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Transgenic Expression of Decoy Receptor 3 Protects Islets from Spontaneous and Chemical-induced Autoimmune Destruction in Nonobese Diabetic Mice

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Abstract

Decoy receptor 3 (DCR3) halts both Fas ligand– and LIGHT-induced cell deaths, which are required for pancreatic β cell damage in autoimmune diabetes. To directly investigate the therapeutic potential of DCR3 in preventing this disease, we generated transgenic nonobese diabetic mice, which overexpressed DCR3 in β cells. Transgenic DCR3 protected mice from autoimmune and cyclophosphamide-induced diabetes in a dose-dependent manner and significantly reduced the severity of insulitis. Local expression of the transgene did not alter the diabetogenic properties of systemic lymphocytes or the development of T helper 1 or T regulatory cells. The transgenic islets had a higher transplantation success rate and survived for longer than wild-type islets. We have demonstrated for the first time that the immune-evasion function of DCR3 inhibits autoimmunity and that genetic manipulation of grafts may improve the success and survival of islet transplants.

Key words: NOD • Fas ligand • LIGHT • T helper 1 cell • T regulatory cell

Introduction

Decoy receptor 3 (DCR3), also known as TR6 or M68, was cloned from the human-expressed sequence tag database because its conserved sequences belong to the TNFR superfamily. DCR3 lacks the transmembrane domain of conventional TNFRs and is believed to be a secreted protein. Elevated DCR3 expression is observed in many kinds of tumors, including lung and colon cancers (1), gastrointestinal tumors (2), and malignant glioma (3). Elevated DCR3 expression might benefit tumors by helping them avoid immune attacks by lymphocyte infiltration and Fas ligand (FasL)/LIGHT-mediated apoptosis. Although the biological significance of DCR3 is not fully understood, DCR3 clearly interacts with FasL (1), LIGHT (4), and TNF-like molecule 1A (TL1A; reference 5), and neutralizes the apoptotic signals through Fas receptor, lymphotoxin β receptor (LTβR), and death receptor 3, respectively. DCR3 also serves another regulating function by directly modulating the differentiation and function of dendritic cells and macrophages (6, 7). Moreover, DCR3 ameliorates the rejection response to mouse heart allograft (8). Because development of autoimmune diabetes requires lymphocyte infiltration into islets and β cell apoptosis mediated by FasL (9, 10) and LIGHT (11, 12), we evaluated the therapeutic potential of DCR3 in an organ-specific transgenic mouse model.

The nonobese diabetic (NOD) mouse is an inbred strain that spontaneously develops autoimmune diabetes, which resembles human type 1 diabetes (13, 14) and is characterized by progressive autoimmune destruction of the insulin-producing β cells in the pancreatic islets of Langerhans. Both genetic predisposition and environmental factors contribute to the pathogenesis of autoimmune diabetes. NOD serves as an important model for dissecting immunopatho-

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Abbreviations used in this paper: DCR3, decoy receptor 3; FasL, Fas ligand; IDDM, insulin-dependent diabetes mellitus; LTβR, lymphotoxin β receptor; NOD, nonobese diabetic; PD, pancreatic DCR3; TL1A, TNF-like molecule 1A; Treg, T regulatory cell.

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logical mechanisms and designing preventive and therapeutic strategies in type 1 diabetes.

To explore the therapeutic potential of DCR3 to prevent autoimmune diseases, we generated transgenic NOD mice, which overexpressed DCR3 in their β islets. Our results indicate that islet-specific expression of DCR3 not only protects β cells from autoimmune or chemical-induced destruction, but also improves the transplantation success rate and prolongs the survival period after transplantation. Our data also show that islet-restricted expression of DCR3 does not alter the integrity of islets and is not likely to induce any abnormal phenotype or overgrowth activity seen in tumor cells, which can harm transgenic mice. More importantly, in NOD mice, local expression of DCR3 did not affect systemic immune responses, such as T cell–mediated diabetogenesis, Th1 differentiation, and T regulatory cell (Treg) development, indicating that the therapeutic potential is restricted and organ specific. We have shown for the first time that the immune-escaping property of DCR3 can apply to organ-specific autoimmunity and that this genetic modification of β cells improves the survival of transplanted islets. This may contribute to alleviating the rejection response common in allogenic islet transplantation in autoimmune diabetes.

Materials and Methods

Mice. NOD/Sytwu (K^d, D^b, L^d, I-A^{g7}) and NOD/SCID mice were purchased from The Jackson Laboratory and subsequently bred at the Animal Center of the National Defense Medical Center in Taipei, Taiwan, under specific pathogen-free conditions. The spontaneous incidence of diabetes in the colony was 80–90% in females and 20–30% in males by 25 wk of age. The linear DNA fragment (see Fig. 1 a) was microinjected into the pronuclei of one-cell NOD embryos.

Detection of DCR3 Transgene. Genomic DNA was prepared and hybridized with DCR3 cDNA (sequence data are available from GenBank/EMBL/DDBJ under accession no. AF104419). TRIzol reagent (Invitrogen) was used to extract pancreatic RNA and protein. Isolated RNA was reverse transcribed by Superscript II reverse transcriptase (Invitrogen) with the backward primer 5′-TAGGTGGGTGTTTCTGCCAC-3′, and amplified by the PCR reaction together with the forward primer 5′-GGATCCTGAGAACTTCAGG-3′ at an annealing temperature of 58°C for 30 cycles. PCR examination of genomic DNA was performed with the same primers described above and internally controlled with another pair of primers, 5′-ATCTCCTACCCCAAACCTC-3′ and 5′-ACCATCTTTCCCCTCTTCC-3′. To standardize the amount of mRNA in each sample, the RT-PCR product of *hgprt* was detected by the forward primer 5′-CTCGAAGTGTTGGATACAGG-3′ and backward primer 5′-TGGCCTATAGGCTCATAGTG-3′. Western blot analysis was probed by primary mouse monoclonal antibody against human DCR3 (Anawrahta) and then detected with horseradish peroxidase–conjugated goat anti–mouse Ig antibody and ECL reagent (Amersham Biosciences).

