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The roles of sepsis-induced myeloid derived suppressor cells in mice corneal, skin and combined transplantation

Yan He [a], Jia Bei [c,d], Hui Zeng [c,d], Zhiqiang Pan [b,*]

[a] Department of Ophthalmology, The Second Xiangya Hospital of Central South University, Eye Institute of The Second Xiangya Hospital of Central South University, Changsha 410010, China
[b] Beijing TongRen Eye Center, Beijing Tongren Hospital, Capital Medical University, Beijing Ophthalmic and Visual Science Key Laboratory, Beijing 100730, China
[c] Institute of Infectious Diseases, Beijing Ditan Hospital, Capital Medical University, Beijing 100015, China
[d] Beijing Key Laboratory of Emerging Infectious Diseases, Beijing 100015, China

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ABSTRACT

Purpose: To explore the effects of adoptive transferring sepsis induced myeloid-derived suppressor cells (iMDSCs) in mice corneal, skin, and combined corneal–skin survival.
Methods: Allogeneic full-thickness corneal transplantation, fully mismatched skin transplantation, and corneal–skin combined transplantation (donor C57BL/6 to recipient Balb/c mice) were performed. Sepsis-induced infectious-MDSCs (iMDSCs), were purified from bone marrow of cecal ligated and punctured (CLP) Balb/c mice. Recipient-derived iMDSCs were adoptively transferred into different recipient groups by retro-orbital injection after surgeries. Corneal and skin grafts were examined and photographed routinely for a period of 45 days. Histopathology was performed to evaluate corneal-graft inflammation. Bone marrow and/or corneal grafts in each group were harvested from executed recipients on postoperative days 15, 25, 35. Corneal cells and bone marrow cells were stained with CD11b-PE and Gr1-FITC, analyzed by FACS.
Results: iMDSCs were able to significantly prolong allograft survival in both corneal and corneal–skin combined transplant groups. A substantial expansion of MDSCs was observed in recipients' bone marrow, particularly in combined groups at an early stage postoperatively, and accordingly the concentration of MDSCs in corneal grafts increased significantly in adoptive transferred groups.
Conclusions: Sepsis-induced MDSCs may suggest a novel cellular therapeutic approach for preventing various types of allograft rejection.

1. Introduction

A myeloid-derived suppressor cell (MDSC) is defined as a novel heterogeneous population of immature myeloid cells with immunosuppressive properties, including macrophages, granulocytes, dendritic cells (DC), and myeloid cells at earlier stages of differentiation [1,2]. In mice, they express the common myeloid cell markers CD11b (αM chain of β2 integrin) and Gr1 (Ly6G and Ly6C) [3]. In general, CD11b + Gr1 + myeloid cells represent about 30–40% of normal bone marrow cells and only 2–4% of all nucleated normal splenocytes [4,5]. Under pathological conditions, such as infection, tumor, and trauma, they accumulate in large numbers in bone marrow, blood, spleen and other lymphoid organs [6,7]. In our previous studies, a dramatic accumulation of CD11b + Gr1 + cells was detected in bone marrows of septic mice models and tumor-bearing mice models [8]. The functions of MDSCs are highly dependent on the circumstances in which their expansion occurs. Significantly cell-mediated immunosuppressive capacities were observed in infectious-MDSCs (iMDSCs) and tumor-bearing MDSCs (tMDSCs) in vitro. However, CD11b + Gr1 + cells from naïve mice showed few immunosuppressive functions.

The role of MDSCs in transplantation tolerance was wildly described in allograft models in recent years [9,10]. Our previous studies [8] were focused on the potential application of MDSCs from different sources to prolong allograft survival, and reported that MDSCs from septic mice models and tumor-bearing mice models were adoptively transferred to allogenic recipients after corneal transplantations, and significantly prolonged corneal allograft survival in vivo. Moreover, iMDSCs transferred significantly reduced neovascularization that was comparable to tMDSCs and naïve MDSC. However, the additional adoptive transfer of MDSCs did not further ameliorate corneal survival. Therefore, rather than a simplex immunosuppressive response, the function of MDSC is more than likely a complex balance between increased immune surveillance and decreased adaptive immune responses.

Corneal allotransplant is an extreme situation, because the anterior chamber of the eye is an immunologically privileged site [11]. Corneal

Abbreviations: MDSC, Myeloid-derived suppressor cell; DC, Dendritic cell; CLP, Cecal ligated and punctured; iMDSC, Inflammation-induced myeloid-derived suppressor cell; tMDSC, Tumor-induced myeloid-derived suppressor cell.

* Corresponding author at: Beijing Tongren Eye Center, Beijing Tongren Hospital, Capital Medical University, Dongjiaominxiang, Beijing 100730, China.
E-mail address: panyj0526@sina.com (Z. Pan).

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vascularization has been reported to be associated with a significantly increased risk of corneal graft rejection [12,13], which is primarily a cell-mediated indirect T cell response mediated by CD4 + T cells. The fully mismatched allogenic skin transplant, as another kind of typical tissue transplantation, was reported to be recognized by recipients via both the direct and indirect pathways by CD4 + T cells in BALB/c recipient's lymphoid organs [14,15]. Moreover, both MHC and minor antigens induced an indirect alloresponse in skin transplantation. However, the entire indirect CD4 + T cell alloresponse in cornea-transplanted mice was directed exclusively to minor antigens [14–17].

As an immunomodulatory strategy to prevent corneal allograft rejection, MDSCs have been used in experimental corneal transplantation by suppressing the proliferation and cytokine production of effector T cells [1,18]. It is not clear whether there is the same positive effect when used in skin transplant, and even more interesting, in corneal–skin combined transplantation models. Moreover, the postoperative infiltration of MDSCs in graft remains unknown.

