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Role of myeloid-derived suppressor cells in mouse pre-sensitized cardiac transplant model

Weihua Gong[a,b,*], Fangmin Ge[a], Dahai Liu[c], Yan Wu[b], Fangbing Liu[d], Beom Seok Kim[e], Tao Huang[b], Maria Koulmanda[b], Simon C. Robson[b,**], Terry B. Strom[b]

[a] *Department of Surgery and Medicine, Transplant International Research Centre (TIRC), Second Affiliated Hospital of School of Medicine, Zhejiang University, Hangzhou City, People's Republic of China*
[b] *Departments of Medicine, Transplant Institute, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA*
[c] *Center for Stem Cell and Translational Medicine, School of Life Sciences, Anhui University, Hefei City, People's Republic of China*
[d] *Department of Hemotology/Oncology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215, USA*
[e] *Department of Internal Medicine, College of Medicine, Yonsei University, Seoul, Korea*

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Abstract Harness of sensitized transplantation remains a clinical challenge particularly in parallel with prolonged cold ischemia time (PCI)-mediated injury. Our present study was to test the role of myeloid-derived suppressor cells (MDSCs) in mouse pre-sensitized transplantation. Our findings revealed that CD11b + $Gr1^{low}$ MDSC was shown to have strong suppressive activity. MDSCs subsets from the tolerated mice exhibited higher suppressive capacities compared with counterparts from naive (untreated) mice. Depletion of Tregs could not affect splenic CD11b + $Gr1^{-low}$ MDSC frequency, but increase peripheral and intragraft CD11b + $Gr1^{-low}$ frequency. Intriguingly, boost of Tregs remarkably caused an increase of CD11b + $Gr1^{-low}$ frequency in the graft, peripheral blood, and spleen. Furthermore, peripheral CD11b + $Gr1^{-low}$ cells were massively accumulated at the early stage when allogeneic immune response was enhanced.

Abbreviations: CFSE, Carboxyfluorescein succinimidyl ester; FACS, Fluorescence-activated cell sorting; HTK, Histidine–tryptophan-ketoglutarate; IACUC, The Institutional Animal Care and Use Committee; MDSCs, Myeloid-derived suppressor cells; MR1, Anti-CD154 monoclonal antibodies; PC61, Monoclonal anti-CD25 antibodies; PCI, Prolonged cold ischemia time; Tregs, Regulatory T cells; WT, Wild-type.

* Correspondence to: W. Gong, Jiefang Road #88, Hangzhou City, Zhejiang Province 310009. Fax: +86 571 87783581.
** Correspondence to: S.C. Robson, The Transplant Institute and Division of Gastroenterology, Beth Israel Deaconess Medical Center, 330 Brookline Avenue – E/CLS 612, Boston, Massachusetts 02215, USA. Fax: +1 617 735 2930.
E-mail addresses: weihua.gong@gmail.com (W. Gong), srobson@bidmc.harvard.edu (S.C. Robson).

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Taken together, MDSCs could prevent grafts from PCI-mediated injury independent on Tregs in the pre-sensitized transplant recipients. Utilization of MDSC subset particularly CD11b + Gr1^{-low} might provide a novel insight into improving graft outcome under such clinical scenarios.

1. Introduction

Injury mediated by prolonged cold ischemia time (PCI) remains a critical clinical challenge, affecting early graft dysfunction and jeopardizing long-term graft survival [1]. PCI can boost early intragraft CD8+ T cells infiltration leading to an increased incidence of rejection episodes [2,3]. Conversely, pre-sensitized immune condition of host can exaggerate PCI-mediated injury of cardiac graft involving in regulatory T cells [4]. Therefore, targeting host's immune system might shed light on preventing graft injury. Accumulating evidences exhibited that induced regulatory T cells (Tregs) could protect kidney/liver from ischemia-reperfusion injury and participate in repair of ischemic acute injury [5–7]. Herein, it is of great interest in testing the role of another type of immune-regulatory cells, myeloid-derived suppressor cell (MDSC) subsets.