Immunohistochemical Analysis. The pancreas was removed from each mouse and fixed with periodate-lysine-paraformaldehyde. Sections of each sample were probed with biotin-conjugated anti-DCR3 monoclonal antibody (Anawrahta) followed by streptavidin horseradish peroxidase, and then stained with AEC reagent (DakoCytomation) for 10 min. In another experiment, transgenic islet-bearing kidneys were removed from mice at different time points after transplantation and the graft sections were fixed and further stained for DCR3 or insulin (guinea pig anti–porcine insulin; DakoCytomation) as described above.

Assessment of Insulitis and Diabetes. The severity of insulitis was scored on hematoxylin- and eosin-stained sections of pancreas from mice 14–16 wk of age. Investigators were blind to the identity of the sections. At least 50 islets from each pancreas were randomly chosen for microscopic examination. Severity of insulitis was classified as previously described (15). Urine glucose concentration was measured weekly using Chemstrips (Boehringer). Animals that scored positively (>250 mg/dL) on two consecutive tests were classified as diabetic. In some experiments, groups of mice were treated intraperitoneally with 200 mg/kg cyclophosphamide twice at the ages of 8 and 9 wk to accelerate diabetic process, and then the urine glucose concentration was measured every other day for 4 wk.

T Cell Proliferation. Splenocytes from 8-wk-old mice were treated with Tris-buffered ammonium chloride to eliminate the erythrocytes, washed, and resuspended at a cell concentration of 2.5×10^6/ml in RPMI 1640 supplemented with 1% Nutridoma-

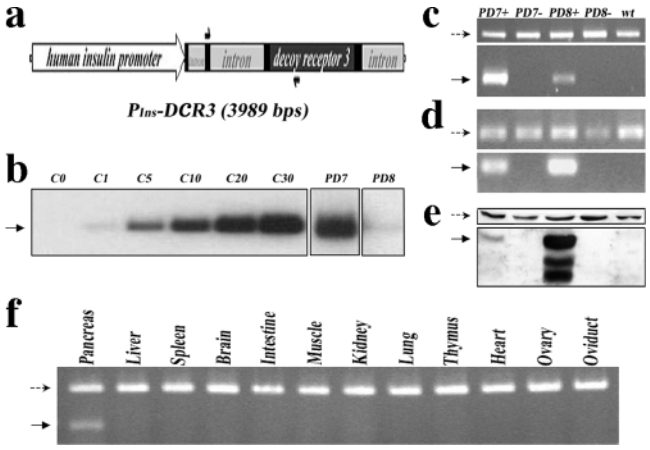

Figure 1. Generation of transgenic mice. (a) Schematic diagram of the whole transgene construct. The black areas represent exons and the gray areas represent introns. The entire first noncoding exon followed by the first intron and 16 bp of the second exon of human *insulin* gene, which are not translated into protein, were preserved to ensure the stringency of the insulin promoter. To enhance the expression of the transgene in this construct, the introns of rabbit *β-globin* gene were introduced adjacent to these. A forward primer located in the second exon of the *insulin* gene (top arrow) and a backward primer in the coding region of *DCR3* (bottom arrow) were designed to determine the transcription of transgene in RT-PCR and to detect the existence of *DCR3* transgene from genomic PCR. (b) Southern blot analysis proved the transgenic *DCR3* signals in PD7 and PD8 founders and provided the relative copy number of each transgenic line, indicated by the solid arrow. (c) The transgene detected by genomic PCR analysis is indicated by the solid arrow and the internal control of *IL-12 p35* is indicated by the dashed arrow. (d) The spliced transcript of transgenic *DCR3* determined by pancreatic RT-PCR is indicated by the solid arrow and internal control of *hgprt* is indicated by the dashed arrow. (e) DCR3 protein was detected by Western blot of pancreatic extracts (solid arrow). Actin signals served as a control (dashed arrow). (f) Tissue-specific expression of the transgene was confirmed by RT-PCR analysis of multiple organs. The positive signal of *DCR3* transcript appeared only in pancreas (solid arrow). *hgprt* transcripts are internal controls (dashed arrow).

SP (Boehringer; reference 16). Cells were stimulated with synthetic GAD peptides (p524–543; reference 17) or control ovalbumin peptide (p323–336). The incorporated [^{3}H]methyl thymidine was detected with TopCount (Packard Instrument Co.). SEM was <15% of the mean.

Adoptive Transfer. Spleens of female transgenic or nontransgenic NOD donors were removed at 13 wk, erythrocytes were depleted with Tris-buffered ammonium chloride, and 2×10^7 cells were injected into 8-wk-old female NOD/SCID via the retro-orbital plexus. Diabetes was assessed as described above. In another experiment, 10-wk-old female transgenic NOD mice or their nontransgenic control littermates were selected as recipients and injected with 2×10^7 cells from 14-wk-old female NOD donors with the same procedure as described above. Diabetes was then followed as described above.

Flow Cytometry. Splenocytes of doubly or triply transgenic mice were stained with anti–mouse CD4 (L3T4, clone RM4-5), anti–mouse CD8α (Ly2, clone 53-6.7), anti–mouse CD45R: B220 (clone RA3-6B2), anti–mouse CD25 (clone PC61), anti–human CD90 (hThy1, clone 5E10), and anti–mouse CD90.1 (mThy1.1, clone OX-7). Flow cytometric analysis was performed with a FACSCalibur™ (BD Biosciences).