Therefore, we utilized infectious MDSCs in the mice cornea, skin and cornea–skin combined transplant models, compared the therapeutic efficiency, and discussed the underlying mechanisms by analyzing the MDSC distribution and survival rate of allograft.

2. Materials and methods

2.1. Mice

C57BL/6(H-2b) and Balb/c(H-2d) mice were purchased from the Experimental Center of Capital Medical University (Beijing, China) and housed in a specific pathogen-free facility. Female C57BL/6 mice (8 to 12 weeks old) were used as donors and female Balb/c mice (8 to 12 weeks old) were used as recipients. Balb/c mice underwent cecal ligation and puncture, as described previously [19], and were prepared at the Institute of Infectious Diseases of Beijing Ditan Hospital. All procedures performed on animals were approved by the Animal Care Research Ethics Committee of the Capital Medical University of China.

2.2. Flow cytometry and purification of MDSCs from bone marrow

Monoclonal antibodies used for fluorescence-activated cell sorting staining were: phycoerythrin (PE) conjugated anti-mouse CD11b and fluorescein isothiocyanate (FITC) conjugated anti-mouse Gr-1 (BD Bioscience, San Diego, CA, USA). Bone marrow cells from cecal ligated and punctured (CLP) Balb/c mice were stained with the above antibodies at 4 °C for 15 min and washed in PBS. The cells were isolated by fluorescence-activated cell sorter (FACSAria, BD, CA, USA). The purity of CD11b + Gr1 + cells from CLP Balb/c mice (iMDSC) was routinely more than 94%. For FACS analysis, the data were acquired on a FACS Calibur flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA) and was analyzed using FlowJo Software v 5.7.2–2 (Tree Star, Ashland, OR, USA).

2.3. Corneal penetrating keratoplasty and skin transplantation

Corneal penetrating keratoplasty was performed as described previously in the right eyes of the mice [20]. In brief, the central full thickness cornea with a diameter of 2.0 mm from the mice C57BL/6 were excised and secured in Balb/c mice graft beds with a diameter of 1.5 mm by eight interrupted 11–0 nylon sutures. 48 h later, the grafts were examined by a slit lamp. Grafts with severe complications, such as infection or hyphema, were excluded from the study. The sutures of the grafts were removed 7 days after surgery.

Skin allografts of 1 cm × 1 cm in size were taken from hair removed from the dorsum of C57BL/6 mice, and were sutured in Balb/c mice graft beds with the same area by 12 interrupted 6–0 silk sutures.

2.4. Group and iMDSC adoptive transferring

The recipient mice were divided randomly into 6 groups, as shown in Table 1 (18 recipients in each group), and 5×10^6 iMDSCs suspended in 150 μL PBS were transferred to the recipients' left eyes, or non-surgery eyes, at the end of operation, via retro-orbital injection. The same volume of PBS was given to the Iso SCT, Allo CT, Allo ST, and Allo SCT groups as the control.

2.5. Assessment of graft survival

Corneal grafts were assessed by slit lamp biomicroscopy every two days after surgery for 45 days. As described previously, the grafts were scored in a range for opacity (0 to 5), as shown in Table 2. Meanwhile, the extent of corneal edema and growth of neovessels were also brought under consideration. Edema and neovessels were scored within a range from 0 to 3, minimum to maximum. When the total scores of opacity, edema, and neovessels reached 6, or opacity score > 2, the grafts were rejected.

Square skin grafts of 1 cm × 1 cm were excised from the backsides of C57BL/6 mice and sutured to the same regions of Balb/c mice with 12 interrupted 8–0 nylon sutures. Corneal and skin transplantation were performed at the same day in the corneal–skin combined transplantation group (SCT group). Skin grafts were examined at daily intervals. Rejection was defined as complete dissolution of graft, and wound completely healed by recipient skin.

2.6. Flow cytometric analysis of grafts

On days 15, 25, and 35 after surgeries, 3 recipients for each group were executed. Corneal grafts were sheared into small pieces, and immersed in 1 mg/mL collagenase IV (Sigma) for 2 h at 37 °C in a rocking device. Bone marrow cells were harvested from executed recipients. The collected suspensions were separately forced through a 100-Mm nylon mesh. Corneal cells and bone marrow cells were stained with CD11b-PE and Gr1-FITC (BD Biosciences, USA). Samples were analyzed by FACS.

2.7. Statistical analysis

Results are expressed as means ± SD. Graft survival time was compared between various groups by the Mantel–Cox Log Rank Test and the Kaplan–Meier survival curve. P values less than 0.01 were considered to be statistically significant.

3. Results

3.1. Adoptive transfer of iMDSCs has similar inhibitory properties in corneal and corneal–skin combined transplantation

Mice in different groups were treated as shown in Table 1 and iMDSC suspensions (5×10^5 cells/150 μL) and were adoptively transferred separately to iMDSC CT and iMDSC SCT groups by retrobulbar injection after corneal transplantation. The clinical characteristics of the graft, including edema, opacity and neovascularization, were examined and scored by slit lamp examination regularly up to 45 days after transplantation.

Compared to the untreated allograft control group (Fig. 1A, Allo CT), no remarkable differences were observed in mice with corneal–skin combined transplantation (Fig. 1A, Allo SCT), while the grafts in the Iso SCT group remained clear and survived until the end of observation. By contrast, mice adoptively transferred with iMDSC (Fig. 1A, iMDSC CT, iMDSC SCT) exhibited reduced mean opacity, edema, and neovessel scores from the control group, particularly at an early period after surgeries. The most remarkable differences were observed in corneal–skin combined transplantation, which developed neovascularization