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Donor bone marrow cells play a role in the prevention of accelerated graft rejection induced by semi-allogeneic spleen cells in transplantation

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Abstract

Spleen or spleen plus bone marrow cells from (BALB/c × C57Bl/6)F1 donors were transferred into BALB/c recipients 21 days before skin or cardiac transplantation. Prolonged graft survival was observed on recipients treated with the mixture of donor-derived cells as compared to those treated with spleen cells alone. We evaluated the expression of CD45RB and CD44 by splenic $CD4^+$ and $CD8^+$ T cells 7 and 21 days after donor cell transfer. The populations of $CD8^+CD45RB^{low}$ and $CD8^+CD44^{high}$ cells were significantly decreased in mice pre-treated with donor spleen and bone marrow cells as compared to animals treated with spleen cells only, although these cells expanded in both groups when compared to an earlier time-point. No differences were observed regarding $CD4^+$ T cell population when recipients of donor-derived cells were compared. An enhanced production of IL-10 was observed seven days after transplantation in the supernatants of spleen cell cultures of mice treated with spleen and bone marrow cells. Taken together these data suggest that donor-derived bone marrow cells modulate the sensitization of the recipient by semi-allogeneic spleen cells in part by delaying the generation of activated/memory $CD8^+$ T cells leading to enhanced graft survival.

Keywords: Skin graft; Cardiac graft; Microchimerism; $CD8^+CD45RB^{low}$; IL-10

1. Introduction

It has been over 50 years since the first report that tolerance to alloantigens could be acquired after donor cell administration in neonatal mice [1]. Since then, a great number of reports showed that adult recipients could tolerate organ or tissue allografts when transplanted after the infusion of donor-derived spleen [2–6], bone marrow [7–10] or immature dendritic cells [11]. Because the development of microchimerism is an important feature in transplantation [12,13] and demonstrated by many authors as being predictive for long-term survivors [14–18], donor cells might have a role in downregulating effector T cell responses. Modulation of allo-specific T cell activation has been described as involving regulatory mechanisms in the periphery [19,20] and/or elimination of potentially alloreactive clones in the thymus after contact with alloantigens [20–23]. However there are conflicting reports regarding this issue since the development of microchimerism has been shown as a feature not necessarily correlated to graft survival [24–29].

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Itabashi et al. showed that the combination of allogeneic spleen and bone marrow cells infused in sub-lethally irradiated hosts was effective in prolonging skin allograft survival [30]. In these receptors, the degree of microchimerism in the spleen and in the blood was greatly enhanced when compared to rejecting animals. In our hands, preliminary results showed that the administration of a mixture of spleen and bone marrow cells from semi-histocompatible donor twenty-one days before skin or cardiac transplantation was able to enhance graft survival compared to non-manipulated transplanted controls. Unpublished reports (Mengel, JO et al.) showed that in the same experimental model the injection of donor-derived spleen cells previously to the skin transplant induced accelerated graft rejection. Taken together, these data suggest that allogeneic bone marrow cells could have a role in down-regulating activation induced by donor spleen cells when injected together in non-immunosuppressed recipients. Our data show that prolonged graft survival in this model is in part due to delayed generation of alloreactive effector/memory $CD8^+$ T cells.

2. Materials and methods

2.1. Animals

Six to 10 week-old BALB/c (H-2^d), (BALB/c × C57Bl/6)F1 (BaB6; H-$2^{d/b}$), C57Bl/6 Il-$10^{-/-}$ and C57Bl/6 wild type (H-2^b) female mice were obtained from our own animal facilities at the University of São Paulo and kept in microisolator cages under specific-pathogen free conditions. In all experiments BALB/c mice were used as recipients and BaB6 were used as donors. All procedures were carried out following the principles of laboratory animal care (Department of Health and Human Services, Publication No. [NIH] 86–23, revised 1985) and were approved by the animal care and users committee at the Institute of Biomedical Sciences from the University of São Paulo.

2.2. Skin transplantation

BaB6 tail skin was grafted on the back of the BALB/c recipient. Skin was removed from the donor tail, cut into 1 cm^2 pieces and kept in PBS at room temperature until use. Grafts were placed on a bed prepared by removing an area on the back dermis of the receptor, sutured and covered with plaster. Donor skin was considered as rejected when 90% of the area was destructed.

2.3. Cardiac transplantation

Heart from BaB6 mice was transplanted in the abdominal cavity of BALB/c animals. Abdominal vascularized heart transplants were performed heterotopically according to the technique described by Corry et al. [31]. Cardiac function was evaluated daily by scoring the heart beating, and graded from +4 (excellent) to 0 (failure), according to the intensity of cardiac pulse.