Islet Transplantation. Pancreatic islets were isolated from mice at 6 wk by the standard collagenase digestion method (18). Wild-type female NOD mice that spontaneously developed diabetes within 1 wk were collected as recipients. 600 isolated islets were transplanted under the left renal capsule of diabetic recipients. After transplantation, recipients' blood glucose concentration was monitored every day, and the transplantation procedure was considered a success when the nonfasting blood glucose concentration returned to normal (<11.1 mM) on 2 consecutive days. The survival of islets corrects hyperglycemia and the rejection of islets leads to recurrence of diabetes in recipients. The recurrence of diabetes among the recipients was defined as a blood glucose concentration >16.7 mM in two consecutive evaluations. The day of diabetic recurrence was considered the end of islet survival.

Results

Generation of Transgenic Mice. To study the organ-specific protection of DCR3 against autoimmune diabetes, we generated the transgenic mice by injecting DNA construct (Fig. 1 a) into fertilized NOD eggs. This construct contained the insulin promoter, the first and partial second noncoding exons of *insulin*, an intron from rabbit β *globin*, human *DCR3*, and a poly A signal of rabbit β *globin*. The expression of the *DCR3* transgene was controlled by the human insulin promoter (P_{Ins}), which is highly specific to the pancreas and

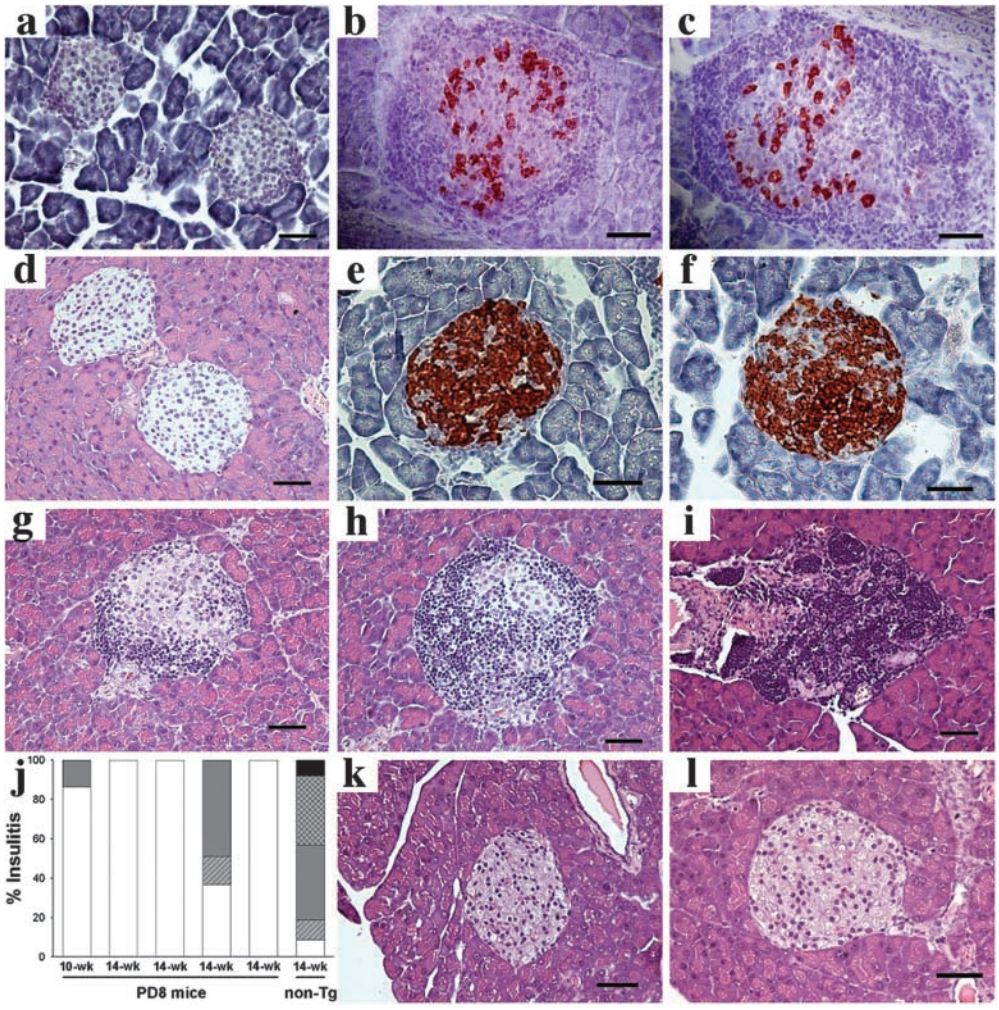

Figure 2. Reverse correlation of transgene expression and severity of insulitis. Immunohistochemical analysis of DCR3 on pancreatic sections was performed on wild-type (a), PD7 (b and c), and PD8 mice (e and f). Differential level of DCR3 expression was observed on islets of PD7 (low expression; b and c) and PD8 mice (high expression; e and f), in agreement with the results of RT-PCR and Western blot analysis shown in Fig. 1. Hematoxylin and eosin staining of islets from PD7 mice (g–i) displayed variable degrees of insulitis at the age of 12 wk, whereas islets of PD8 mice of the same age (k and l) were nearly free of infiltration and maintained their integrity. Cumulative results of insulitis severity were classified and scored (j) as follows: intact islets, without infiltrating cells (open bars); peri-insulitis, <25% of the area of an islet was occupied by infiltrating cells (hatched bars); intra-insulitis, >25 and <50% (gray bars); severe insulitis; >50% (bar with crisscross pattern); and destructive insulitis, whole islet was occupied (solid bar). Data represent five individual PD8 mice (each bar for one transgenic mouse, from left, 1–5) and averaged results from six nontransgenic littermates (right) at the age of 14 wk. Bar, 0.05 mm.

leads to good levels of protein production (19, 20). To determine the transcription of the transgene and to distinguish any possible PCR or RT-PCR products from potential murine homologues of *DCR3*, we designed a forward primer that locates to the second exon of the *insulin* gene (Fig. 1 a, top arrow) and a backward primer that locates to the coding region of *DCR3* (Fig. 1 a, bottom arrow).

