Myeloid-derived Suppressor Cells Recruit CD4$^+$/Foxp3$^+$ Regulatory T Cells in a Murine Cardiac Allograft

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ABSTRACT

Introduction. Myeloid-derived suppressor cells (MDSCs) play an important role in regulating allograft rejection in organ transplantation. On the other hand, CD3$^+$/CD4$^+$/FOXP3$^+$ regulatory T cells (Tregs) also are of vital importance in immunological tolerance. We previously revealed that adoptive transfer of MDSCs recruited Tregs in the spleen. However, it is still uncertain whether MDSCs are capable of recruiting Tregs to an allograft in vivo.

Objectives. We conducted adoptive transfer experiments of MDSCs to clarify the effects of MDSCs on Tregs in vivo.

Methods. Gr-1$^+$/CD11b$^+$ MDSCs were isolated from rapamycin-treated cardiac transplant (CTx) recipients (3 mg/kg, intraperitoneally on postoperative days [POD] 0, 2, 4, and 6) on POD 7 by magnetic-activated cell sorting (purity >95%). In murine heterotopic cardiac transplantation, 2×10^6 MDSCs were transferred into the graft aorta 5 minutes before reperfusion.

Results. Flow cytometric analyses of a cardiac allograft on POD 7 showed that MDSCs derived from rapamycin-treated CTx mice (MDSCs-Rap) transfer led to significant recruitment of Tregs compared with a PBS-injected allograft. The level of programmed death ligand-1 (PD-L1) on MDSCs-Rap was higher than those from non-treated recipients. Furthermore, pathological findings also confirmed accumulation of Foxp3$^+$ Tregs in an allograft.

Conclusion. Induced PD-L1 on MDSCs might result in recruitment of Tregs. These results suggested that functional MDSCs possessed an ability to induce Tregs in a cardiac allograft and developed a tendency to immunological tolerance.

STRONG attention has been paid to the adaptive immune system as an attractive target in terms of forming immunologic tolerance in organ transplantation. Ideal control of regulatory T cells (Tregs) appears to be especially important [1]. Although lymphocytes are necessary components, conditions are not sufficient to induce immune tolerance. In fact, donor-specific transfusion, including a variety of nonlymphocytes, is required for many regimens of inducing alloantigen specific tolerance [2,3]. Simultaneously, increased evidence suggests that myeloid-derived suppressor cells (MDSCs) have a key role in controlling the host immune system in the context of cancer [4], autoimmune diseases [5], or organ transplantation [3,6]. However, MDSCs alone are also not sufficient for producing transplantation tolerance. Thus, interaction between MDSCs and lymphocytes appears to be crucial. Given the fact that MDSCs interact in a contact-dependent manner, it is worthwhile investigating their interaction in allografts.

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Our results demonstrate that $CD4^+/Foxp3^+$ Tregs are efficiently induced in allografts by transcoronary adoptive transfer of MDSCs. Interaction of these cells might result in synergistic tolerance induction.

MATERIAL AND METHODS

Animals

Six- to 8-week–old male C3H and C57BL/6 (B6N) mice were purchased from Shimizu Laboratory Supplies (Kyoto, Japan). All mouse experiments were performed under clean conditions in line with Institutional Animal Care protocols approved by Kyoto Prefectural University of Medicine.

Vascularized Cardiac Transplantation

Cardiac grafts, harvested from C57BL/6N (B6N) donors, were transplanted into the abdominal cavity of C3H recipients as previously described [6].

Isolation of Myeloid-derived Suppressor Cells

$Gr\text{-}1^+/CD11b^+$ MDSCs were isolated from single-cell suspensions prepared from the spleens of CTx recipients. Cells were isolated using biotinylated antibodies against Gr-1, magnetic microbeads, and magnetic-activated cell sorting columns (Miltenyi Biotec, Auburn, Calif., United States) according to the manufacturer's protocol. The purity of the $Gr\text{-}1^+/CD11b^+$ MDSC population was >95% as determined by a Calibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ, United States).

Adoptive Transfer of Myeloid-derived Suppressor Cells

2×10^6 MDSCs were transferred into the graft aorta 5 minutes before re-perfusion as previously described [6].

