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Blockade of CD27/CD70 pathway to reduce the generation of memory T cells and markedly prolong the survival of heart allografts in presensitized mice

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ABSTRACT

Background: Alloreactive memory T cells are a major obstacle to transplantation acceptance due to their capacity for accelerated rejection.
Methods: C57BL/6 mice that had rejected BALB/c skin grafts 4 weeks earlier were used as recipients. The recipient mice were treated with anti-CD154/LFA-1 with or without anti-CD70 during the primary skin transplantation and anti-CD154/LFA-1 or not during the secondary transplantation of BALB/c heart. We evaluated the impact of combinations of antibody-mediated blockade on the generation of memory T cells and graft survival after fully MHC-mismatched transplantations.
Results: One month after the primary skin transplantation, the proportions of $CD4^+$ memory T cells/$CD4^+$ T cells and $CD8^+$memory T cells/$CD8^+$ T cells in the anti-CD154/LFA-1 combination group were $47.32 \pm 4.28\%$ and $23.18 \pm 2.77\%$, respectively. In the group that included anti-CD70 treatment, the proportions were reduced to $34.10 \pm 2.71\%$ and $12.19 \pm 3.52\%$ ($P<0.05$ when comparing the proportion of memory T cells between the two groups). The addition of anti-CD70 to the treatment regimen prolonged the mean survival time following secondary heart transplantation from 10 days to more than 90 days ($P<0.001$). Furthermore, allogenic proliferation of recipient splenic T cells and graft-infiltrating lymphocytes were significantly decreased. Meanwhile, the proportion of regulatory T cells was increased to $9.46 \pm 1.48\%$ on day 100 post-transplantation ($P<0.05$).
Conclusions: The addition of anti-CD70 to the anti-CD154/LFA-1 combination given during the primary transplantation reduced the generation of memory T cells. This therapy regimen provided a potential means to alleviate the accelerated rejection mediated by memory T cells during secondary heart transplantation and markedly prolong the survival of heart allografts.

1. Introduction

Transplant patients may develop alloreactive memory T cells after exposure to alloantigens during previous transplantations, blood transfusions, pregnancies, or due to continuous exposure to bacterial and viral pathogens [1,2]. In adult humans, 40–50% of T cells circulating in the peripheral blood have memory phenotypes [3,4]. Memory T cells, including $CD4^+$ and $CD8^+$ subsets, play a key role in accelerated rejection. Furthermore, there is a growing body of evidence that B cell production of alloantibodies is also an important element in the accelerated rejection [5]. Therefore, inhibiting the generation of memory T cells and alloantibodies is a critical step in the prevention of rejection.

A variety of methods to induce memory cells tolerance in mouse models have been identified, such as the use of antilymphocyte serum and antithymocyte globulin for general clearance of T and B lymphocytes [6], anti-CD122 to deplete $CD8^+CD122^+$ memory T cells [7,8], and anti-CD20 to deplete B lymphocytes during treatment of autoimmune diseases [9,10]. Here, we developed a protocol designed to minimize the generation of memory T cells and production of alloantibodies during primary transplantation in order to significantly prolong the secondary allograft survival.

The CD40/CD154 and LFA-1/ICAM-1 pathways have been shown to play an important role in the activation of T cells [11–13]. The CD40/CD154 pathway also provides important signals regulating B lymphocytes clonal expansion, antibody production and isotype switching, as well as the development of humoral memory [14,15]. CD70 (CD27 ligand) is a type II transmembrane glycoprotein belonging

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to the TNF family that is induced upon T and B cell activation [16]. CD27/CD70 engagement has been implicated in T cell development, T cell activation, and T cell-dependent antibody production by B cells [17,18]. CD70 blockade can inhibit clonal expansion of $CD8^+$ T cells and reduce the generation of memory $CD8^+$ T cells [19,20].

In this study, we attempted to reduce the generation of memory T cells and alloantibodies by treating mice with anti-CD70 added in combination with an anti-CD154/LFA-1 regimen during primary transplantation. We provided evidence that this approach could alleviate the accelerated rejection mediated by memory T cells during secondary heart transplantation, and we explored the possible mechanism of the obviously prolonged survival.

2. Materials and methods

2.1. Animals

Female C57BL/6 (B6, H-2^b) and BALB/c (H-2^d) mice (8–12 weeks old) were purchased from Slac Laboratory Animal Co. Ltd. (Shanghai, China) and used as graft recipients and donors, respectively. All animals were maintained and bred in a pathogen-free facility, and all procedures were performed according to the Institutional Animal Care and Use Committee (IACUC) guidelines.

2.2. Antibodies

All administered antibodies were produced by Bioexpress (West Lebanon, NH, USA), including anti-CD70 (FR-70), anti-CD154 (MR-1), anti-LFA-1(M17/4), and their respective isotype controls. Antibodies used for flow cytometric analysis, including FITC-anti-CD4 (GK1.5), FITC-anti-CD8 (53–6.7), PE-anti-CD44 (IM7), PECy5-anti-CD62L (MEL14), PE-anti-IgM (RMM-1), FITC-anti-IgG1 (RMG1-1), FITC-anti-IgG2a (RMG2a-62), and their isotype controls were purchased from Biolegend (San Diego, CA, USA). $CD4^+Foxp3^+$ regulatory T cells (Tregs) were detected using the Mouse Regulatory T cell Staining Kit from eBioscience (San Diego, CA, USA).

