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Role of NKT cells in allogeneic islet graft survival

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Abstract Although NKT cells expressing CD1d-reactive TCR exerted protective role in autoimmune diseases, the regulatory function of CD1d-dependent NKT cells in alloimmune responses has not been investigated thoroughly. Here, we demonstrated the regulatory effects of NKT cells using a pancreas islet transplantation model. CD40/CD154 blocking induced long-term graft survival in most B6 recipients, but B6.CD1d$^{-/-}$ recipients showed co-stimulation blockade-resistant rejection. Adoptive transfer of NKT cells into B6.CD1d$^{-/-}$ restored tolerizing capacity of co-stimulatory blockade. Activation of NKT cells was effective for the prolongation of graft survival and up-regulated membrane-bound TGF-β expression transiently on their cell surface. The activated CD1d-dependent NKT cells inhibited alloantigen-driven cell proliferation through cell contacts and the beneficial effect of CD154 blocking for allograft survival was related to TGF-β pathway. Thus, we can conclude that NKT cells are essential for the stable allograft survival and the regulatory function is dependent on, at least in part, TGF-β engagement.

Introduction

During the last four decades, there has been a marked improvement in short-term survival of allograft; however, long-term graft survival has remained relatively unchanged [1,2]. The discovery of a variety of potent immunosuppressive agents capable of preventing acute graft rejection have not been effective against chronic graft loss. Furthermore, most allograft recipients suffer from toxicities and complications arising from the use of immunosuppressive agents, which range from susceptibility to opportunistic infection, to fatal malignancies. The induction of donor-specific allograft tolerance is considered to be the definitive goal for scientists working in this field.

CD1d is a nonclassical MHC class I-like, β2-microglobulin-associated protein constitutively expressed by antigen presenting cells and some epithelial cells. CD1d is recognized by a subpopulation of T cells, many of which express markers typical of NK cells and have been identified as a unique population NKT cells [3–5]. A major fraction of CD1d-

Abbreviations: HMC, hepatic mononuclear cell; CD1d$^{-/-}$, CD1d deficient; B6, C57BL/6; rTGF-β, recombinant TGF-β; MR1, anti-CD154 mAb; αGalCer, α-galactosyl ceramide.

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restricted T cells recognize glycolipid antigen through an invariant T cell receptor α chain. CD1d-dependent NKT cells using this invariant TCR have regulatory functions in innate and adaptive immune responses [5,6]. They secrete large amounts of IL-4 and IFN-γ upon stimulation with their TCRs. In animal models, NKT cells have been reported to affect the development and progression of diabetes mellitus [7], experimental autoimmune encephalitis [8] and systemic lupus erythematosus [9]. NKT cells have been exploited in several organ transplantation systems. They are critical for the induction of antigen-specific tolerance to xenogeneic islet cells induced by anti-CD4 mAbs [10]. NKT cells also mediate the tolerogenic action of anti-LFA-1 and anti-ICAM-1 Ab in an allogeneic heart graft model [11]. The regulatory capacity of NKT cells may be dependent on the quantitative strength of antigenicity [12] and be less potent than that of $CD4^+CD25^+$ regulatory T cells [13,14]. However, the functional mechanisms of CD1d-dependent NKT cells in the initiation and progression of alloimmune responses have not been investigated thoroughly.

The objective of this study was to evaluate the regulatory capacity and molecular interactions of CD1d-dependent NKT cells in allogeneic immune responses using a murine islet allograft model.

Materials and methods

Animals

Male C57BL/6 (B6; H-2^b, 8 weeks old) and DBA/2 (H-2^d, 8 weeks old) mice were purchased from Shizuoka Institute for Laboratory Animals, Inc. (Hamamatsu-shi, Shizuoka-ken, Japan) and used as recipients or donors of transplantation. CD1d deficient ($CD1d^{-/-}$) mice were purchased from Jackson laboratories and backcrossed to B6 background for 10 generations (B6.$CD1d^{-/-}$) and used as allograft recipients at the age of 8 weeks (H-2^b). All the animal experiments were performed under the approval of the Institutional Animal Care and Use Committee of Clinical Research Institute at Seoul National University Hospital and according to the 'Guidelines for the Care and Use of Laboratory Animals' of the National Research Council.

