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S. Hayashi
M. Guang-Lin
I. Yokoyama
Y. Namii
H. Hamada
A. Nakao

Adenovirus-mediated gene transfer of CTLA4Ig gene results in prolonged survival of heart allograft

S. Hayashi (✉) · M. Guang-Lin · I. Yokoyama · Y. Namii · A. Nakao
Department of Surgery II,
Nagoya University, School of Medicine,
65 Tsurumai-cho, Showa-ku,
Nagoya 466, Japan
e-mail: shayashi@tsuru.med.nagoya-u.ac.jp
Tel.: + 81-52-7442245
Fax: + 81-52-7442255

H. Hamada
Department of Molecular Biotherapy Research, Cancer Chemotherapy Center,
Tokyo, Japan

Abstract It has been demonstrated that the administration of CTLA4Ig protein can induce the suppression of allograft and xenograft rejection. The purpose of this study is to determine the effect of adenovirus-mediated gene transfer with CTLA4Ig gene on the transgene expression and suppression of alloimmune response in allogeneic cardiac transplantation of rats. Adenoviral vectors with β galactosidase or human CTLA4Ig cDNA (Adex/LacZ, Adex/hCTLA4Ig) were constructed. These vectors were transduced to the liver of Lewis (LEW) rats by intravenous injection. Then LEW rats received heterotopic cardiac transplantation from Dark Agouti rats. Experiments were performed using both CTLA4Ig gene transduction and/or immunosuppressant FK506. The transgene expression after adenovirus-mediated transfer with CTLA4Ig cDNA lasted for several months, compared with several weeks after that in controls, which was proportional to the blood concentration of CTLA4Ig protein. By the administration of 1×10^9 PFU of Adex/hCTLA4Ig, the survival of cardiac grafts was significantly prolonged, compared with controls or the use of 1×10^8 PFU of Adex/hCTLA4Ig. In the rats with beating grafts over 100 days, the blood concentrations of CTLA4Ig were undetectable. The combination therapy using a low titer Adex/hCTLA4Ig and low-dose of FK506 was synergistically effective on this cardiac transplantation model. In conclusion, adenovirus-mediated gene transfer with CTLA4Ig gene was efficient for the prolongation of both transgene expression and allograft survival.

Key words CTLA4Ig · Transplantation · Gene therapy

Introduction

The interaction of T cells with alloantigen presented by antigen-presenting cells via T-cell receptor usually results in T cell activation, as manifested by cellular proliferation and lymphokine production. This interaction leads to the recruitment and activation of immune effector mechanisms that play the central role in allograft rejection. The B7 molecules expressed on antigen-presenting cells may deliver a critical costimulatory signal through CD28 on the T cell and play an important role during the T cell response to alloantigen. CTLA4Ig, a recombinant fusion protein containing the extracellular domain of human CTLA4 fused to the human Ig1 region, effectively binds the B7 molecule, interrupting the costimulatory pathway [1]. Recent investigations have shown that the administration of CTLA4Ig protein can induce the suppression of allograft and xenograft rejection [2, 3].

It is well known that adenovirus-mediated gene transfer is an excellent strategy for in vivo gene therapy because of high transduction efficiency to various organs, especially to the liver [4]. To confirm the effect of adenovirus-mediated gene transfer with CTLA4Ig

cDNA on the suppression of alloimmune response, we performed the fully allogeneic cardiac transplantation in the Dark Agouti (DA)-to-Lewis(LEW) rat combination.

Materials and methods

Animals

LEW (RT-1^l) and DA (RT-1^a) rats served as transplant recipients and donors, respectively. The animals were maintained under standard conditions and were fed water and rodent chow ad libitum. For the experiments, the "Principles of laboratory animal care" (NIH Publication No. 86–23, revised 1985) were followed, as well as the regulations of the Animal Research Laboratory of Nagoya University, School of Medicine.

Heart transplantation

Heterotopic cardiac transplantation was performed according to the technique described by Ono and Lindsey [5]. The heterotopic cardiac transplant survival was monitored by daily palpation and the rejection reaction, considered as the time of cessation of a palpable heart beat, was confirmed by pathological examination.

