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Effect of Triple Costimulation Blockade on Islet Allograft Survival in Sensitized Mice

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Abstract

Background—Islet allograft rejection in sensitized recipients is difficult to control by costimulation blockade using anti-CD154 and cytotoxic T-lymphocyte antigen-4 immunoglobulin (CTLA4Ig). Because leukocyte function antigen (LFA) 1 is highly expressed on memory T cells, adding an LFA-1 blockade may inhibit memory T-cell activities. We examined the effects on islet allograft survival of triple costimulation blockade in presensitized recipient mice.

Methods—C57BL/6 mice were sensitized by transplantation under the kidney capsule or intraperitoneal injection of Balb/c islets. Four weeks after transplantation, sensitization was confirmed by flow-cytometric detection of alloreactive antibodies. Diabetes was induced by a single intravenous injection of streptozotocin. Recipients were transplanted with 200 Balb/c islets under the right kidney capsule. Graft function was assessed by daily blood glucose and body weight records. Transplanted animals were divided into 3 treatment groups: group 1, control antibody; group 2, anti-CD154 and CTLA-4 Ig double therapy; group 3, anti-CD154, CTLA4Ig, and anti-LFA-1 triple therapy. Injections were administered every second day from day – 2 to day 8.

Results—Naïve mice rejected islet allografts between days 7 and 29 (mean 16 ± 6 d; $n = 5$), sensitized mice in group 1 between days 0 and 14 (mean 7 ± 5 d; $n = 8$), in group 2 between days 4 and 16 (mean 8 ± 4 d; $n = 7$), and in group 3 between days 4 and 26 (mean 11 ± 7 d; $n = 10$).

Conclusion—Triple costimulation blockade with anti-CD154, CTLA4Ig, and anti-LFA-1 was not sufficient to improve islet allograft survival in sensitized recipients.

Memory T cells are induced after antigen exposure in the context of viral/bacterial infections, vaccinations, blood transfusions, pregnancies, and previous transplantations. Transplant recipients may display T cells that cross-react with their allografts, increasing the risk for rejection and making it more difficult to achieve long-term graft survival. Costimulation blockade with anti-CD154 and cytotoxic T-lymphocyte antigen 4 immunoglobulin (CTLA4Ig) prolongs allograft survival in naïve mice, but not in immunized mice or in larger animals such as humans.¹

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We have previously shown that addition of antileukocyte function antigen-1 (LFA-1) antibodies improved pig islet graft function in mice with long-term transplant acceptance induced with CTLA4Ig/anti-CD154.² LFA-1 is expressed on most hematopoietic cells, including memory T cells. Anti-LFA-1 antibodies have been shown to block the cytotoxicity of activated T and natural killer cells. Therefore, we hypothesized that if anti-LFA-1 antibodies block memory T-cell responses, anti-CD154 antibody and CTLA4Ig may induce peripheral tolerance even among immunized recipients. Herein, we have investigated the effects of costimulation blockade using anti-CD154 and CTLA4Ig with or without anti-LFA-1 antibodies on allogeneic islet graft survival in sensitized mice.

MATERIALS AND METHODS

The local Animal Ethics Committee approved this study. We adhered to the requirements of the Animal Welfare Act and the National Institutes of Health guidelines for the care and use of laboratory animals.

Antibodies

Antibodies included anti-CD154 (MR1, hamster IgG), CTLA4Ig (fusion protein of human CTLA4 and Fc fragment of human IgG), anti-LFA-1 antibody (M-17.5.2, rat IgG2a), and isotype control antibodies for human IgG, hamster IgG, and rat IgG2a. All of the antibodies were purchased from Bio Express (West Lebanon, NH, USA).

Islet Isolation

Islets were isolated from the pancreata of Balb/c mice aged 12–20 weeks and weighing >24 g by injection of collagenase P (0.75 mg/mL; Roche Diagnostics, Mannheim, Germany) into the organ via the bile duct. They were purified using a Histopaque 1077 and 1119 gradient (Sigma), followed by hand picking. The islets were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin for 1–2 days before transplantation.

