Bone Marrow Transplantation as a Strategy for Treatment of Non–Insulin-dependent Diabetes Mellitus in KK-Ay Mice

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Summary

The effects of allogeneic bone marrow transplantation (BMT) on non–insulin-dependent diabetes mellitus (NIDDM) were examined using KK-Ay mice. KK-Ay mice reconstituted with KK-Ay bone marrow cells showed glycosuria, hyperinsulinemia, and hyperlipidemia. However, KK-Ay mice (H-2^b) that had been lethally irradiated (9.0 Gy) and then reconstituted with T cell–depleted bone marrow cells from normal BALB/c mice (H-2^d) showed negative urine sugar with decreases in serum insulin and lipid levels 4 mo after BMT. Morphological recovery of islets and glomeruli was also noted after allogeneic BMT. These findings suggest that BMT can be used to treat not only a certain type of NIDDM but also its complications such as hyperlipidemia and diabetic nephropathy.

Based on the degree of risk of patients developing ketoacidosis in the absence of insulin, diabetes mellitus has been classified into two types (type I, insulin-dependent diabetes mellitus [IDDM]; and type II, non–insulin-dependent diabetes mellitus [NIDDM]). Using an animal model for IDDM (NOD mouse), we have demonstrated that allogeneic bone marrow transplantation (BMT) can be used to prevent IDDM (1), and that the combination of BMT and pancreas grafts can be used as a treatment (2).

The etiology of NIDDM is an enigma, since various abnormalities (obesity, glucose intolerance, hyperinsulinemia and peripheral insulin resistance, hyperlipidemia, the deposit of amyloid polypeptides in the islets, the production of insulin receptor tyrosine kinase inhibitors, etc.) can be found in patients with NIDDM (3).

In this report, we examine the effects of allogeneic BMT on NIDDM in KK-Ay (H-2^b) mice, which are considered to be the animal model for NIDDM (4–10). The KK mouse is an inbred strain established by Kondo et al. (4) from native Japanese mice in 1957. Several abnormalities have been found in this mouse strain, including impaired glucose tolerance, hyperglycemia, insulin resistance of peripheral tissue, hyperinsulinemia, and glomerular changes (5). Although KK mice develop NIDDM, the appearance of glycosuria is influenced by age, sex, and season. In 1969, Nishimura (6) introduced the yellow obese gene of Ay mice into the KK mice by repeatedly crossing these two strains (6). The congenic mice established by this procedure have been termed "yellow KK" or

"KK-Ay" mice. These mice show no sexual or seasonal variation in the appearance of glycosuria, but show hyperglycemia and hyperinsulinemia at the age of 5 wk. Degranulation and glycogen infiltration of β cells are first observed at this age, followed by hypertrophy and central cavitation of islets (7). Renal glomerular changes, which are similar to the diffuse or exudative type of glomerular sclerosis in human diabetes, were also recognized at the age of 16 wk (8). Insulin sensitivity of adipose tissue decreased with age and completely disappeared by the age of 16 wk (9, 10). In this paper, we show that allogenic BMT leads to treating not only NIDDM in KK-Ay mice but also its complications, such as hyperlipidemia and diabetic nephropathy.

Materials and Methods

Mice. BALB/c, C57BL/6J (B6), and KK-Ay mice were obtained from CLEA Japan (Osaka).

Bone Marrow Transplantation. Lethally irradiated (9 Gy) KK-Ay mice were reconstituted with intravenous injection of 2 × 10^7 T cell–depleted bone marrow cells from 8-wk-old BALB/c (in allogeneic group) and 8-wk-old KK-Ay (in syngeneic group) mice. In the allogeneic group, 19-wk-old KK-Ay mice with glycosuria (+ + + +) were used as recipients, whereas in the syngeneic group, 8-wk-old KK-Ay mice without glycosuria were used as recipients. The bone marrow cells collected from tibias and femurs of donor mice were pretreated with anti-Thy-1.2 mAb (F7D5; Olac, Bicester, England) plus rabbit complement to remove T cells. All mice were given tetracycline (2 mg/ml) in acidified drinking water (pH 2.7) for 2 wk after cell transfer.

