exposed to agonistic paired immunoglobulin-like receptor B monoclonal antibodies prolonged skin allograft survival in mice.¹³ Bone marrow-derived DCs generated *in vitro* with granulocyte-macrophage colony-stimulating factor, interleukin-10, and transforming growth factor- β , followed by pulsing with lipopolysaccharide prevented lethal graft-versus-host disease following allogeneic bone marrow transplantation in sublethal-irradiated mice.¹⁴ These tolerogenic DCs are termed DCregs. DCregs also promote murine heart transplantation survival.¹⁵

USE OF TOLEROGENIC DCS FOR CORNEAL TRANSPLANTATION

We previously reported that the administration of donor-derived tolerogenic DCs suppressed corneal transplantation rejection.¹⁶ We performed a DCreg adoptive transfer experiment in which DCregs isolated from B6 mice were transferred intravenously to corneal allograft recipients (BALB/c) before surgery. mDCs and iDCs were transferred as control cells. The adoptive transfer of 1×10^6 DCregs significantly increased the allograft survival compared with iDC- and mDC-treated recipient mice, as well as untreated recipient mice (Fig. 2). In addition to our study, another group also reported the efficacy of tolerogenic DCs for corneal transplantation. Glucocorticoid-treated donor bone marrow-derived DCs prolonged corneal allograft survival.¹⁷ Khan et al. established novel tolerogenic DCs that inhibited the expression of CD80/86 using a fusion protein, CTLA4-KDEL.¹⁸ Administration of CTLA4-KDEL-expressing DCs resulted in the long-term survival of corneal allografts.¹⁸

MECHANISM OF GRAFT REJECTION SUPPRESSION BY TOLEROGENIC DCS

Impairment of T cell immune reactions

The mechanisms by which tolerogenic DCs suppress allogeneic response have been studied. Rapamycin-treated DCs are poor producers of the cytokines interleukin-12p70 and tumor necrosis factor, and impair T cell immune reactions.⁹ These rapamycin-treated DCs can stimulate naturally occurring Tregs, in addition to their ability to inhibit expansion of CD4⁺ effector T cell populations.¹⁹ The administration of donor-derived DCs treated with 1 α , 25(OH)₂D₃ promoted the survival of mouse pancreatic islet allografts by inducing CD4⁺CD25⁺Foxp3⁺ Tregs.¹² DCregs also promote CD4⁺CD25⁺Foxp3⁺ Tregs and suppress antigen-specific autologous T-cell activation.^{15, 20} We also reported that the administration of donor-derived DCregs suppressed Th1 responses and expanded CD4⁺CD25⁺Foxp3⁺ Tregs in a mouse corneal transplantation model.¹⁶ The percentage of CD4⁺IFN- γ ⁺ cells in cervical lymph nodes after 3 weeks of corneal transplantation was approximately 1.0% in iDC- and mDC-treated recipient mice, as well as untreated recipient mice. Numbers of CD4⁺IFN- γ ⁺ cells in DCreg-treated recipient mice (0.6%) were decreased compared with iDC- and mDC-treated recipients, as well as untreated recipients. To determine whether DCregs generated Tregs *in vivo*, we detected the mRNA expression of Foxp3 in draining lymph nodes after corneal transplantation. The expression of Foxp3 in DCregs was higher than in untreated and mDC-treated groups (Fig. 3B).

Suppression of direct and indirect pathways of allorecognition

Organ graft rejection is caused by two different types of allosensitization: the direct and indirect pathways.²¹ The direct pathway involves T cells that respond directly to foreign MHC molecules expressed by donor-derived APCs (Fig. 4). In the indirect pathway, recipient APCs present donor-derived allopeptides in the context of self-MHC (Fig. 4). The direct pathway has classically been considered to be the most powerful mechanism involved in acute graft rejection. The indirect pathway is predominant at later timepoints following transplantation and is the main mechanism of allorecognition that triggers chronic rejection. Tolerogenic DCs have been used to interfere with the direct and indirect pathways of allorecognition with the aim of prolonging allograft survival.⁷ We reported that donor-derived DCregs suppressed indirect pathway-type allosensitization in mouse corneal transplant recipients.¹⁶ We measured the post-transplantation alloreactive T cell frequencies of untreated versus DCreg-infused recipients using an ELISPOT assay system (IFN- γ). We found that untreated recipients had increased direct and indirect pathway-type allosensitization compared with naïve mice (Fig. 5C, D). However, the magnitude of the increase was much higher in the indirect pathway (28-fold increase) than in the direct pathway (1.4-fold increase), suggesting that corneal graft rejection is indirect pathway-dominant. This result was in accord with previous studies.^{22–25} Strikingly, DCreg-infused recipients showed decreased direct ($P=0.007$) and indirect ($P=0.0007$) pathway-type allosensitization relative to untreated recipients; the magnitude of reduction was significantly higher in the indirect pathway (30-fold reduction) compared with the direct pathway (1.5-fold reduction) (Fig. 5C,D). Therefore, taken together, these data suggest that

donor-derived DCregs suppress indirect pathway-type allosensitization in corneal transplant recipients.