Construction of recombinant adenovirus

The replication-defective recombinant adenovirus encoding the *Escherichia coli* LacZ gene, capable of producing β-galactosidase (gal) was used as control vector (Adex/LacZ). Generating recombinant adenovirus was performed according to the paper described by Miyatake et al. [6]. Briefly, E1- and E3-deficient adenoviral vectors Adex/LacZ and Adex/hCTLA4Ig, which encode β-gal, human CTLA4Ig cDNA, were constructed by homologous restriction between the expression cosmid cassette and the parental virus genome. The expression cosmid cassette and adenovirus DNA-terminal protein complex were cotransfected into 293 cells by calcium phosphate precipitation. After the incorporation of the expression cassette into the recombinant adenoviruses was confirmed by the digestion with appropriate restriction enzymes, the recombinant adenoviruses were subsequently propagated with 293 cells. The titers of recombinant adenoviruses were determined by plaque-forming assay. These recombinant adenoviruses were purified and concentrated through two cycles of CsCl step gradients followed by dialysis against Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline – 10% glycerol buffer.

Experimental group

Experiment 1

To examine the effect of Adex/hCTLA4Ig on the transgene expression, LEW rats were divided into two groups, one with 1×10^9 PFU of Adex/LacZ and the other with 1×10^9 PFU of both Adex/LacZ and Adex/hCTLA4Ig. The rats in each group were killed at 1, 2, 4, 6, 8, 10, 12, 14, and 16 postoperative weeks, and the liver and blood samples were preserved at –80 °C.

Experiment 2

To examine the effect of Adex/hCTLA4Ig on the suppression of alloimmune response, LEW rats were divided into six groups. Seven days before transplantation, group 1 was administered with 1×10^9 PFU of Adex/LacZ, group 2 with 1×10^9 PFU of Adex/hCTLA4Ig, and group 3 with 1×10^8 PFU of Adex/CTLA4Ig. Group 4 was injected with FK506 (Fujisawa, Japan) 1 mg/kg per day i.m. for 7 days and group 5 with FK506 0.1 mg/kg per day i.m. for 7 days, starting on the transplant day. For group 6, the combination of 1×10^8 PFU of Adex/hCTLA4Ig and 0.1 mg/kg per day i.m. for 7 days of FK506 was used. Graft rejection was confirmed by the cessation of heart beat. The blood samples were collected just before transplantation and just after beating stopped.

Detection of β-gal protein

Tissue samples were homogenized with 1.0 mM phenylmethylsulfonylfluoride in 0.25 M TRIS-HCl, and then the extractions were preserved in –70 °C for use. β-gal protein was detected by ELISA (5 Prime → 3Prime, Colo., USA). Briefly, the extractions were incubated in a 96-well flat plate precoated with β-gal-coating antibody at 37 °C for 2 h, and then incubated with biotinylated antibody to β-gal. After incubating with streptavidin-conjugated alkaline phosphatase, diethanolamine buffer with 5 mg para-nitrophenylphosphate was added. The chemiluminescence was analyzed at A405 by a microwell reader, and the concentration of β-gal protein was measured.

Determination of blood concentration of CTLA4Ig

The blood concentration of hCTLA4Ig was determined by binding assay with NIH/3T3 cells transduced with B7.1cDNA (B7.1 transfectants) [7]. Briefly, 15×10^5 B7.1 transfectants were incubated with 100 μl rat sera for 1 h. Then the cells were mixed with FITC-conjugated goat anti-human Ig polyclonal antibody (Ig-FITC 50 mg/ml, 100 μl; Dako, Denmark) and then applied to the flowcytometer. A standard curve was designed using purified hCTLA4Ig (supplied by Bristol-Myers Squibb Pharmaceutical Research Institute, Wash., USA).

Statistics

Transplant survival of experimental groups was compared using the Mann-Whitney-Wilcoxon test.

Results

Transgene expression

The expression of LacZ antigen in the liver using Adex/LacZ was 115 ng/ml total protein at the 1st postoperative week, 12 at 2 weeks, and disappeared by 4 weeks, whereas that using both Adex/LacZ and Adex/hCTLA4Ig lasted up to 12 weeks, and gradually disappeared by 16 weeks. In proportion to the expression of LacZ in the liver using both Adex/LacZ and Adex/hCTLA4Ig, the blood concentration of CTLA4Ig was maintained over 20 μg/ml for 12 weeks.