2.3. Skin transplantation

Full-thickness skin grafts were prepared from the lateral thoracic skin of BALB/c mice, cut into circular pieces (~1.2 cm^2 in area), and engrafted onto the lumbar region of B6 mice. After the fully MHC-mismatched transplantation, the B6 mice received a 2-antibody treatment regimen consisting of 0.25 mg of anti-CD154 and 0.1 mg of anti-LFA-1, or a 3-antibody treatment regimen consisting of 0.25 mg of anti-CD154, 0.1 mg of anti-LFA-1, and 0.25 mg of anti-CD70. Control group mice were treated with isotype antibodies (Table 1). The drugs were intraperitoneally (i.p.) administered on days 0 and 2 post-transplantation.

2.4. Alloantigen-primed heart transplantation model

Four weeks after skin grafting, the B6 mice were defined as alloantigen-primed mice. Vascularized heterotopic heart transplantations from BALB/c donors to B6 recipients were performed with anastomosis to the vessels of the neck using a non-suture cuff technique as described previously [21]. The mice were treated with 0.25 mg anti-CD154 and 0.1 mg of anti-LFA-1 or isotype controls on days 0, 2, 4, and 6 post-transplantation (Table 1). Graft survival was monitored by daily palpation. Rejection was defined as complete loss of palpable heart beat.

Table 1
The meanings of abbreviation in this study.

Abbreviation	Treatment	Days post-transplantation
S0	Skin transplantation + isotype antibodies	At days 0 and 2
S2	Skin transplantation + anti-CD154/LFA-1	At days 0 and 2
S3	Skin transplantation + anti-CD154/LFA-1/CD70	At days 0 and 2
H0	Heart transplantation + isotype antibodies	At days 0, 2, 4 and 6
H2	Heart transplantation + anti-CD154/LFA-1	at days 0, 2, 4 and 6

Table legend: mAbs were pooled and administered i.p. after the transplantation procedure as follows: 0.25 mg/dose anti-CD154, 0.1 mg/dose anti-LFA-1, and 0.25 mg/dose anti-CD70. Heart transplantations were performed one month after primary skin transplantation.

2.5. Mixed lymphocyte reactions (MLR)

T lymphocytes were isolated from spleens of B6 mice using nylon wool columns (Wako, Osaka, Japan) and used as responder cells. Donor spleen cells were used as stimulator cells and treated with mitomycin (40 lg/ml, Amresco, Solon, OH, USA) before used in the MLR assay. For proliferation assays, 10^5 stimulator cells were cultured with 5×10^5 responder cells in RPMI1640 media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin and streptomycin in 96-well plates. Three separate wells were dedicated to each responder–stimulator combination and each experiment was repeated three times. Cells were incubated for 72 h at 37 °C in 95% humidified air mixed with 5% carbon dioxide. After 72 h of culture, cell proliferation was quantified using a BrdU kit (Roche Applied Science, Mannheim, Germany).

2.6. Enzyme-linked immunosorbent assay (ELISA)

ELISAs were performed using commercially available kits (NeoBioscience Technology Limited Company, Shenzhen, China) to detect the concentrations of IL-2, IFN-γ, IL-10, and TGF-β in the supernatants from MLR according to the manufacturer's instructions. A standard curve was generated using known amounts of purified recombinant murine cytokines.

2.7. Graft pathological analysis

The heart allografts were resected from the recipient mice on day 7 or day 100 post-transplantation. Tissues were fixed in 10% buffered formalin solution, embedded in paraffin, cut into 5 μm sections and stained with hematoxylin and eosin (H&E). Graft rejection was graded on the extent of infiltration and the anatomical localization of inflammatory cells according to the International Society of Heart and Lung Transplantation (ISHLT) standard [22,23].

2.8. qRT-PCR

RNA was isolated from the heart allografts using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Reverse transcription and qRT-PCR were performed using commercially available reagents (TOYOBO, Osaka, Japan) with the StepOne Real-Time PCR System (ABI, Foster, UK). Syber Green I was used to detect amplification and β-actin was used as a normalizing control. Calculations were performed using the $2^{-\Delta\Delta CT}$ method. Each reaction was carried out in triplicate. The primer sequences used for the qRT-PCR were listed in Table 2.

2.9. Extraction of lymphocytes from heart allografts

The harvested heart allografts were minced with a sterile blade and incubated in 10 ml buffered saline with 2% bovine serum albumin (BSA) and 2 mg/ml collagenase at 37 °C for 2 h. The cells were strained through a 70 μm nylon cell strainer (Becton Dickinson, Franklin Lakes, NJ, USA). Lymphocytes were isolated from these cells using EZ-Sep^{TM} Mouse lymphocyte separation medium (Dakewe Biotech Company, Shenzhen, China) with centrifugation for 20 min at 1600 rpm. After washed twice in RPMI 1640, lymphocytes were resuspended in phosphate buffered saline (PBS) with 10% fetal bovine serum for the Flow cytometry.