Two lines of P_{Ins}-DCR3 transgenic NOD mice, denoted as pancreatic DCR3 (PD)7 and PD8, were obtained within two independent microinjections. Southern blot analysis revealed that PD7 carries ~10–15 and PD8 carries ~1–5 copies of the transgene in their genomes (Fig. 1 b) and that transgenic mice could be distinguished from their nontransgenic littermates by genomic PCR (Fig. 1 c). Although PD7 mice had more copies of the transgene inserted in the genome, they expressed lower levels of both RNA (Fig. 1 d) and protein (Figs. 1 e and 2, b, c, e, and f) than PD8 mice. Transgene-based and islet-specific expression of *DCR3* was further confirmed by RT-PCR (Fig. 1 f) and immunohistochemical staining (Fig. 2, a–c, e, and f). Except for the transgenic pancreas, none of the organs tested (liver, spleen, brain, intestine, muscle, kidney, lung, thymus, heart, ovary, and oviduct) from the transgenic mice transcribed RNA or expressed protein of DCR3, nor did islets from wild-type NOD mice. These results strongly indicate that the expression of DCR3 is strictly transgene based and islet specific.

Transgene Expression and the Disease Process. To investigate the idea that higher expression of transgenic DCR3 may render stronger protection, we compared the severity of insulitis and spontaneous or chemical-induced disease incidence between PD7 and PD8 mice. High expresser PD8 mice expressed transgene in a diffuse and extended manner throughout nearly the entire islet (Fig. 2, e and f). In contrast, PD7 mice secreted DCR3 in a spot-like pattern within limited areas (Fig. 2, b and c). Expression of the transgene in islets significantly ameliorated the severity of insulitis, especially in PD8 mice (Fig. 2 j). Interestingly, mononuclear cell infiltration was lower in areas expressing high levels of DCR3 in transgenic islets than in areas with lower levels of transgene expression. This was consistent with the observation that low expresser PD7 mice still exhibit variable degrees of insulitis (Fig. 2, g–i), whereas high expresser PD8 mice are almost free from insulitis (Fig. 2, j–l). This inverse correlation between DCR3 expression and insulitis severity is supported by previous data showing that DCR3 by itself can counteract FasL-mediated chemotaxis and therefore reduce the infiltration of immune cells in gliomas (3). Moreover, a recent study demonstrated that reverse signaling through LIGHT could also inhibit T cell chemotaxis both in vitro and in vivo (21).

To further investigate the efficacy of the protection of transgenic DCR3 in PD7 and PD8 mice, we compared the incidence of diabetes in spontaneous or chemical-induced disease (Fig. 3). PD7 mice developed less severe diabetes than their nontransgenic littermates ($P < 0.05$), suggesting that weak transgenic expression of DCR3 mediates a certain degree of protection (Fig. 3 a). More importantly, PD8 mice with higher DCR3 expression were completely protected from developing autoimmune diabetes ($P < 0.01$), demonstrating the strong immunoprotective potential of DCR3 in an organ-specific manner (Fig. 3 b). Similarly, transgenic DCR3 protected mice from cyclophosphamide-induced diabetes in a dose-dependent manner (Fig. 3 c). After two shots of cyclophosphamide injection (200 mg/kg) at the ages of 8 and 9 wk, all control NOD mice became diabetic within 3 wk, whereas only 50% of PD7 ($P < 0.05$) and none of the PD8 mice ($P < 0.01$) became dia-