Glucose Tolerance Tests (GTTs). Peripheral blood was collected from mice in allogeneic and syngeneic groups using heparinized PCV tubes (Monoject, St. Louis, MO). The levels of fasting blood sugar levels in each group were measured 16 h after starting fasting using test tapes (Lilly, Indianapolis, IN) and a dextrometer. The mice were intraperitoneally injected with glucose (0.5 g/kg), and the blood sugar levels were then measured at 30, 60, and 120 min.

Renal Biopsy. Mice were anesthetized with sodium pentobarbital (0.05 mg/g body weight; Somnopentyl; Pitman-Moore, Inc., Washington Crossing, NJ). After laparotomy, small pieces of the renal cortex were obtained and used for immunofluorescence (IF) and light microscopical studies.

Histological Studies. Pancreases were removed for histological study. They were fixed with 10% formalin, embedded in paraffin, and sectioned. Aldehyde fuchsin staining was carried out. Immunohistochemical staining of β cells using the antiinsulin antibody (Dako Corp., Santa Barbara, CA, USA) was also carried out, as previously described (2).

Measurement of Insulin and Lipid Levels. Immunoreactive insulin was measured using a radioimmunoassay with the polyethylene glycol method, as previously described (11). Serum cholesterol and triglyceride levels were measured using an autoanalyzer (Hitachi, Tokyo, Japan).

All experiments were performed more than three times. Each experiment consists of more than five mice. Because reproducible results were obtained, only representative data are shown.

Results and Discussion

Nontreated KK-Ay mice showed age-dependent impaired glucose tolerance in contrast to BALB/c and (BALB/c→ C57BL/6J) mice (Fig. 1). We performed allogeneic BMT on 19-wk-old KK-Ay (H-2^b) mice when they showed glycosuria (+ + + +); T cell–depleted BALB/c bone marrow cells (2 × 10^7) were injected intravenously through the tail veins of lethally irradiated (9 Gy) KK-Ay mice 1 d after irradiation. As a control group, urine sugar–negative 8-wk-old female KK-Ay mice were lethally irradiated (9 Gy) and then recon-
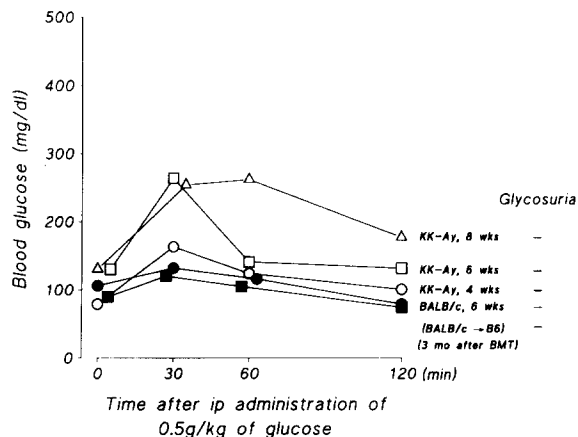
stituted with 2 × 10^7 bone marrow cells of age- and sex-matched KK-Ay mice. Body weight, food and water consumption, and urine sugar were monitored daily. All mice in both groups that received BMT showed negative urine sugar 1 wk after BMT. Food and water consumption were similar, and a weight loss of ∼10% was observed in both groups 2 wk after BMT. (KK-Ay→KK-Ay) mice showed a gradual appearance of glycosuria from 4 wk after BMT and developed urine sugar (+ + + +) 8 wk after BMT (16 wk of age), whereas (BALB/c→KK-Ay) mice continued to show negative urine sugar. We performed intraperitoneal GTTs monthly on both groups, starting 1 mo after BMT in (BALB/c→KK-Ay) mice, and 2 mo after BMT in (KK-Ay→KK-Ay) mice. Fig. 2 shows the significant difference between these two groups in the glucose tolerance curve. (BALB/c→KK-Ay) mice showed negative urine sugar and a normal glucose tolerance curve even 4 mo after BMT. FACS® analyses, using the spleen cells of (BALB/c→KK-Ay) mice, revealed that donor (H-2^d)-derived cells had become dominant (>95%). In contrast, glucose tolerance was abnormal and urine sugar positive in the (KK-Ay→KK-Ay) mice 2 mo after BMT.