Interestingly, Divito et al. showed that injected donor-derived DCregs were processed by the recipient's quiescent conventional DCs in a mouse model of heart transplantation.²⁶ These host DCs in turn promoted the deletion of donor-specific effector T cells with consequent increases in the relative number of donor-specific CD4⁺CD25⁺Foxp3⁺ Tregs.²⁶ Processing of the injected donor-derived DCregs by host DCs can explain why donor-derived DCregs suppress the rejection of corneal allografts that mostly occurs via the indirect pathway of allorecognition. Thus, the tolerogenic status of host conventional DCs that process injected tolerogenic DCs appear to be key for the success of tolerogenic DC therapies.⁷

LCS IN NORMAL CORNEA

The cornea is thought to be an immune privileged tissue²⁷ because of the absence of APCs in the normal cornea.²⁸ However, recent studies revealed that a significant number of APCs reside in normal cornea.²⁹ Furthermore, significant numbers of DCs exist in the corneal epithelium and anterior stroma, and the posterior stroma contains high numbers of macrophages.²⁹

Langerin, a c-type lectin expressed by specific DCs such as LCs, recognizes pathogenic mycobacteria.³⁰ We previously reported the presence of CD11c⁺Langerin⁺ LCs in normal corneal epithelium.³¹ Most Langerin⁺ LCs also express MHC-II, but some are MHC-II-negative.

Functions of corneal LCs

The importance of LCs in the induction of immunoreactions against corneal microbial infection has been reported. The administration of LCs to the central cornea before *Pseudomonas* infection in BALB/c mice led to severe microbial keratitis.³² The presentation of Herpes simplex virus antigen on LCs favored the activation and accumulation of CD4⁺ T lymphocytes in Herpes simplex virus-1-infected mouse corneas.³³ Thus, LCs in the cornea are associated with the induction and amplification of immunoinflammatory responses in microbial and bacterial keratitis. However, how corneal LCs recognize these microbes is still unclear.

The mechanism by which skin LCs uptake skin surface antigen have been determined using three-dimensional (3D) images of skin LCs. Beneath the stratum corneum (SC), tight junctions (TJs) seal the paracellular spaces between keratinocytes at the stratum granulosum layer of the epidermis in mice.³⁴ Skin LCs form a network within the epidermis underneath TJs³⁵. SC damage in mice activates LCs, whose dendrites elongate, penetrate into the TJ barrier, take up antigens from the extra-TJ environment,³⁵ and trigger protective T helper 2 responses.³⁶ Based on these findings, we wanted to understand how corneal LCs uptake ocular surface antigen. We tried to establish 3D images of corneal LCs using the same methods used for skin LCs³⁵. We facilitated the 3D visualization of whole-mount corneas by confocal microscopy. We also studied the physiological interaction between the physical corneal barrier and corneal LCs. Corneas were harvested from naïve C57BL/6 mice and

fixed with methanol. Anti-ZO-1 antibody (Abcam, Cambridge, MA, USA) was used to stain the corneal epithelial TJs, and anti-MHC-II antibody (BioLegend, San Diego, CA, USA) was used to stain corneal LCs. The images of stained corneas were captured by confocal microscopy (LMS700, Carl Zeiss, Thornwood, NY, USA) and analyzed by an image analyzer (ZEN, Carl Zeiss). 3D reconstruction images of ZO-1 staining demonstrated that TJs sealed the paracellular pathway of double or triple layers of superficial cells (Fig. 6). We evaluated the positioning of LC dendrites in relation to TJs. 3D reconstruction images showed that most LC bodies existed in the basal cell layer, and an upward projection of LC dendrites toward the corneal surface, which stopped at the wing cell layer with obvious spaces between dendrite tips and TJs, was observed (Fig. 6). This result indicates that corneal LCs in normal cornea do not uptake foreign materials to the corneal surface.

SUMMARY, CONCLUSION, AND FUTURE DIRECTIONS

Previous studies, including our own, have demonstrated the therapeutic effect of donor-derived tolerogenic DCs on corneal transplant survival. Moreover, we showed that donor-derived DCregs suppressed the indirect pathway-type allosensitization, in addition to the direct pathway. It is necessary to prevent both direct and indirect pathway-type allosensitization to promote long-term organ transplantation survival. Thus, the suppression of both direct and indirect pathway-type allosensitization is a great advantage of using donor-derived DCregs for the promotion of long-term transplant survival.

We also determined the position of LC dendrites in relation to TJs in the normal cornea. 3D reconstruction images demonstrated an upward projection of LC dendrites toward the ocular surface, which stopped at the wing cell layer without interacting with the TJs, indicating that normal corneal LCs do not uptake ocular surface antigens. It is still unclear how corneal LCs uptake ocular surface antigen in immunopathogenic conditions. Our experimental methods using 3D reconstruction images of corneal LCs might help our understanding of how corneal LCs uptake ocular surface antigen in immunopathogenic conditions such as infectious keratitis or allergic keratoconjunctivitis.

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FIGURE 1.