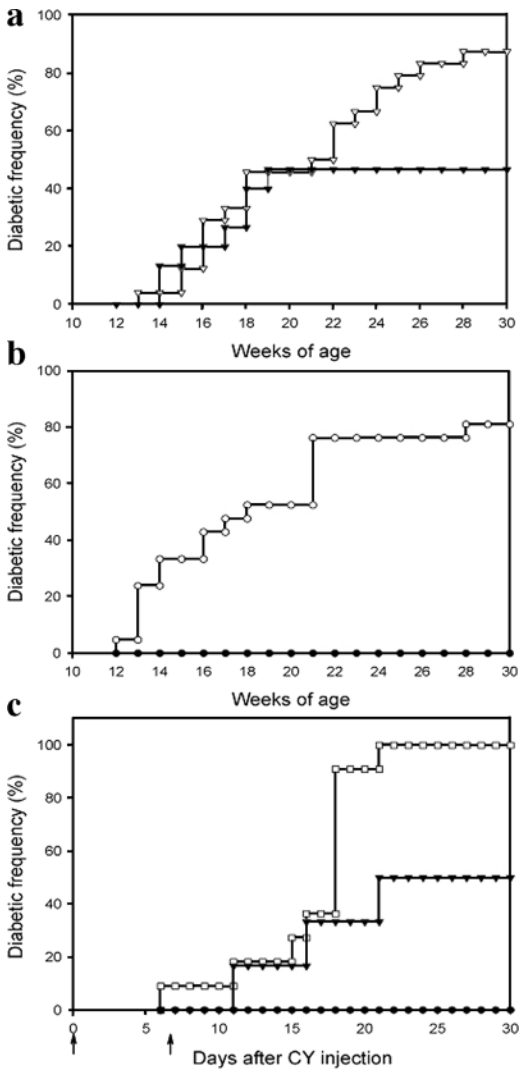

Figure 3. Spontaneous and chemical-induced diabetes in transgenic mice. Female transgenic mice and their control littermates were monitored by weekly measurements of glycosuria and blood sugar for spontaneous diabetic incidence (a and b). There were 18 PD7 mice (▼) and 24 nontransgenic littermates (▽). a, Kaplan-Meier method, $P < 0.05$. There were 22 PD8 mice (●) and 21 control littermates (○). b, $P < 0.01$. Frequency of chemical-induced diabetes by two shots of cyclophosphamide (indicated by arrows at days 0 and 7) was monitored in PD7 (▼, $n = 12$, $P < 0.05$), PD8 (●, $n = 12$, $P < 0.01$), and nontransgenic control mice (□, $n = 16$).

betic. We further observed that none of the PD8 mice, regardless of sex, developed diabetes at any age thus far studied, even those older than 70 wk or throughout pregnancy and several breeding cycles (unpublished data). Our results indicate that sufficient expression of DCR3 strongly protects islets from autoimmune or chemical-induced destruction and completely prevents development of diabetes. The difference in protection between PD7 and PD8 mice does not arise simply by random integration of transgenes because the protective efficacy between these lines displays a dose-dependent manner and correlates with the quantity of transgenic DCR3 expression in islets.

Diabetogenic Properties of T Cells from Transgenic Mice. Our data showed that transgenic DCR3 in PD8 mice completely protected them from either spontaneous or cyclophosphamide-induced diabetes. To investigate whether the protective mechanism works through down-regulation of diabetogenic T cells, we examined the GAD65-specific T cell response in PD8 mice. GAD65 is one of the key autoantigens in insulin-dependent diabetes mellitus (IDDM). 8-wk-old PD8 mice exhibited levels of T cell proliferative responses to synthetic GAD65 peptide (524–543) similar to those observed in age-matched control NOD mice (Fig. 4 a). This result indicates that PD8 mice protected from autoimmune diabetes retain a significant autoantigen-specific T cell response. To further examine the diabetogenic ability of PD8 lymphocytes, we performed an adoptive transfer experiment in the NOD/SCID system. We isolated lymphocytes from PD8 mice or their nontransgenic littermates at the age of 13 wk and transferred these cells into NOD/SCID mice. The two groups of recipients developed diabetes with similar onset, diabetic process, and severity (Fig. 4 b). This result clearly indicates that DCR3 most likely protects islets in situ instead of down-regulating the diabetogenicity of lymphocytes by systematically altering T cell function in PD8 mice. To further investigate this point, we performed another adoptive transfer experiment. Splenocytes from female diabetic NOD mice were transferred into DCR3 transgenic mice or their control littermates. 4 of 6 control recipients became diabetic within 3 wk after transfer, whereas none of the 11 transgenic recipients developed diabetes ($P < 0.05$; Fig. 4 c). This result further confirms that expression of *DCR3* transgene in situ effectively protects islets from destruction by diabetogenic splenocytes. This organ-specific modulation is further supported by our recent findings that soluble DCR3 is barely detectable in the circulation of transgenic mice (unpublished data).

Development of Th1 and Treg Cells in Transgenic Mice. An imbalance between Th1 and Th2 responses predisposes NOD mice to developing autoimmune diabetes (22, 23). To investigate whether down-regulation of Th1 effector cells or induction of Th2 cells protects against diabetes in PD8 mice, we crossed PD8 transgenic mice with T1 and T2 doubly transgenic mice, and monitored the development of Th1 or Th2 cells in these triply transgenic NOD mice. Originally from a BALB/c background, T1 and T2 doubly transgenic mice bear two transgenes, which express