It is well-studied that MDSCs are also capable of regulating a variety of inflammatory, innate and adoptive immune responses, even promoting allograft survival [8–10]. In tumor model, MDSC's suppressive function is mediated through Tregs [11]. Furthermore, an interaction between CD152 expressed on Tregs and CD80 expressed on MDSCs is required [11]. MDSCs can expand Tregs and promote cross-tolerance by cross-presenting antigen specifically to Tregs [12]. In the allograft, the proliferation of Tregs in transplant recipients can be inhibited by MDSCs [13]. Nevertheless, it remains unclear of the role and function of MDSCs in the settings of pre-sensitized transplantation. Their relationship with Tregs is required to be identified. Are MDSCs and Tregs synergistic or antagonistic regulators in protecting graft from PCI-mediated injury in the presensitized transplant recipients?

To the best of our knowledge, our present study is the first reporting the role of MDSCs in PCI-mediated injury and interplay between MDSCs and Tregs in the pre-sensitized transplant recipients. We revealed that CD11b + Gr1^{-low} MDSC subset rather than CD11b + Gr1^{-int} or CD11b + Gr1^{-high} subset bears immunosuppressive activity in the sensitized transplant condition, and it is independent of Tregs.

2. Materials and methods

2.1. Mice

Inbred male wild-type BALB/c (B/c; H-2^{d}) and C57BL/6 (B6; H-2^{b}) mice were obtained from the Jackson Laboratories (Bar Harbor, ME). All mice were housed in the pathogen-free facilities. Animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) at Beth Israel Deaconess Medical Center (Boston, MA).

2.2. Skin and heterotopic cardiac transplantation

Mice were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg). Allogeneic tail skin grafts (~0.5 cm in diameter) from Balb/c were sutured onto the flank of C57BL/6 recipients. These recipients were challenged 7 days later with heterotopic Balb/c or C57BL/6 heart transplants using standard microsurgical techniques [14–16]. Grafts were perfused with Custodiol HTK (histidine–tryptophan-ketoglutarate) solution (Bensheim, Germany) for procurement [17]. The graft was preserved at 4 °C for 25–35 min or 5.8–8 h. The graft function was daily assessed by the observation of donor heartbeat palpation. Rejection was defined as the date on which the donor heartbeat ceased completely. The heart grafts unfunctioning at day 1 post-transplant were excluded.

Experimental groups are shown in Table 1. Briefly, the following groups of pre-sensitized + PCI (G1, as control), pre-sensitized + PCI + PC61 (G2), pre-sensitized + PCI + Tregs (G3) and pre-sensitized + PCI + Rapamycin (G4) isotransplantations were performed to investigate the effects of regulatory T cells and prolonged cold ischemia per se. To study the role and function of peripheral CD11b + Gr1^{low}, three different groups of presensitized (G5), pre-sensitized + Rapamycin (3 mg/kg BW) (G6), pre-sensitized + Rapamycin (3 mg/kg BW) + MR1 (anti-CD154 monoclonal antibodies, 0.5 mg/mouse) treatment (G7) were done.

2.2.1. Administration of rapamycin

Non-tolerizing dose of Rapamycin (3 mg/kg, Henry Schein, Melville, NY) [18] was intraperitoneally administered daily on transplant days −3–6 and then every other day until day 13 [19].

2.2.2. CD25+ T cells depletion

Cardiac graft recipients were treated with anti-CD25 monoclonal antibodies (Clone PC61, BioExpress, West Lebanon, NH) intraperitoneally on transplant day −1 (0.5 mg), followed by 0.25 mg at day 1 post-transplant. Tregs depletion was confirmed by analyzing CD4 + CD25+ cells in the peripheral blood at day 3 post-transplant via flow cytometry.

2.2.3. Cell sorting and adoptive cell transfer

Foxp3 + Tregs were collected from the lymph nodes and spleen from Foxp3-GFP reporter mice and single-cell suspension prepared in complete RPMI-1640 medium. The pooled cells were labeled with FITC-anti-CD4 and PE-anti-CD44 (eBioscience, San Diego, CA). CD4 + CD44 + Foxp3-GFP + cell population was gated and sorted by MoFlo high-speed cell sorter (DakoCytomation, Ft Collins, Co). The purity is consistently >96%, as we previously reported [20]. For adoptive cell transfer, skin-presensitized (without the Foxp3-GFP reporter gene) B6 mice were used as recipients. The sorted Foxp3-GFP + Tregs were adoptively transferred