Rapid Publications

Prevention of Recurrence of IDDM in Islet-Transplanted Diabetic NOD Mice by Adjuvant Immunotherapy

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Insulin-dependent diabetes mellitus (IDDM) involves the destruction of the insulin-producing cells in the islets of Langerhans. One possible cure is by transplanting the islet cells; however, transplanted islets, even between identical twins, are subject to autoimmune destruction by the disease process, resulting in diabetes recurrence. We recently reported that complete Freund's adjuvant (CFA), an immunomodulating agent, prevented development of autoimmune diabetes in the NOD mouse. In this study, we evaluated adjuvant therapy in prevention of autoimmune destruction and rejection of transplanted islets in diabetic NOD mice. After transplantation, untreated syngeneic islet recipients (n = 16) initially became normoglycemic and then hyperglycemic, with a median survival time (MST) of the graft of 17 days. When CFA was administered at the time of transplantation, 11 of 13 CFA-treated syngeneic islet recipients remained normoglycemic long term (>100 days) with an MST >107 days. Ten of 11 mice maintained indefinite normoglycemia until the conclusion of follow-up (101 to 172 days). When adjuvant therapy was used in conjunction with allogeneic islet transplantation, graft survival was not extended, with MST being similar to the untreated allogeneic islet recipients (12 [n = 5] and 13 [n = 5] days, respectively). The extended acceptance of second syngeneic islet grafts by CFA-treated mice indicates that the persistent autoimmunity against the transplanted islets can be reversed in the diabetic NOD mice after CFA treatment. *Diabetes* 41:114–17, 1992

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Received for publication 16 September 1991 and accepted in revised form 21 October 1991.

Insulin-dependent diabetes mellitus (IDDM) is an organ-specific autoimmune disease (1). The NOD mouse is an excellent animal model of human IDDM (2). Recently, pancreatic islet cell transplantation has induced long-term insulin independence in IDDM patients (3,4). In addition to recognition of transplanted islets as an allograft, a confounding problem facing all islet-transplanted IDDM patients is autoimmune destruction of the islet graft by the disease process (autoimmunity), resulting in recurrent IDDM. This was first observed in IDDM patients receiving MHC-matched pancreases (5) and has been demonstrated in diabetic NOD mice transplanted with syngeneic (6) or immunomodulated allogeneic (7) islets. We have shown that IDDM can be prevented by a single injection of CFA in young NOD mice (8). In that study, CFA treatment preserved most of the islet tissue long term. In this study, we administered CFA treatment in conjunction with islet transplantation in diabetic NOD mice to determine whether such a therapy could protect the newly transplanted islets from destruction by diabetic autoimmunity.

RESEARCH DESIGN AND METHODS

NOD mice (K^d, $I\text{-}A^{NOD}$, D^b) were kindly provided by E. Leiter of Jackson Laboratory (Bar Harbor, ME) and bred by brother-sister mating in our facility at the University of Alberta. NOD mice were screened for diabetes by weekly monitoring of blood glucose. Diabetic mice (blood glucose > 11.11 mM) were maintained by daily injection of 1 U beef insulin until the day before transplantation. Five- to 10-wk-old female CBA/J mice ($H\text{-}2^k$) were also purchased from Jackson Laboratory. All mice were cared for according to the guidelines of the Canadian Council on Animal Care.

The syngeneic islet donors were 4- to 6-wk-old prediabetic NOD mice (sex matched with recipients). The

TABLE 1
Effect of single injection of complete Freund's adjuvant (CFA) at time of islet transplantation on survival of syngeneic and allogeneic islet grafts in diabetic NOD mice

Islet donor	Individual graft survival time (days)	
	Without CFA	With CFA
NOD	3,10,12,16*,16*,17†,18,20,21,24,27,56 (17)‡	24,77,107,>101†,>103,>109,>128*,>158,>172* (>107)‡
CBA/J	10,12,13,14,17 (13)	10,12†,14 (12)

*n = 2.
†n = 3.
‡$P < 0.01$ by Student's t test. Median survival time given in parentheses.