Sensitization of Recipients

C57B1/6 mice (aged 10–16 weeks) were used as recipients that had been immunized by transplanting 200 Balb/c islets under the left kidney capsule or by intraperitoneal injection of 50–100 µL of an allogeneic pancreatic digest including islets. Four weeks after immunization, recipient sera were examined for the presence of alloreactive antibodies using Balb/c spleen cell targets in flow cytometry. Mice that had developed alloreactive antibodies were used as allo-islet sensitized recipients.

Diabetes Induction and Retransplantation

Between 5 and 8 weeks after immunization, diabetes was induced by a single intravenous injection of streptozotocin (Sigma-Aldrich, CO; 200 mg/kg body wt). Three to 4 days after induction of diabetes (blood glucose >20 mmol/L), 200 Balb/c islets were transplanted under the right kidney capsule. Blood glucose and body weight were recorded daily. Recipients were divided into 4 groups: group 0, naïve recipients transplanted with 200 allogeneic islets; group 1, mice receiving isotype control antibodies (human IgG, hamster IgG, and rat IgG2a); group 2, mice treated with anti-CD154 (MR1; 0.5 mg) and CTLA4Ig (0.5 mg); and group 3, injected with anti-CD154, CTLA4Ig, and anti-LFA-1 (0.2 mg). Injections were performed every second day from day –2 to day 8. Graft function was estimated by daily monitoring of blood glucose and body weight. Non-fasting blood glucose levels of <10 mmol/L in transplanted recipients reflected functional grafts. Blood glucose levels of >20 mmol/L (360 mg/dL) for >2

consecutive days was considered to be indicative for graft rejection, in which case the mouse was sacrificed.

RESULTS

Graft survivals are summarized in Table 1. Naïve mice rejected islet allografts between 7 and 29 days (mean 16 ± 8 d; $n = 5$), immunized mice in the control group 1 between days 0 and 14 (mean 7 ± 5 d; $n = 8$), the double therapy group 2 between days 4 and 16 (mean 8 ± 4 d; $n = 7$), and the triple therapy group 3 between days 4 and 26 (mean 11 ± 7 d; $n = 10$). Two naïve mice transplanted with islet allografts and receiving triple therapy accepted their allografts (>180 days; data not shown).

DISCUSSION

Combined treatment with anti-CD154, CTLA4Ig, and anti-LFA-1 antibodies could not prolong islet allograft survival among sensitized recipients. The sensitized mice showed both alloreactive antibodies and memory T cells. Therefore, rejections in the sensitized recipients may be caused by alloreactive antibodies, by the memory T-cell compartment, or by a combination of both factors. If the antibody-mediated effect is strong enough to cause graft destruction, costimulation blockade that curbs only T cell responses may not be sufficient to prevent graft rejection.

In xenotransplantation rodent models, xenospecific antibodies have been shown to enhance islet rejection.³ Also clinically, the presence of donor-specific anti-HLA antibodies has been shown to negatively influence islet allograft survival.⁴

Anti-CD154 or CTLA4Ig treatment alone or the combination of these 2 antibodies was not sufficient to prolong allograft survival in sensitized hosts. Recently, it has been shown that in mouse models accelerated heart allograft rejection mediated by alloantigen-primed memory T cells was inhibited by a combination of anti-CD154, CTLA4Ig, anti-LFA-1, and anti-OX40-ligand antibodies.⁵ OX40, also known as CD134, is known to be expressed on memory T cells. Blocking interactions between OX40 and its ligand can reduce memory T-cell activities.⁶ However, in these models no alloreactive antibodies were present.

In the clinical situation, some type 1 diabetes recipients are sensitized, suggesting that both donor-reactive memory T cells and antibodies may be present. The effect of graft-reactive antibodies can be as important as T-cell responses. In future studies, we will seek to dissect the effects of allospecific antibodies versus memory T cells on islet allo- and xenograft survivals, continuing to explore the therapeutic effects of various combinations of costimulation blockade agents.

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Table 1

Graft Survival After Islet Allotransplantation

Group	Graft Survival (d)									
Naïve (n = 5)	7	14	29	15	14					
Group 1 (n = 8)	0	0*	4	5	8	9	13	14		
Group 2 (n = 7)	4	4	6*	7*	8	8	16			
Group 3 (n = 10)	4	5*	6	7*	7*	8	13	14	17	26

* Immunized by intraperitoneal injection of a Balb/c pancreatic digest (including islets); others immunized by transplantation of 200 islets under the kidney capsule.