Effect of immature DCs (iDCs), mature DCs (mDCs) and tolerogenic DCs on T cell immunity. The steady state iDCs remain quiescent after capturing and processing exogenous antigen. These quiescent iDCs express low levels of co-stimulatory molecules and therefore cause a deficient activation of naive T cells, and induce T cell apoptosis or anergy and probably the generation and/or expansion of regulatory T cells (Treg). In contrast, after exposure to danger signals and/or activation and maturation stimuli, iDCs increase the surface expression of MHC and co-stimulatory molecules, which enhances their ability to stimulate naive or memory T cells. Tolerogenic DCs present antigen to antigen-specific T cells but fail to deliver adequate co-stimulatory signals for effector T cell activation and proliferation. This may manifest as T-cell death, T cell anergy, or Treg expansion or generation.

FIGURE 2.

Donor-derived regulatory dendritic cells (DCregs) prolong corneal graft survival. Immature DCs (iDCs), mature DCs (mDCs), and DCregs were generated from C57BL/6 mouse bone marrow cells as described previously by Sato et al.¹⁴ Donor-derived mDC (1×10^6), iDC (1×10^6), or DCreg (0.2×10^6 , 1×10^6)-infused BALB/c mice or untreated mice were transplanted with C57BL/6 corneas and graft survival was followed microscopically for 8 weeks (n = 6 per group). Kaplan–Meier survival curves indicate that infusion of 1×10^6 donor-derived DCregs prolonged graft survival (* $P = 0.043$). Reproduced and modified from Hattori et al¹⁶ with permission from the *Journal of Leukocyte Biology*.

FIGURE 3.

Direct and indirect allorecognition in transplantation. Allorecognition can occur by two distinct, but not mutually exclusive, pathways: the direct and indirect pathways. The direct pathway results from the recognition of intact foreign major histocompatibility molecules (MHC) on the surface of donor cells. Indirect allorecognition occurs when donor histocompatibility molecules are internalized, processed, and presented as peptides by host antigen presenting cells (APC)—this is how the immune system normally encounters antigen.

FIGURE 4.

Infusion of donor-derived regulatory dendritic cells (DCregs) impairs T cell differentiation in corneal graft recipients. C57BL/6 corneas were transplanted to BALB/c mice. For the infusion of DCs into recipients, 1×10^6 immature DCs (iDCs), mature DCs (mDCs), or DCregs, generated from C57BL/6 mouse bone marrow cells were injected intravenously via the lateral tail vein 7 days before transplantation. (A) T cells were isolated from draining lymph nodes 3 weeks post-transplantation ($n = 3$ per group). Isolated T cells obtained from DCreg/mDC-infused recipients or untreated recipients were stimulated with PMA/ionomycin and IFN- γ^+ ($CD4^+$ and $CD4^-$ fractions) and were subsequently measured by flow cytometry. (B) mRNA was isolated from draining lymph nodes 3 weeks post-transplantation ($n = 3$ per group). Expression of Foxp3 in draining lymph nodes post corneal transplantation was measured by real-time PCR. * $P = 0.03$. Reproduced and modified from Hattori et al.¹⁶ with permission from the *Journal of Leukocyte Biology*.

FIGURE 5.

Donor-derived regulatory dendritic cells (DCregs) suppress the indirect pathway in corneal transplant recipients. C57BL/6 corneas were transplanted to BALB/c mice. For the infusion of DCregs into recipients, 1×10^6 DCregs generated from C57BL/6 mouse bone marrow cells were injected intravenously via the lateral tail vein 7 days before transplantation. T cells were isolated from draining lymph nodes of untreated and DCreg-infused recipients ($n = 4$ per group) 3 weeks post-transplantation, and were measured by IFN- γ ELISPOT assay. Isolated T cells were cocultured with allogeneic antigen-presenting cells to measure the

direct pathway or cocultured with syngeneic antigen-presenting cells in the presence of alloantigen to measure the indirect pathway. (A, B) Triplicate enzyme-linked immunospot wells for IFN- γ production responder T cells after 48 h of culture from each group (direct pathway: A, indirect pathway: B). (C) Donor-derived DCregs reduced the frequency of direct pathway-type alloreactivity compared with untreated recipients. $*P = 0.007$. (D) Donor-derived DCregs also reduced the frequency of indirect pathway-type alloreactivity relative to untreated recipients. $**P = 0.0007$. Moreover, the reduction (30.2-fold) was much higher than in the direct pathway (1.5-fold). Data are representative of two experiments. Reproduced and modified from Hattori et al.¹⁶ with permission from the *Journal of Leukocyte Biology*.

FIGURE 6.

3D visualization of epithelial tight junctions and Langerhans cells (LCs) in normal mouse cornea. Corneas were harvested from naïve C57BL/6 mice and stained with anti-ZO-1 antibody (red) and anti-MHC-II antibody (green). Images of stained corneas were captured by confocal microscopy and analyzed by an image analyzer. (A) Reconstituted 3D images of epithelial tight junctions and LCs are shown. (B) *En face* imaging is demonstrated by confocal microscopy. (C) A 90° rotated image of the area in (B) is shown. The white arrow indicates the tight junctions of the corneal epithelium. The gray arrows indicate dendrites of corneal LCs.