allogeneic islet donors were 4- to 6-wk-old female CBA/J mice ($H\text{-}2^k$). Islets were isolated according to the method of Gotoh et al. (9), with stationary collagenase digestion and Ficoll density purification followed by handpicking. The NOD recipients (either sex) were diabetic for 1–3 wk before islet transplantation. Over a 3-mo period, 39 diabetic recipients were transplanted with syngeneic islets (n = 29) and allogeneic islets (n = 10), respectively, with a spinning-catheter method developed in our laboratory. The recipients were anesthetized with Avertin (2,2,2,-tribromoethanol), a flank incision was made, and the left kidney was mobilized. A small incision was made in the upper pole of the kidney, and a pouch was created by separating the capsule from the kidney parenchyma with a fine glass probe toward the lower and anterolateral aspect of the kidney. Four hundred freshly isolated and purified islets (>95% purity) were brought to the center of the 10×35-mm petri dish in RPMI-1640 supplemented with 25 mM HEPES, 10% fetal calf serum, and 1% penicillin and streptomycin and then drawn up into a PE-50 catheter (0.76-mm ID, 30 cm long) with an attached micromanipulator syringe (developed at the Surgical-Medical Research Institute, Univ. of Alberta). The catheter tip was closed with a hemoclip, and the whole syringe and catheter was spun at 350 rpm for 15–20 s to pellet the islets at the tip. After removal of the hemoclip, the tip was inserted under the kidney capsule, and the islet pellet was slowly advanced into the pouch. The catheter was removed, and the entrance was sealed with an ophthalmic cautery. Transplantation was considered technically successful if the nonfasting blood glucose returned to normal (<9.4 mM) within 2–3 days (10). Islet graft function was evaluated as follows. Random blood glucose levels were monitored daily on tail vein blood samples for 21 days posttransplantation with an ExacTech glucose monitor (MediSense Canada, Mississauga, Ontario) and subsequently every 2nd day until conclusion of the study. Autoimmune destruction or allorejection of the islet grafts was diagnosed by return to hyperglycemia (>16.66 mM) on 2 consecutive days and also confirmed by histology.

To evaluate the effect of adjuvant therapy on autoimmune destruction or allorejection of grafted islets, 18 mice were randomly selected from the syngeneic (n = 13) and allogeneic (n = 5) islet recipients and treated once with CFA at the time of islet transplantation. CFA (Difco, Detroit, MI) was freshly mixed with saline (1:1 vol/vol), and 50 μl was injected into each hind footpad. The above groups were compared with nontreated syngeneic (n = 16) and allogeneic (n = 5) islet recipients.

RESULTS

Results of islet transplantation are shown in Table 1. All untreated syngeneic islet recipients (n = 16) initially became normoglycemic, and then hyperglycemic, with a graft MST of 17 days. Histological examination of the graft from mice that became hyperglycemic showed massive mononuclear cell infiltration of the islets accompanied by β-cell degranulation (Fig. 1, *A* and *B*). When CFA was administered at transplantation, 11 of 13 CFA-treated recipients remained normoglycemic long term (>100 days), with an MST >107 days. Ten of these 11 mice have maintained indefinite normoglycemia until the conclusion of follow-up (101–172 days). When adjuvant therapy was used in conjunction with allogeneic islet transplantation, graft survival was not extended with MST being similar to the untreated allogeneic islet recipients (12 and 13 days, respectively).

To confirm conclusively that the transplanted islets were functioning, we removed the islet-bearing kidney from four syngeneic islet recipients that were treated with CFA and were normoglycemic for >100 days (101, 103, 128, and 172 days, respectively). These mice developed immediate onset of hyperglycemia (>25 mM) (Fig. 2), and mean ± SD body weight declined rapidly from 29.0 ± 5.4 to 23.3 ± 5.1 g, resulting in death within 2–3 days. The histological examination of kidneys bearing islet grafts showed well-granulated islets with no intraislet mononuclear infiltration (Fig. 1, *C* and *D*).

In the remaining six long-term survivors (2 >101, 1 >109, 1 >128, 1 >158, and 1 >172 days), a second syngeneic islet graft was transplanted to determine whether tolerance to islets was established. These mice were transplanted with 400 islets to the right kidney, and 7 days later, the left kidney bearing the first islet graft was removed. Histological examination of the first graft showed no intraislet mononuclear infiltration, and the islets were well-granulated, similar to Fig. 1 (*C* and *D*). All six mice remained persistently normoglycemic for >40 days after removal of the first graft. Representative blood glucose levels of one of these mice are shown in Fig. 2.