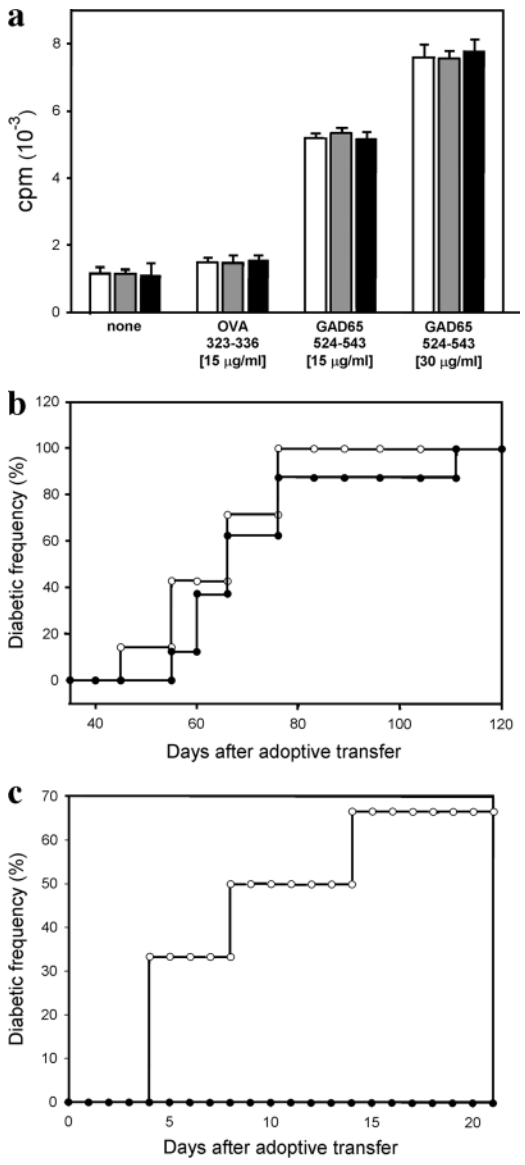

Figure 4. Diabetogenic properties of T cells from transgenic mice. (a) GAD65-specific T cell response in PD8 mice. Splenocytes from 8-wk-old female PD8 (two individual mice are indicated as gray and solid bars) and nontransgenic control (open bars) mice were stimulated with GAD65 antigenic peptide p524–543 or control peptide ovalbumin p323–336 in vitro. The incorporated [^{3}H]methyl thymidine was detected with TopCount. SEM was <15% of the mean. (b) Adoptive transfer of lymphocytes of PD8 mice. Splenocytes from 13-wk-old female PD8 mice (●) or their nontransgenic littermates (○) were intravenously transferred into NOD/SCID mice. Recipients were monitored for diabetes by weekly measurements of glycosuria and blood sugar. The number of recipients for PD8 and control mice was 10 and 8, respectively. (c) Protection from diabetogenic lymphocyte-transferred disease. Splenocytes from 14-wk-old female diabetic NOD donors were intravenously transferred into 10-wk-old female transgenic NOD mice (●) or their nontransgenic control littermates (○). Recipients were monitored for diabetes every other day by measurements of glycosuria and blood sugar. The number of transgenic recipients and control mice was 11 and 6, respectively.

two distinct cell surface markers: one human Thy1 transgene (hThy1) under control of the murine *IFN-γ* promoter, and one murine Thy1.1 (mThy1.1) under control

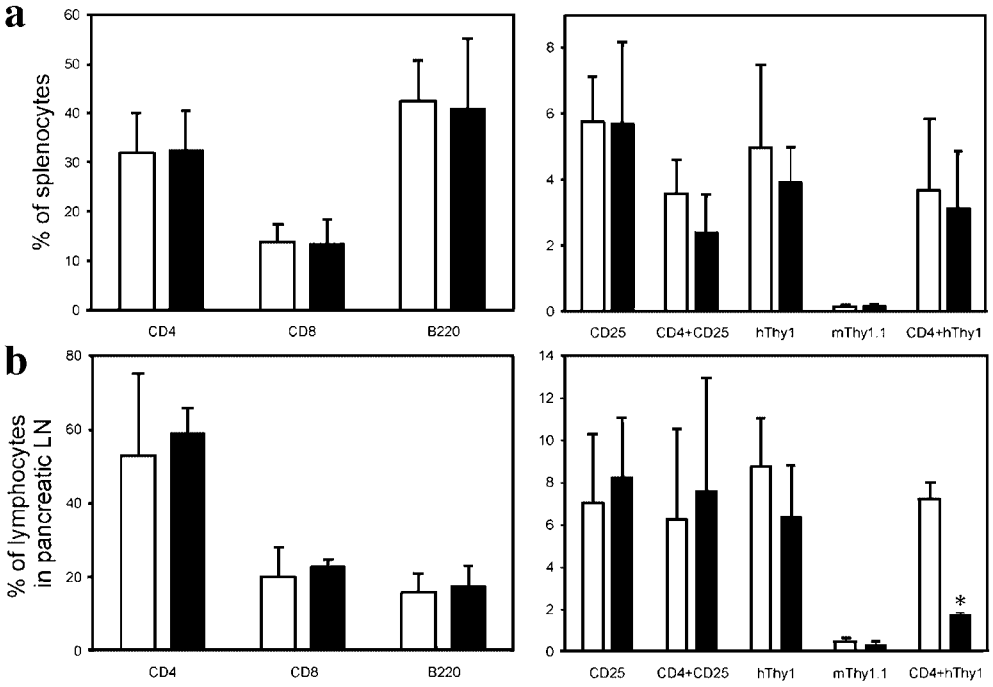

Figure 5. Development of Th1 and Treg cells in transgenic mice. The composition of lymphocytes in spleen (a, left) and pancreatic lymph node (b, left) was analyzed by flow cytometry from 10–14-wk-old mice (triply transgenic mice are indicated with the solid bars, and control mice are indicated with the open bars). Th1 ($CD4^+$ $hThy1^+$) and Treg ($CD4^+$ $CD25^+$) cells in spleen (a, right) and pancreatic lymph node (b, right) were further analyzed from triply (solid bars) or doubly (open bars) transgenic mice. Data represent five independent experiments and standard errors were calculated.

of the murine *IL-4* promoter. We designated these as T1 and T2 transgenes, respectively (24). These doubly transgenic mice were backcrossed to NOD mice for more than 12 generations, which provided the best model to study the differentiation of helper or cytotoxic T cell subsets during the diabetogenic process in IDDM. In the spleen or pancreatic lymph node, the absolute cell number and lymphocyte subpopulation distribution were similar in regular and triply transgenic NOD mice, indicating that transgenic DCR3 in islets does not affect lymphocyte development in peripheral lymphoid organs (Fig. 5, a and b, left). Interestingly, the number and percentage of IFN-γ–producing ($hThy1^+$) or IL-4–producing ($mThy1.1^+$) cells of spleen or pancreatic lymph node in triply transgenic mice were not different from those observed in doubly transgenic mice (Fig. 5, a and b, right), suggesting that transgenic DCR3 in islets does not suppress the development of systemic IFN-γ–producing or induce IL-4–producing cells in mice. However, the pancreatic lymph node, but not the spleen, of triply transgenic mice had significantly fewer Th1 cells ($CD4^+$ $hThy1^+$) than that of doubly transgenic mice. This result further confirms the essential role of Th1 cells in IDDM. These data also suggest that Th1 cell number in pancreatic lymph node in DCR3 transgenic mice declines because of significantly reduced infiltration of mononuclear cells in nearby islets. The similar populations of Th1 cells in spleens in triply and doubly transgenic mice further suggest that DCR3-mediated protection is more likely to occur through modulating local rather than systemic effects.