Significant histological differences were also noted between the two groups. In aldehyde fuchsin staining, the (KK-Ay→KK-Ay) mice showed hyperplastic islets with degenerated β cells (Fig. 3 *A, d*), whereas the (BALB/c→KK-Ay) mice showed hyperplastic islets with active β cells (Fig. 3 *A, b*). The islet cells in the (KK-Ay→KK-Ay) mice showed the same morphology as those of untreated KK-Ay mice (Fig. 3 *A, c*). When the β cells were stained with antiinsulin antibodies (Dako Corp.) the (BALB/c→KK-Ay) mice showed strongly positive staining (Fig. 3 *B, b*), as seen in the control BALB/c mice (Fig. 3 *B, a*). The staining patterns in the (KK-Ay→KK-Ay) mice (Fig. 3 *B, d*) were, however, almost the same in the untreated KK-Ay mice (Fig. 3 *B, c*). These findings may be explained as follows: NIDDM results from an imbalance between insulin secretion and sensitivity. The earliest detectable abnormality in NIDDM is an impairment of the
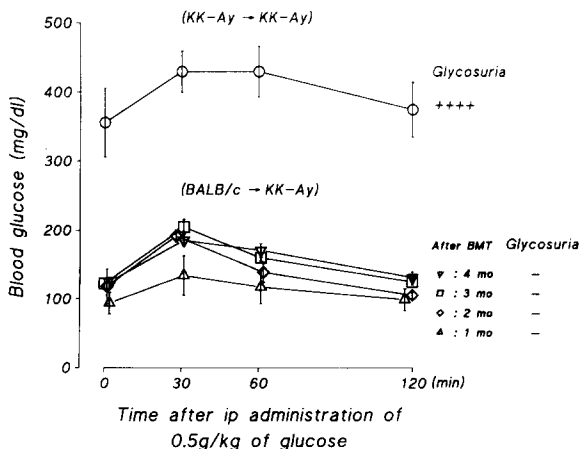

Figure 1. GTTs in normal BALB/c, (BALB→C57BL/6J), and different-aged nontreated KK-Ay mice: BALB/c (●), (BALB/c→C57BL/6J) (■), 4-wk-old KK-Ay (○), 6-wk-old KK-Ay (□), 8-wk-old KK-Ay (△). The fasting blood sugar levels were measured 16 h after starting fasting, the mice were intraperitoneally injected with glucose (0.5 g/kg), and the blood sugar levels were then measured after 30, 60, and 120 min. Because each mouse showed similar GTT patterns, only representative data are shown.