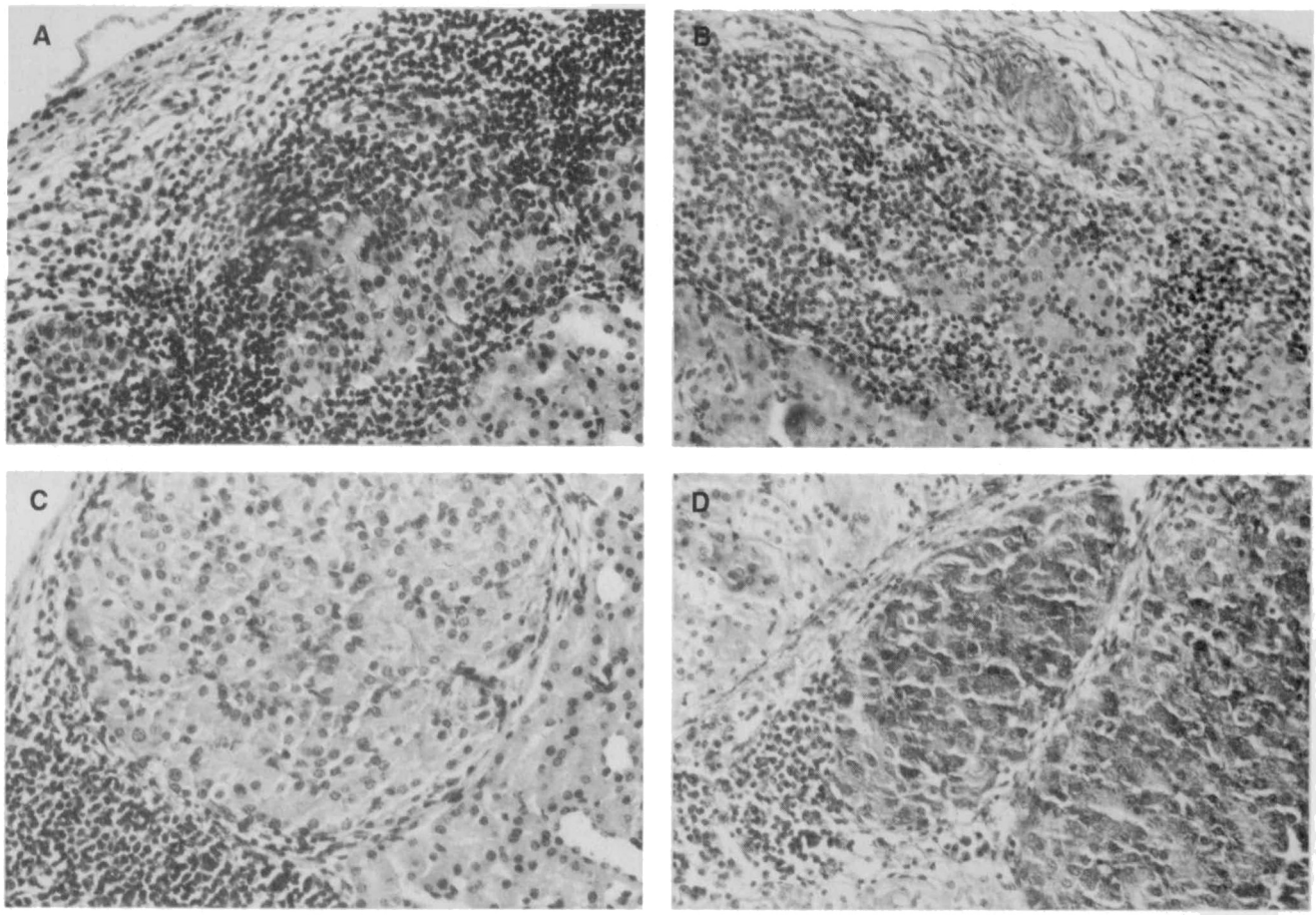

FIG. 1. Photomicrographs of kidneys from diabetic NOD mice that received renal subcapsular syngeneic islet grafts (*arrows*, kidney capsule). Sections were stained with hematoxylin-eosin or aldehyde-fuchsin (x260 original, scale, 100 μm). *A*: section (hematoxylin-eosin stain) from a mouse that received a syngeneic islet graft without complete Freund's adjuvant (CFA) treatment and developed hyperglycemia 21 days posttransplantation. The graft was removed on day 3 after onset of hyperglycemia. The islets became eroded by infiltrating mononuclear cells from surrounding connective tissue. Intraislet infiltration can be found in all islets. *B*: section (aldehyde-fuchsin stain) from same mouse as *A* shows markedly reduced intensity of aldehyde fuchsin stain, indicating β-cell degranulation. *C*: section (hematoxylin-eosin stain) from CFA-treated syngeneic islet recipient that had been normoglycemic for 172 days posttransplantation. An islet with regular spherical shape can be seen in the renal subcapsular space. It is surrounded but not infiltrated by mononuclear cells. *D*: section (aldehyde-fuchsin stain) from same mouse as *C* shows that islet β-cells were well granulated as indicated by aldehyde-fuchsin stained dark areas.

DISCUSSION

In this study, we demonstrated that hyperglycemia in diabetic NOD mice can be reversed by transplantation of 400 islets from adult syngeneic or allogeneic donors. However, the nontreated syngeneic islet recipients became hyperglycemic again with an MST of graft being 17 days. This MST slightly exceeded that reported by Matsuo et al. (6) for diabetic NOD recipients of pancreases from newborn NOD mice. This return to hyperglycemia by 17 days indicates that the existing autoimmunity in these mice destroys the transplanted islets quickly. A single