Islet transplantation

Islet transplantation was performed on wild-type or B6.$CD1d^{-/-}$ mice as previously described [15]. Briefly, DBA/2 donor pancreata were perfused with 3.5 ml HBSS containing collagenase (0.8 mg/dL, Sigma-Aldrich, St. Louis, MO) through the common bile duct and incubated at 37 °C for 14 min. Islets were released from the pancreata and purified in discontinuous Ficoll (Sigma-Aldrich) gradients. Harvested islets were washed in HBSS and more than 400 islets were transplanted under the renal capsule of each recipient rendered diabetic by a single intraperitoneal injection of streptozotocin (250 mg/kg; Sigma-Aldrich). Allograft function was monitored by serial blood glucose measurements. Primary graft function was defined as a blood glucose level <200 mg/dL on 2 days after transplantation and graft rejection was defined as two consecutive elevations in blood glucose >300 mg/dL following a period of primary graft function. Some of islet allograft recipients were treated with anti-CD154 mAb, anti-TGF-β mAb, rTGF-β protein and α-galactosyl ceramide (α-GalCer) as indicated. Sorted NKT cells or hepatic mononuclear cells enriched with NKT cells from wild-type B6 mice were adoptively transferred to some B6.$CD1d^{-/-}$ recipients.

Treatment schedule

Administration of mAb and cytokine

To test the tolerogenic effect of CD40/CD154 blockage, 0.25 mg anti-CD154 mAb (MR1, Taconic biotechnology, Germantown, NY) was introduced intraperitoneally three times every other day after transplantation. An irrelevant, isotype-matched hamster anti-keyhole limpet hemocyanin (KLH) mAb provided by Biogen (Cambridge, MA) was used for control treatment (DBA/2→C57BL/6). For blocking TGF-β-mediated signals, 500 μg of anti-TGF-β mAb (isolated from hybridoma; clone 1D11.16.8 purchased from ATCC) was introduced intraperitoneally on days −2, 0, 2 and 4 of transplantation. To test the effect of TGF-β, rTGF-β (500 ng, R&D Systems, Minneapolis, MN) was injected intraperitoneally on days −4, 0, 2, 4, 6, 8 of transplantation.

Administration of α-GalCer

α-GalCer, a glycosphingolipid originally isolated as a natural product of marine sponge, was kindly provided by Dr. Sanghee Kim (Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul, Korea) [16]. α-GalCer (2 μg) or vehicle was introduced to mice intraperitoneally on days −7, −3, 0, 3, 7, 14 and 21 of islet transplantation.

Adoptive transfer of hepatic mononuclear cells or NKT cells

For reconstitution of NKT cells, hepatic mononuclear cells (HMCs) from B6 mice were adoptively transferred into B6.$CD1d^{-/-}$ mice (5×10^6/mouse, 1 day before islet transplantation). HMCs were prepared as followings. Mice were sacrificed and the liver was smashed and dispersed in loading buffer (1× PBS plus 10% FBS and 1 mM EDTA), passed through a stainless steel mesh, overlaid onto lymphocyte M (Cedarlane, Hornby, Ontario, Canada), and centrifuged at 900×*g* at 25 °C for 18 min. More than 20% of isolated HMCs were NKT cells defined by double positivity against NK1.1 and TCR-β. NKT cell sorting was conducted as followings. NKT cells were determined using rat anti-mouse PE-conjugated anti-NK1.1 and CyChrome-conjugated anti-TCRβ (PharMingen, San Diego, CA). Flow cytometric sorting was performed with a FACSCalibur instrument using CellQuest software (BD Biosciences, Franklin Lakes, NJ) and the purity of sorted cells was >98%. Sorted NKT cells were transferred into B6.$CD1d^{-/-}$ mice intravenously (1×10^6 in 300 μL of PBS per mouse) on days −1 and 0 (day 0 being the day of islet transplantation).

Antibodies and Flow cytometric analysis

Expression of membrane-bound TGF-β on DN32.D3 NKT cells ($V\alpha14^+$ TCR mouse CD1d-specific NKT cell hybridoma) was