Figure 2. GTTs in (KK-Ay→KK-Ay) and (BALB/c→KK-Ay) mice; syngeneic group (○) and allogeneic group; 1 mo (△), 2 mo (◇), 3 mo (□), and 4 mo (▽) after BMT.
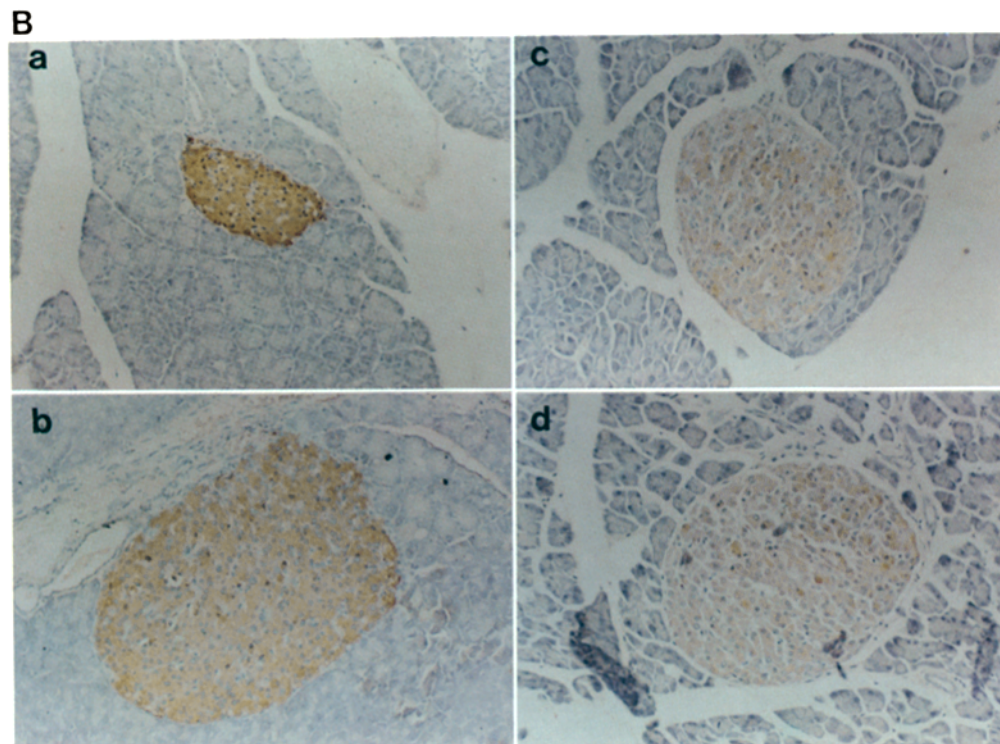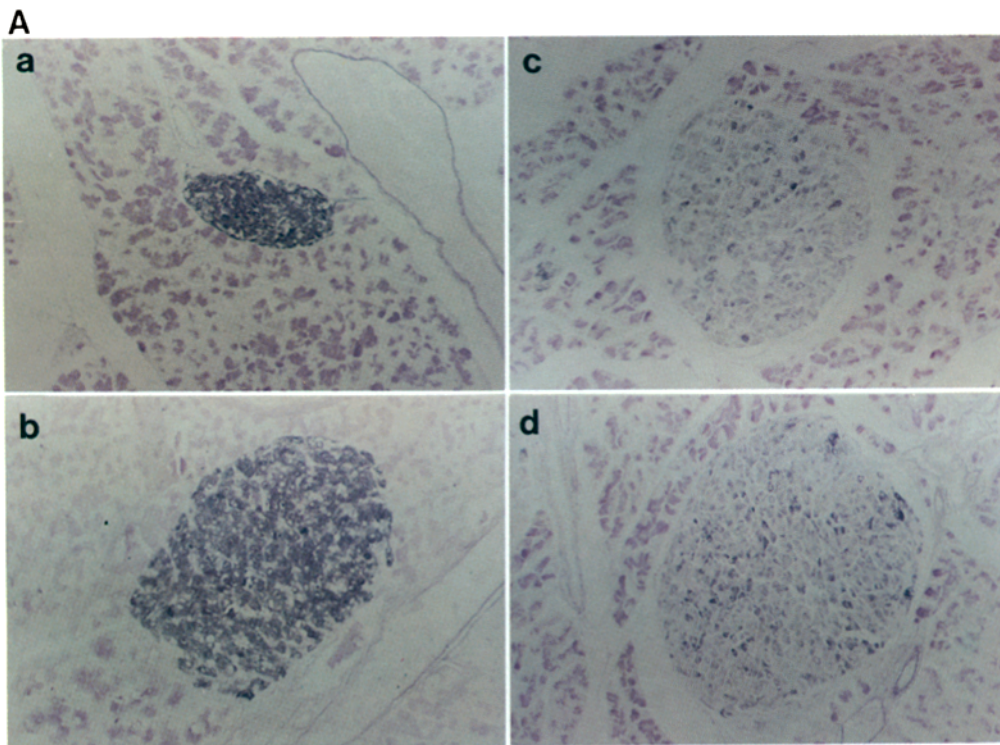
Figure 3. (A) Aldehyde fuchsin stainings of β cells. Morphological similarities are noted in the islets between (a) BALB/c and (b) allogeneic group, or between (c) untreated mice and (d) syngeneic group. (B) Immunohistochemical stainings for insulin. Staining patterns are similar to those in A.

body's ability to respond to insulin. Therefore, the islets show hyperplasia to secrete insulin. However, once the insulin levels increase in the sera, the β cells do not need to secrete as much insulin. Thus, the pancreatic islets of nontreated KK-Ay or (KK-Ay→KK-Ay) mice show hyperplasia but less insulin staining than normal BALB/c or (BALB/c→KK-Ay) mice.