Digestion of Cardiac Allograft

Cardiac grafts were irrigated by HBSS (Kojin Bio, Saitama, Japan) containing 1% heparin, then harvested, cut into tiny pieces, and digested by 400 U/mL collagenase IV (Sigma Aldrich, St. Louis, Mo., United States) in the presence of 0.01% DNase I (RNase-free) (Life Technologies, Carlsbad, Calif., United States). Following the digestion, Tregs were stained in a manner described below.

Flow Cytometory Analysis

Fc receptors were blocked with an anti CD16/CD32 (Becton Dickinson) antibody and the cells were then stained with antimouse antibodies against Gr-1 (clone: RB6-8C5) (eBioscience, San Diego, Calif., United States), Ly6G (1A8), Ly6C (AL-21), CD11b (M1/70), CD3 (17A2), CD4 (RM4-5), CD25 (PC61), and Foxp3 (MF23) (Becton Dickinson). Samples were run in a Calibur flow cytometer (Becton Dickinson) and data were analyzed using FlowJo software (TreeStar Inc., Ashland, Ore., United States).

Histopathology

Heart grafts were excised, fixed in 10% buffered formaldehyde, paraffin embedded, sectioned (3 μm), and stained with hematoxylin and eosin (H&E). Sections were blocked in Blocking 1 solution (Nakarai-tesque, Kyoto, Japan) to prevent nonspecific antibody reaction Foxp3 antibodies, followed by reaction with a horseradish peroxidase polymer-conjugated system and 3,3'-diaminobenzidine. Isotype-matched IgG antibodies were used as negative controls. Images were captured under an Olympus DP72 microscope. In terms of fluorescent immunohistochemistry, MDSCs were isolated and stained with carboxyfluorescein succinimidyl ester (CFSE, 2.5 μM). Then these MDSCs were adoptively transferred to the allograft through the coronary arteries 5 minutes before reperfusion. The allograft was harvested and frozen. Sections of 5 μm were prepared, fixed, and blocked in Blocking 1 solution (Nakarai-tesque). Subsequently, the sections were stained with iNOS (Wako, Osaka, Japan), followed by reaction with an Alexa Fluor 594 chicken antirabbit IgG (Life Technologies). Images were captured under a BIOREVO BZ9000 (Keyence, Osaka, Japan).

RESULTS

$CFSE^+/Gr\text{-}1^+/CD11b^+/iNOS^+$ MDSCs Localize in Cardiac Allografts After Being Adoptively Transferred

To confirm whether adoptively transferred MDSCs localized in allografts, CFSE-labeled MDSCs were transferred through the coronary artery 5 minutes before reperfusion. On POD 4, immunofluorochemistry confirmed successful MDSC migration by the presence of CFSE/iNOS double-positive MDSCs in the subintimal space (Fig 1). Thus,

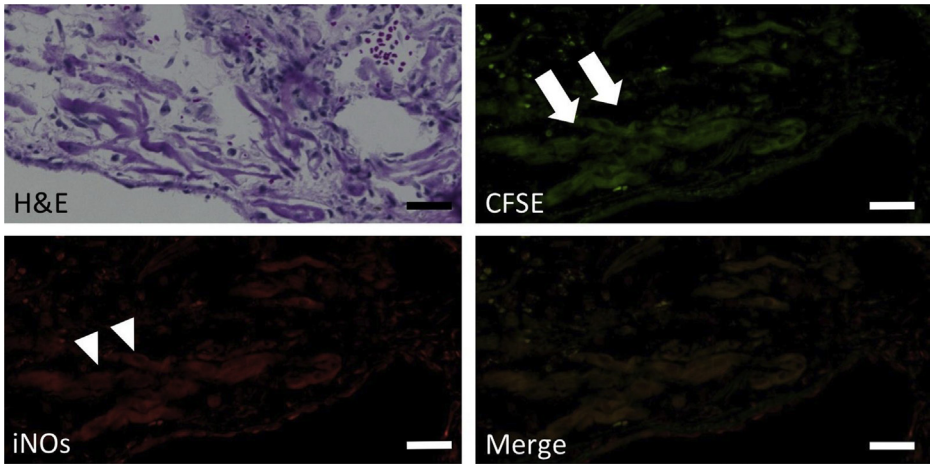

Fig 1. Transferred MDSCs localize in a cardiac allograft. CFSE-labeled (white arrows) adoptively transferred M-MDSCs stained with iNOs (white arrowheads) were observed by fluorescent immunohistochemistry. Original magnification × 40, Scale bars: 20 μm.