NOD mice have a relative deficiency of $CD4^+$ $CD25^+$ Tregs, which could cause an inability to maintain peripheral tolerance (25). To investigate whether transgenic DCR3 mice can generate these regulatory cells and protect PD8 mice from autoimmune diabetes, we compared the number and percentage of $CD4^+$ $CD25^+$ cells in transgenic and control mice. PD8 transgenic mice and their nontransgenic littermates exhibited similar numbers and percentages of $CD4^+$ $CD25^+$ cells in the spleen and pancreatic lymph node (Fig. 5, a and b, right), suggesting that the protective effect of DCR3 is not likely to be mediated by inducing the population of Tregs.

Transplantation of Transgenic Islets. DCR3 protein can ameliorate the rejection response to a mouse heart allograft and reduce the primary nonfunction of an islet isograft (26). A stringent way to test whether overexpression of DCR3 could protect β cells against immune attack is to examine whether transgenic islet grafts could reverse diabetes in spontaneously diabetic mice. We isolated islets from PD8 mice and transplanted them into kidney capsules of diabetic NOD recipients. DCR3-secreting islets had a higher rate of successful transplantation and survived for longer than wild-type islets (9.1 and 3.1 d, respectively; $P < 0.05$; Table I). To further investigate whether transplantation processes disturb the transgene expression and how long transgenic islets continue to express DCR3, we examined the production of DCR3 in graft islets by immunohistochemical staining. Transgenic islet grafts from mice at day 6 after transplantation still expressed DCR3 with preservation of islet architecture (Fig. 6 a). However, as compared with pancreatic islets in PD8 mice, islet grafts from transplanted mice showed a different expression pattern. In contrast to a diffuse and extended manner throughout nearly the entire pancreatic islet (Fig. 2, e and f), DCR3 expression in islet graft was significantly diminished and limited in central area (Fig. 6 a). Interestingly, the area of DCR3-producing cells was almost confined to that of insulin-secreting cells (Fig. 6 b), suggesting a coexpression of these two proteins in graft islets. However, these transgenic islets were destroyed by autoimmune

Table I. *Transgenic DCR3 Enhances the Success Rate and Prolongs the Survival Period of Grafts in Isogenic Islet Transplantation*

Donor	PD8 mice	Control mice	p-value
Number of recipients	20	16	—
Number of successes	13	6	—
Success rate	65%	38%	—
Mean survival period (days)	9.1	3.1	<0.05

Pancreatic islets isolated from 6-wk-old mice were transplanted into recipients that were newly diagnosed with diabetes. Recipients' blood glucose concentration was monitored every day and the transplantation procedure was considered a success when the nonfasting blood glucose concentration returned to normal (<11.1 mM) for 2 consecutive days after transplantation. The recurrence of diabetes among the recipients was defined as a recipient blood glucose concentration >16.7 mM in two consecutive evaluations. The day of diabetic recurrence was considered the end of islet survival.

attack after longer days. Islet grafts removed from mice 20 d after transplantation showed a massive mononuclear cell infiltration with disrupted islet architecture (Fig. 6 c). In summary, expression of transgenic DCR3 was potentially effective in prolonging islet graft survival, but did not provide permanent protection from diabetes recurrence.

Discussion

The biological function of DCR3 is still being evaluated. Its natural ligands include FasL (1), LIGHT (4), TL1A (5), and unknown molecules present on certain antigen-presenting cells such as dendritic cells and macrophages (6, 7). Studies have increasingly focused on the best-characterized function: the ability of DCR3 to counteract FasL/LIGHT-mediated apoptosis (1, 4) and block FasL-mediated chemotaxis (3, 21). Our data are the first to demonstrate that islet-restricted expression of DCR3 in NOD mice protects them from chemical-induced or autoimmune diabetes. This organ-specific modulation does not appear to affect systemic T cell development and function. Moreover, the transgenic islets had a higher transplantation success rate and survived for longer than control islets, suggesting a potential of DCR3 in organ transplantation.

The role of Fas in autoimmune diabetes is still controversial. Both NOD-*lpr/lpr* (deficient in Fas; reference 27) and NOD-*gld/gld* (deficient in FasL; reference 10) mice are free from insulitis and diabetes, suggesting a pathogenic role of Fas in β cell destruction. However, the difficulty in detecting Fas on the surface of β cells in NOD mice complicates understanding the role of Fas in autoimmune diabetes (28). One report demonstrated that the expression of Fas on β cells was induced by lymphocytes infiltrating into islets, supporting its pathogenic role in autoimmune diabetes (9). Another more recent study using rat insulin promoter–driving dominant negative Fas transgenic NOD mice revealed that early interference in the Fas pathway of the disease process could retard or even prevent autoimmune diabetes (29). These data indicate that although β cells do not physiologically express Fas, the Fas-mediated pathway still plays an initial and subsequent role in β cell destruction when triggered by the autoimmune diabetogenic process. Moreover, transgenic expression (11) or treatment with LTβR–Ig fusion protein (12), which blocks the native interaction between LTβR and LIGHT, could also inhibit the diabetic process in NOD mice. The observation that P_{Ins}-DCR3 transgenic NOD mice exhibit the same phenotype supports this concept. Our data suggest two possible mechanisms underlying DCR3-mediated protection. First, islet-specific expression of DCR3 may neutralize chemotaxis induced by soluble FasL, which is secreted by mononuclear cells infiltrating into islets in situ. Therefore, we observed significant depression of the insulitis score in PD8 mice. Second, DCR3 may counteract β cell apoptosis directly caused by FasL- or LIGHT-bearing cells that infiltrate into islets, thereby protecting the integrity and function of islets.