This finding may alternatively be explained by the exhaustion that results from the excess secretion of insulin.

Serum lipid and insulin levels were examined before and after BMT. As shown in Table 1, significant differences were noted between the two groups; serum lipid and insulin levels decreased after allogeneic BMT. The levels were between 4-

Table 1. *Serum Insulin and Lipid Levels*

Mice	Treatment (age)	Insulin	Cholesterol	Triglyceride
		ng/ml	*mg/dl*	*mg/dl*
BALB/c	Nontreated (16 wk old)	0.463 ± 0.313	72.33 ± 18.29	17.33 ± 16.9
KK-Ay	Nontreated (4 wk old)	3.40 ± 0.53	85.00 ± 11.41	18.25 ± 28.97
	Nontreated (6 wk old)	15.76 ± 14.67	110.00 ± 11.53	287.00 ± 58.95
(KK-Ay→KK-Ay)	Before BMT (8 wk old)	43.201 ± 8.150	ND	ND
	8 wk after BMT (16 wk old)	81.157 ± 10.636	111.0 ± 12.72	207.0 ± 17.66
(BALB/c→KK-Ay)	Before BMT (19 wk old)	50.924 ± 25.597	169.25 ± 26.32	273.5 ± 26.75
	4 mo after BMT (9 mo old)	7.727 ± 4.088*	84.25 ± 6.02*	160.75 ± 19.01*

Immunoreactive insulin was measured using a radioimmunoassay with the polyethylene glycol method, as previously described (11). The data are expressed as the mean ± SD of 10 mice.
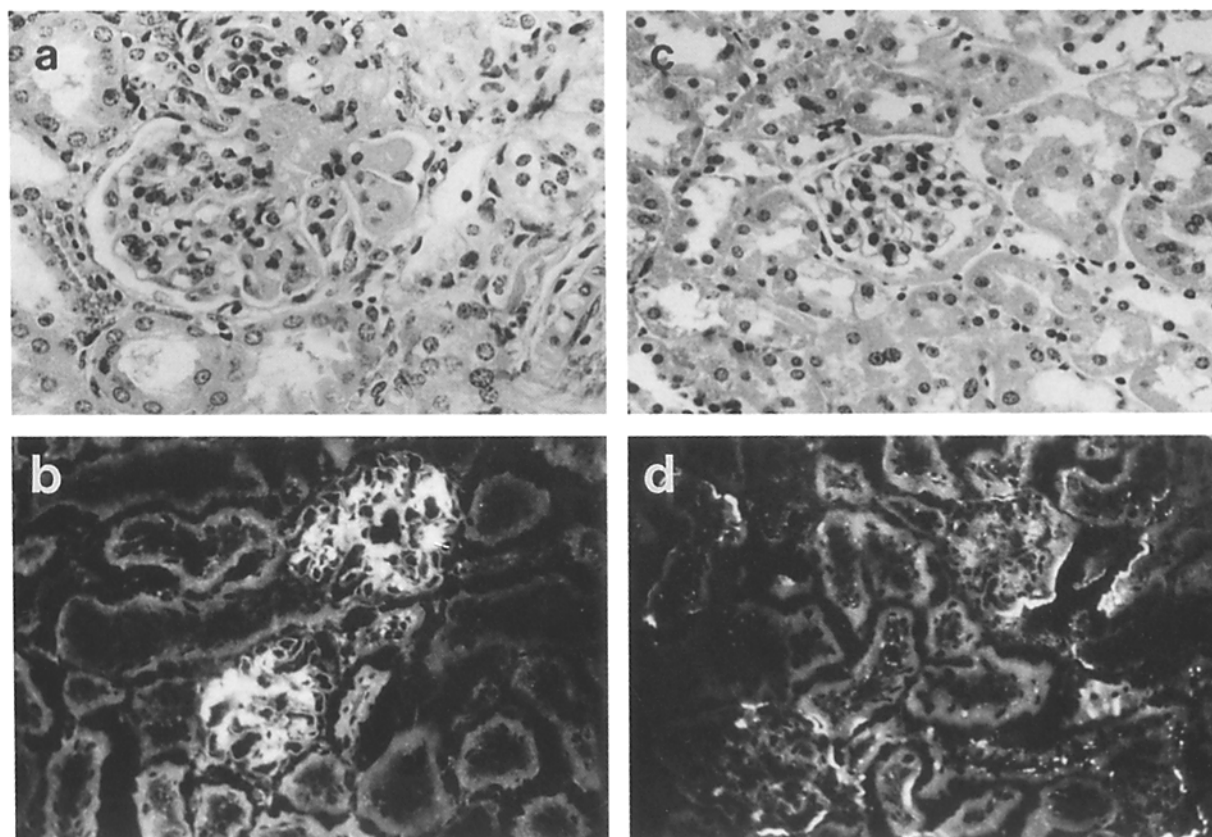* $p < 0.01$ vs. the date of (BALB/c→KK-Ay) mice before BMT.

