Role of Natural Killer Cells in the Innate Immune System After Intraportal Islet Transplantation in Mice

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ABSTRACT

Background. Both liver natural killer (NK) and NK T cells of the innate immune system play a crucial role in islet graft loss after intraportal islet transplantation, although a relationship between NK and NK T cells in islet loss has not been proven. In this study, we investigated the role of NK cells in the innate immune system in islet graft loss after intraportal islet transplantation.

Methods. To investigate the involvement of liver NK cells in islet destruction, we assessed the differences in graft survival after intraportal islet transplantation between $CD1d^{-/-}$ diabetic mice and NK cell–depleted $CD1d^{-/-}$ diabetic mice.

Results. The transplantation of 400 islets into the liver was sufficient to reverse hyperglycemia in wild-type diabetic mice (100%, 4/4). However, normoglycemia could not be achieved when 200 islets were transplanted (0%, 0/4). In contrast, intraportal transplantation of 200 islets in NK cell–depleted $CD1d^{-/-}$ diabetic mice ameliorated hyperglycemia in 71% of cases (5/7), whereas transplantation of the same number of islets in $CD1d^{-/-}$ diabetic mice did not (0%, 0/4). Histologic findings also confirmed that intact islets were observed in NK cell–depleted $CD1d^{-/-}$ diabetic mice, but were difficult to observe in $CD1d^{-/-}$ diabetic mice.

Conclusions. The involvement of liver NK cells in the innate immune system related to islet graft loss after intraportal islet transplantation is revealed by improved graft survival and function in NK cell–depleted $CD1d^{-/-}$ diabetic mice. Our data reveal that regulation of NK cell activity is particularly important when insufficient islet numbers are used for transplantation.

ISLET transplantation is regarded as a potential treatment for type 1 diabetes (T1D) patients who suffer from severe and persistent hyperglycemia [1]. However, a current drawback of islet transplantation is that multiple transplantations are often required to achieve insulin independence because the number of islets supplied by a single donor is limited and the majority of islets are rejected within a short period of time for various reasons. These include innate inflammatory responses referred to as instant blood-mediated inflammatory reaction (IBMIR) [2], toxicity of immunosuppressive drugs [3], and innate immune responses [4–6]. Innate immune responses emerge before adaptive immune responses after transplantation. Macrophages play a key role in early inflammatory events that adversely affect islet engraftment [7]. Natural killer T (NKT) cells are also involved in islet destruction after intraportal islet transplantation in mouse models [6,8].

Furthermore, natural killer (NK) cells are pivotal immune cells of the innate immune system. We have primarily

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focused on liver NK cells after islet transplantation because we had previously shown that human liver mononuclear cells extracted from liver perfusates contain a large population of NK cells that mediate higher cytotoxicity against neoplastic or infected cells via the tumor necrosis factor (TNF)–related apoptosis-inducing ligand (TRAIL)–induced apoptosis pathway than do peripheral blood NK cells [9]. We further demonstrated that NK cell depletion leads to successful intraportal islet transplantation in a mouse model and that the cytotoxicity of NK cells and TRAIL expression on NK cells significantly increased after intraportal islet transplantation [4]. Although it has been reported that interferon (IFN) γ produced by NKT cells activates NK cells of the innate immune system [10,11], the underlying mechanism of NK cell activation has not been fully elucidated.

Consequently, we performed intraportal islet transplantation in NK cell–depleted NKT cell–deficient mice to investigate the role of NK and NKT cells in islet loss after transplantation.

METHODS

Mice

Male C57BL/6J (B6) (H-2^b) mice (8–12 weeks old) were purchased from Clea Japan (Osaka, Japan). CD1d$^{-/-}$ mice on a B6 background, which harbor a specific deletion of CD1d gene loci, were used (kindly provided by Dr K. Seino, Laboratory for Immune Regulation, Riken Research Center for Allergy and Immunology, Yokohama, Japan) [12]. B6 mice and CD1d$^{-/-}$ mice were used as recipients of islet transplantations, and B6 mice were used as syngeneic islet donors. All animals were maintained in a specific pathogen-free microenvironment. All experiments were approved by the Institutional Review Board of Hiroshima University and conducted in accordance with the guidelines of the National Institutes of Health (publication no 86-23, revised 1996).