Although NOD-*lpr/lpr* mice are completely protected from insulitis and diabetes, islets from these mice survive only slightly longer than wild-type islets after transplantation into diabetic recipients (28). This indicates that other killing mechanisms, such as cytokine-induced apoptosis (30), perforin-mediated cytotoxity (31), or elevated oxidative stress (20, 32), may damage islet grafts in diabetic recipients. Interestingly, our transplantation results revealed that DCR3-secreting islets were maintained without any treatment after surgery, indicating that in contrast to Fas-deficient islets, DCR3 protects islets and prolongs graft sur-

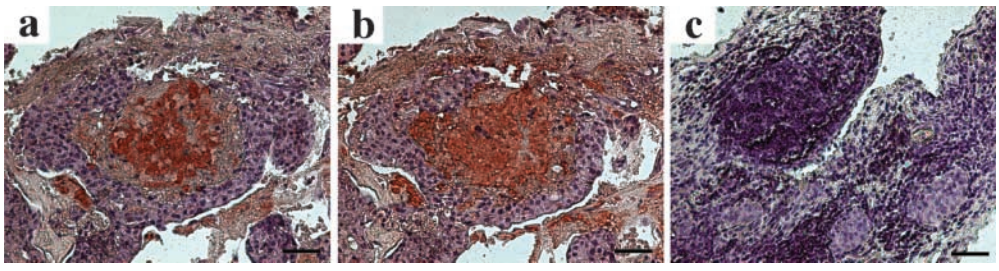

Figure 6. Transgene expression in graft islets. (a) DCR3 expression in early stage after transplantation. Transgenic islet graft-bearing kidneys were removed from mice 6 d after transplantation and immunohistochemically stained for DCR3. (b) Insulin expression in early stage after transplantation. Transgenic islet graft-bearing kidneys were removed from mice 6 d after transplantation and immunohistochemically stained for insulin. (c) Transgene expression in late stage after transplantation. Transgenic islet graft-bearing kidneys were removed from mice 20 d after transplantation and immunohistochemically stained for DCR3 and insulin. Bar, 0.05 mm.

vival from active immune attack in diabetic recipients. We suggest that this results from the secreting function of DCR3 or the net effect of its biological functions other than the FasL- or LIGHT-neutralizing property because DCR3 modulates the local or systemic immune responses by interacting with FasL, LIGHT, TL1A, and other unknown ligands. It is also important that Fas-independent pathways contribute to autoimmune destruction and eventually eliminate both Fas-deficient (28) and DCR3-secreting islet grafts. In our transplantation model, the failure of long-term graft survival correlated with the reduction of DCR3 expression. This may imply two possibilities. One possible explanation might be that *insulin* promoter–driving expression of DCR3 was disturbed by the primary nonfunction of transplanted islets. Another possible explanation is that transplantation processes trigger strong oxidative stress- or cytokine-induced apoptosis of transplanted islets, which cannot be blocked by DCR3. Nevertheless, our data still provide a new aspect for designing preventive and therapeutic approaches to islets or other transplants, which escape from autogenic, allogenic, or even xenogenic immune responses.

Unlike islet-specific expression of TGF-β in transgenic NOD mice, which results in smaller-sized islets (33, 34), DCR3 does not alter the nature and integrity of islets. Both cases described above can effectively protect these mice from autoimmune diabetes, however, lymphocytes from TGF-β transgenic mice are less able to induce diabetes after transfer into NOD/SCID mice. Therefore, the protective effects of TGF-β tend to influence the diabetogenicity of lymphocytes rather than local events. Recent studies of DCR3 demonstrate that the native form of this protein does not exist in the mouse circulation for long enough to bind FasL (35, 36), supporting our conclusion that the DCR3-mediated protection is mainly a local effect. This is consistent with our inability to detect soluble DCR3 in sera from PD8 mice by sandwich ELISA, although we cannot completely rule out the existence of undetectable amounts of DCR3 in the circulation.

Our experiments demonstrate that systemic T cell phenotype and function are maintained in DCR3 transgenic mice. The specific T cell properties maintained include T cell subpopulations, Th1 or Treg cell development, autoantigen-specific immune response, and diabetes transfer ability. We believe that the decrease in the Th1 population in pancreatic lymph nodes is an outcome of reduced insulitis rather than a causal factor that mediates protection. Our results indicate that transgenic DCR3 most likely protects islets in situ rather than by influencing the polarizations of immune responses or induction of Tregs through the circulation. Our data suggest that graft-restricted expression of DCR3 may provide additional advantages without disturbing the immune system in recipients.

The mouse homologue of human DCR3 has not yet been identified. Our experiments explored the biological significance and potential application of DCR3 in immune evasion, which occurs in tumor cells and applies to the tissue-specific, autoimmune attack model. DCR3 has other biological effects besides escape from the immune system. In the human and mouse, DCR3 participates in other immune regulatory functions, such as modulation of dendritic cell and macrophage functions through unknown ligands. We believe that the biological function and physiological role of DCR3 might be more extensive than currently known. Further understanding of the biological function of DCR3 may contribute to development of therapeutic strategies in immune or other diseases.

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