Figure 4. Histopathological and immunofluorescence microscopical findings in the glomeruli of (BALB/c→KK-Ay) mice before and after BMT. Before BMT: (a) the glomerulus of a KK-Ay mouse (19 wk old) shows a diffuse increase in mesangial matrix as well as definite mesangial nodules (H-E staining, ×268); and (b) IgG deposits are noted in the glomerular basement membranes and mesangial matrix (IF staining, ×134). 4 mo after BMT: (c) the glomerulus of a KK-Ay mouse reconstituted with BALB/c bone marrow cells shows normal appearance (H-E staining, ×268); and (d) no IgG deposits are noted in the glomerulus of the (BALB/c→KK-Ay) chimeric mouse (IF staining, ×134).

and 6-wk-old nontreated KK-Ay mice, but not as low as those of normal BALB/c mice, since the islets still show hyperplasia. This suggests that hyperlipidemia and hyperinsulinemia in NIDDM are secondary to insulin resistance.

Glomerular changes before and after BMT were also examined in the two groups. Before BMT, the kidneys of KK-Ay mice showed a global and diffuse increase in the mesangial matrix (Fig. 4 a), and a linear distribution of IgG along the basement membranes of glomerular capillaries was noted (Fig. 4 b). However, after BMT, the (BALB/c→KK-Ay) mice showed normal appearance in the H-E staining (Fig. 4 c), and the IgG deposits had completely disappeared (Fig. 4 d). The (KK-Ay→KK-Ay) mice, however, showed no changes (data not shown).

In this paper, we have demonstrated that BMT can be used to treat NIDDM in KK-Ay mice without pancreas grafts. It should be noted that hyperlipidemia, hyperinsulinemia, and diabetic nephropathy as complications of diabetes can be corrected by BMT. The possibility of a cachectic effect induced by BMT on reduced serum lipid and insulin levels can be excluded, since (KK-Ay→KK-Ay) mice showed glycosuria, hyperlipidemia, and hyperinsulinemia, and there was no statistical difference in body weight between (KK-Ay→KK-Ay) and (BALB/c→KK-Ay) mice. The other possibility, that chronic GVHR reduced the serum lipid and insulin levels in the allogeneic group, can also be excluded, since neither macroscopical wasting syndrome nor microscopical features of chronic GVHR could be seen in any organs in the allogenic group. In humans, Flier et al. (12) have found the presence of antiinsulin receptor antibodies in some cases of NIDDM with insulin resistance. Since we have demonstrated that autoantibodies, such as anti-DNA antibodies, rheumatoid factors, and antiplatelet antibodies, are reduced after BMT (13–15), the most plausible explanation is that BMT suppresses the production of the antiinsulin receptor antibodies in KK-Ay mice, if antiinsulin receptor antibodies are present in KK-Ay mice. We examined this possibility using an assay method for detecting human anti-insulin receptor antibodies (11), since there is as yet no appropriate method for measuring mouse antiinsulin receptor antibodies. Unfortunately, we failed to obtain a positive answer. We are, however, in the process of elucidating how BMT can lead to recovery from these abnormalities. In our preliminary experiments, we have found that KK-Ay and db/db mice (another animal model for NIDDM) show immunological abnormalities, and that the abnormalities can be corrected by BMT (S. Ikehara et al., manuscript in preparation).

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