2.4. Preparation of donor cells

A mixture of donor spleen and bone marrow cells (3:2 respectively) or spleen cells alone were injected at a single dose of 5×10^6 in 200 μl of culture medium (DMEM) in the tail vein of the recipients. Bone marrow cells were obtained by flushing the femurs of BaB6 mice aseptically with culture medium. Cells were washed and tested for viability with trypan blue and kept at 4 °C in serum-free DMEM until use. Spleen cells were isolated by mashing the spleen aseptically and subsequently lysing the red blood cells with NH_4Cl buffer. Cells were washed with phosphate-buffer saline, transferred to serum-free DMEM and tested for viability. In all experiments cells were infused 21 days before transplantation. Control animals received no cells. For evaluation of early microchimerism donor spleen and bone marrow cells were labeled with CFSE according to the manufacturer's instructions (Molecular Probes, Oregon, USA) before injection. Cells were evaluated in the recipient's spleen, bone marrow, thymus and lymph nodes by flow cytometry.

2.5. Mixed leukocyte reaction (MLR)

Mixed leukocyte reaction was performed and supernatants were evaluated for cytokine production. Three million (3×10^6) spleen cells from skin grafted BALB/c mice were cultured with irradiated (3000 rads) spleen cells from BaB6 donors at a 1:1 ratio in a 24-well plate for 96 h (established as the optimal time for this assay). The supernatant was harvested and kept at −20 °C until use. Cytokine was also measured in the supernatant of MLR assays from naïve animals at the same conditions. In other experiments 2×10^5 cells from naïve BALB/c mice were cultured with allogeneic spleen or bone marrow cells or a mixture of spleen and bone marrow cells at a 1:1 ratio in a 96-well U bottom plate for 5 days. ^{3}H-thymidine (1 μCi/well) was added at the last 18 h. Plates were harvested and ^{3}H-thymidine

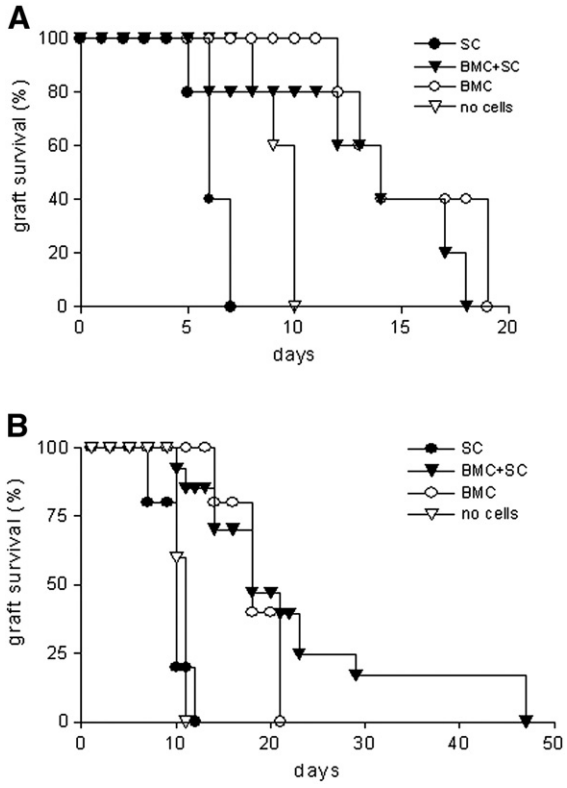

Fig. 1. Skin and cardiac graft survival in recipients pre-treated with semi-allogeneic cells. BALB/c mice were infused intravenously with 5×10^6 cells from different populations obtained from BaB6 donors (spleen cell plus bone marrow cells in a 3:2 ratio, respectively, spleen cells or bone marrow cells only or no cells) twenty-one days before skin (A) or cardiac (B) transplantation. Recipients of spleen plus bone marrow cells presented prolonged skin graft survival ($n=5$; MST=14 days) as compared to mice pre-infused with semi-allogeneic spleen cells ($n=5$; MST=6 days; $p=0.02$) and slightly enhanced graft survival compared to controls ($n=5$; MST=10 days; $p=0.05$). Spleen cell recipients rejected skin grafts earlier as compared to the control ($p=0,002$). Recipients of bone marrow cells alone also presented enhancement of skin graft survival ($n=5$; MST=14 days) compared to controls ($p=0.015$). Cardiac allograft survival was prolonged in mice pre-treated with bone marrow cells ($n=5$; MST=18 days) or with bone marrow plus spleen cells ($n=12$; MST=18 days) as compared to the control group ($n=5$; MST=11 days; $p=0.016$ and $p=0.0016$ respectively). Recipients of spleen cells ($n=5$; MST=10 days) rejected cardiac grafts earlier than recipients of bone marrow plus spleen cells ($n=5$; $p=0.0003$). MST: median survival time; SC: spleen cells; BMC: bone marrow cells.