Graft survival

The mean graft survival time of each experimental group of experiment 2 was as follows: 6.0 days in group 1; more than 80.8 days in group 2; 11 days in group 3; 57.2 days in group 4; 13.4 days in group 5; and 34.4 days in group 6. Three of five rats in group 2 survived longer than 100 days after transplantation with a normal beating heart. The long-surviving grafts proved that there was no immunological rejection reaction with little mononuclear cellular infiltration. When the skin grafts from DA rats were performed in the rats with long-surviving grafts, the survival of the skin grafts was significantly prolonged, compared with those from third-party rats.

Blood concentration of hCTLA4Ig

The blood concentration of hCTLA4Ig in experiment 2 was 0 μg/ml in group 1, 21.46 μg/ml in group 2, and 1.51 μg/ml in groups 3 and 6 before transplantation, and 0 μg/ml in group 1, 0.1 μg/ml in group 2, 0.16 μg/ml in group 3, and 0.46 μg/ml in group 6 after transplantation. In the rats with graft survivals more than 100 days, the analysis of hCTLA4Ig blood concentration was performed using sera on the 60th day after transplantation. The concentration of hCTLA4Ig was greater than 20.0 μg/ml in all rats, which was comparable to that before transplantation. The blood concentration of CTLA4Ig on the day when the heart stopped beating in group 6, which was measured on about 33.0 days after transplantation, was higher than that in group 3 measured 11 days after transplantation, but not significantly higher.

Discussion

CD28 and CTLA4 are structurally related T-cell surface molecules which bind a counterreceptor B7 on antigen-producing cells and deliver a costimulatory signal which is necessary for T-cell proliferation. Previous reports have shown that interference with costimulation through the CD28/CTLA4/B7 receptor system using CTLA4Ig leads to aborted T cell responses, inhibition of T-cell dependent antibody responses, and suppression of allo- and xenorejection [2, 3].

This report demonstrated that the overexpression of hCTLA4Ig in the liver using adenovirus-mediated gene transfer prolonged both transgene expression and graft survival in the DA-to-LEW cardiac allograft model. It has been reported that in the fully allogeneic liver transplantation of rats, the doner livers transduced with hCTLA4Ig cDNA using adenovirus-mediated gene transfer can survive indefinitely, followed by the induction of donor-specific unresponsiveness [8, 9]. Here we report that heterotopic allotransplantation of donor hearts to recipients transduced by adenovirus-mediated gene transfer with CTLA4Ig gene via the peripheral route also significantly prolong the graft survival. Considering that the transgene expression of CTLA4Ig was detected in the liver by RT-PCR, the production of CTLA4Ig from the liver may induce the immunosuppressive status in the recipient rats. Using the rats with 1×10^9 PFU of Adex/hCTLA4Ig, some of the cardiac grafts survived over 100 days, and in these rats with long-surviving grafts, the blood concentration of hCTLA4Ig was not detected and donor-specific immunosuppression was demonstrated by a second challenge of skin grafts. The skin graft examination was equivocal, however, the donor-specific unresponsiveness may possibly be induced.

It has been reported that the transgene expression after adenovirus-mediated gene transfer is transient, the duration of which is within several weeks [10]. Our investigation has shown that using Adex/hCTLA4Ig, the duration of transgene expression can be prolonged from several weeks to several months in proportion to the sustained blood concentration of CTLA4Ig. In the rats with long-surviving grafts, the transgene expression on 100 days after transplantation had disappeared, although it was maintained until 60 days after transplantation with almost similar levels to those before transplantation.

Recombinant CTLA4Ig protein has to be administered intravenously long term to achieve an effective immunosuppression, whereas the single administration of Adex/hCTLA4Ig can successfully perform long-term suppression of transplant reaction. The optimal dose of Adex/hCTLA4Ig and the blood concentration of hCTLA4Ig protein remain to be determined, however, adenovirus-mediated gene transfer using hCTLA4Ig gene can be a promising form of immunosuppression.

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