Antibodies and Flow Cytometry Analysis

Mononuclear cells (MNCs) were stained with the following monoclonal antibodies (mAbs): APC-conjugated anti-NK1.1 (BD Pharmingen, San Diego, California) and APC-Cy7–conjugated anti–T-cell receptor β. All analyses were performed on a FACSCanto II cytometer (BD Biosciences, Mountain View, California). Nonspecific FcγR binding of labeled mAbs was blocked by anti-CD16/32 (2.4G2; BD Pharmingen). Dead cells were excluded from the analysis by means of light scatter and/or propidium iodide staining.

Isolation of Mononuclear Cells From the Liver

Liver mononuclear cells (LMNCs) were prepared as previously described [13]. Briefly, the liver was removed after perfusion via the portal vein with 1 mL phosphate-buffered saline solution (PBS) supplemented with 10% heparin. LMNCs were obtained from the liver by means of perfusion with 50 mL PBS supplemented with 0.1% EDTA (Sigma-Aldrich, St Louis, Missouri).

Induction of Diabetes in Recipient Mice

Diabetes was induced in recipient B6 mice by means of an intraperitoneal injection of streptozotocin (STZ, 200 mg/kg; Sigma-Aldrich). Blood glucose levels were measured with the use of a glucose analyzer (GT-1830; Arkray, Tokyo, Japan). After STZ injection, nonfasting blood glucose levels exceeded 500 mg/dL by day 3, and the mice remained hyperglycemic until the time of islet transplantation. Graft survival was assessed by determining plasma glucose levels after transplantation. The reversal of diabetes was indicated by 2 consecutive plasma glucose level measurements <200 mg/dL.

In Vivo NK-Cell Depletion

To deplete NK cells, CD1d$^{-/-}$ mice were administered an intraperitoneal injection of anti-NK1.1 mAb (300 μg per mouse) 2 times per week, beginning 2 days before islet transplantation. Anti-NK1.1 mAb was prepared in the laboratory from a hybridoma (PK136; ATCC).

Islet Isolation and Transplantation

Islets were isolated by means of our standard procedure. Briefly, the pancreas was distended by infusion of 1 mg/mL collagenase P (Roche Diagnostics, Indianapolis, Indiana) solution and incubated at 37°C for 17 minutes. Pancreatic tissue was dissociated by repeated shaking and washing several times. Islets with >90% purity were obtained by means of purification by Percoll Plus (GE Healthcare) centrifugation, followed by hand picking. Islets that were 100–200 μm in diameter were used for transplantation. Islets were transplanted into the liver of recipient mice via the portal vein [14].

Histology

Sections of liver from transplant recipients were fixed in 4% neutral-buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin-eosin.

Statistical Analysis

The statistical significance of differences between dichotomous variables was compared by means of Fisher exact test. *P* values of <.05 were considered to be statistically significant.

RESULTS

NK1.1 Depletion by Anti-NK1.1 Antibody

To assess the depletion of mononuclear cells (MNCs) in CD1d$^{-/-}$ mice by anti-NK1.1 mAb treatment, MNCs were isolated from the liver and spleen of anti-NK1.1 mAb–treated CD1d$^{-/-}$ mice 2 days after intraperitoneal injection. CD1d$^{-/-}$ mice lack NK1.1$^+$ TCR-β$^+$ NKT cells, and intraperitoneal injection of anti-NK1.1 antibody depleted >99% of both liver and spleen NK and NKT cells of treated CD1d$^{-/-}$ mice (Fig 1).

NKT Cells Are Involved in Islet Destruction

We examined the involvement of both NKT and NK cells in islet destruction by assessing islet graft survival in CD1d$^{-/-}$ mice. Transplantation of 400 islets into the liver was sufficient to reverse hyperglycemia in wild-type mice (100%, 4/4; data not presented). In contrast, 300 islets, considered to be a marginal mass of islets, could achieve normoglycemia in 3 of 9 recipients (33.3%; Fig 2A). To evaluate the role of innate immune cells, 300 islets were transplanted into CD1d$^{-/-}$ mice or CD1d$^{-/-}$ mice treated with anti-NK1.1 mAb. Transplantation of 300 islets reversed hyperglycemia in all CD1d$^{-/-}$ mice (100%, 3/3) and all CD1d$^{-/-}$ mice treated with anti-NK1.1 mAb (100%, 2/2) for up to 14 days