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Original Article

Induction of immune tolerance and altered cytokine expression in skin transplantation recipients

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Abstract Apoptotic lymphocytes can induce specific immune tolerance. This study aimed to investigate the influence of the preoperative transfusion of apoptotic lymphocytes on allograft survival after skin transplantation. In addition, we aimed to determine changes in IL-4, IL-10 and IFN-γ mRNA expression in the grafted skin. A total of 20 New Zealand white rabbits were randomly divided into two groups: lymphocyte preconditioned group ($n = 10$) and control group ($n = 10$). Rabbits in the lymphocyte preconditioned group were intravenously injected with ^{60}Co γ-treated donor lymphocytes at seven days before the surgery. Rabbits in the control group were intravenously injected with normal lymphocytes at seven days before skin transplantation. The mRNA expression of IL-4, IL-10 and IFN-γ in the grafted skin was determined using real-time PCR. Skin allograft rejection time was 72.63 ± 2.65 days in the lymphocyte preconditioned group and 6.52 ± 0.64 days in the control group. IL-4, IL-10 and IFN-γ mRNA expression in the skin graft was 4.32 ± 0.48, 7.86 ± 0.56 and 2.63 ± 0.25 respectively in the lymphocyte preconditioned group, and 0.58 ± 0.07, 0.91 ± 0.14 and 8.68 ± 1.23 respectively in the control group. The preoperative transfusion of apoptotic lymphocytes induced immune tolerance in the grafted skin, as demonstrated by longer survival time of the grafts before rejection. This coincided with the increased mRNA expression of IL-4 and IL-10, and the decreased expression of IFN-γ.

Conflicts of interest: All authors declare no conflicts of interests.

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Introduction

Allogenic skin grafts have become an effective method in the treatment of burns. However, due to rejection, the grafted skin may not survive for a long period. Although the application of immunosuppressive drugs can be effective in preventing acute rejection, their long-term use has been associated with many side effects, and poses a potential risk for transplant patients in terms of infection and increased incidence of tumors [1—5]. In addition, immunosuppressive drugs cannot effectively control chronic rejection.

The induction of specific immune tolerance in recipients of donor organs is one of the most effective measures to prevent rejection. Thymus transplantation and bone marrow chimeras induce immune tolerance, which are very complicated. Apoptotic lymphocytes have previously been shown to induce specific immune tolerance [6,7]. CD4+ T-cells are divided into two types: Th1 and Th2 cells. Th1 cells mainly secrete IL-2, IFN-γ, TNF and GM-CSF, while Th2 cells mainly secrete IL-4, IL-5, IL-6, IL-10 and IL-13. In the present study, we investigated the influence of the preoperative transfusion of apoptotic lymphocytes on allograft survival after skin transplantation, and determined the changes in *IL-4*, *IL-10* (Which represented Th2-type cytokine) and *IFN-*γ mRNA (Which represented Th1-type cytokine) expression in the grafted skin.

Materials and methods

Rabbit skin grafts

These experiments were conducted in accordance with international guidelines, and approved by the Local Animal Ethics Committee. Outbred New Zealand white rabbits (*Oryctolagus cuniculus*, New Zealand strain) and California rabbits (*O. cuniculus*, California strain) weighing 2.5—3.0 kg were obtained from Shandong University Experimental Animal Center. These New Zealand white rabbits ($n = 20$) were randomly and equally divided into two study groups, in accordance with the random number table: lymphocyte preconditioned group and control group. Rabbits in the lymphocyte preconditioned group were intravenously injected with ^{60}Co γ-treated donor lymphocytes at seven days before surgery. Rabbits in the control group were intravenously injected with killed lymphocytes at seven days before skin transplantation. The California rabbits ($n = 8$) were used as donors of full-thickness skin grafts for the transplantation onto the backs of all recipient using standard techniques [3]. Bandages were removed on day three, and the skin grafts were scored twice, daily. Graft rejection was detected through the macroscopic appearance of graft necrosis, while graft necrosis was evaluated using scale that is based on color changes, blister formation, edema and local inflammation. Allograft biopsies were conducted every two days after the operation. The biopsied tissues were fixed in formalin solution (4—5 μM), embedded in paraffin, and sectioned. The sections were stained with hematoxylin and eosin.

Preoperative transfusion of apoptotic lymphocytes

Donor lymphocytes were isolated from heparinized whole blood by density-gradient centrifugation in a lymphocyte separation medium (Pharmacia). The separated lymphocyte suspension was subjected to 2.5 Gy of ^{60}Co γ-ray irradiation and placed in 10 mL of RPMI 1640, and the cells were cultured for 12—16 h at 37 °C in an incubator with CO_2, while control cell were normal lymphocytes. The irradiated cells and control cells were collected separately. Annexin V/PI double staining flow cytometry (BD Biosciences) was used to detect the rate of apoptosis in these lymphocytes.

At 16 h after the irradiation process the lymphocyte were injected into the rabbits, where rabbits in the lymphocyte preconditioned group were intravenously injected with 5×10^8 apoptotic lymphocytes which were treated by ^{60}Co γ-ray irradiation at seven days before surgery, while rabbits in the control group were intravenously injected normal lymphocytes at seven days before skin transplantation.

Real-time quantitative PCR

Allograft biopsied tissue was acquired at the sixth day in both the lymphocyte preconditioned group and control group. Total RNA was extracted from 50 to 100 mg of the grafted skin tissue using TRIzol (Invitrogen). The RNA was briefly exposed to RNAase-free DNAase I, and 1 μg of RNA was reverse transcribed into cDNA using the M-MLV Reverse Transcriptase (Promega).

IL-4, *IL-10* and *IFN-*γ mRNA expression was detected using the Light Cycler 2.0 real-time quantitative PCR machine. All PCR reagents were from the SYBR green real-time PCR kit (TaKaRa). The cDNA was amplified using the following primer sequences: *IL-4*: 5′-GCTATTGATGGGTCTCACCC-3’ (forward) and 5′-CAGGACGTCAAGGTACAGGA-3’ (reverse); *IL-10*: 5′-AGGGCACCCAGTCTGAGAACA-3’ (forward) and 5′-CGGCCTTGCTCTTGTTTTCAC-3’ (reverse); *IFN-*γ: 5′-TTTTGGGTTCTCTTGGCTGTT-3′ (forward) and 5′-CTCCTTTTTCGCTTCCCTGT-3′ (reverse); β-actin (used as an internal control): 5′-CGTGCGGGACATCAAGGA-3’ (forward) and 5′-AGGAAGGAGGGCTGGAACA-3’ (reverse). The PCR cycling parameters used were as follows: 10 s at 95 °C and 1 min at 60 °C for 40 cycles, followed by a thermal denaturation protocol. The mRNA expression of *IL-4*, *IL-10* and *IFN-*γ, compared to *β-actin*, was determined using the $2^{\Delta\Delta CT}$ method.

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