injection of CFA at transplantation significantly prolonged the syngeneic islet grafts with an MST >107 days. In our laboratory, a single injection of CFA in NOD mice at an early age (4 wk) prevented progression of insulitis and onset of diabetes in NOD mice (8), whereas, this immunotherapy has been ineffective in preventing onset of diabetes when administered to prediabetic NOD mice >10 wk old (H.-Y. Qin and B. Singh, unpublished observations). Therefore, the CFA protection of an islet graft from autoimmune destruction in established dia-

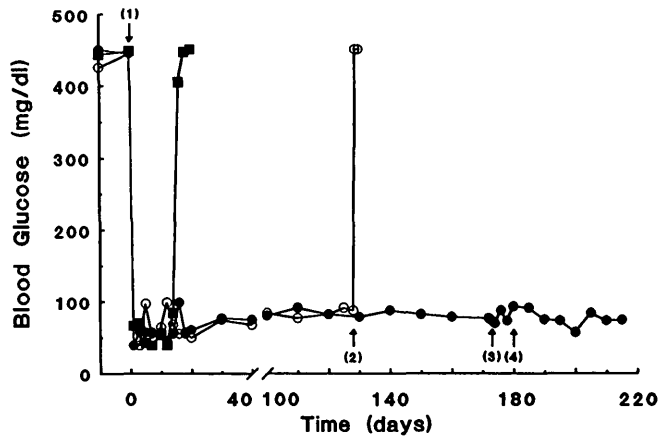

FIG. 2. Representative blood glucose profiles of diabetic NOD mice transplanted with freshly isolated syngeneic islets with or without complete Freund's adjuvant treatment. *1*, transplantation of islets. ■, Untreated; ○ and ●, 1 injection CFA at transplantation. *2*, Removal of islet-bearing kidney. *3*, Second renal capsular transplantation of NOD islets. *4*, Removal of kidney bearing 1st islet graft.

betic recipients was unexpected. It is possible that CFA prevented recurrent diabetes in islet recipients but did not prevent onset of diabetes in older prediabetic mice, because CFA therapy prevents lymphocyte infiltration into newly transplanted islets but may not protect islet β-cells from destruction by autoimmune effector cells that have already infiltrated the islets, as in older prediabetic mice. The mononuclear cell infiltration and insulitis take place as early as 4–6 wk of age in NOD mice (11–14), therefore, we suspect that the induction of diabetic autoimmunity must take place even earlier. However, the mice do not become overtly diabetic until 80–90% of the islet mass is destroyed by infiltrating cells. In a previous study (8), we observed mild mononuclear infiltration around the islets but not within the islets when the NOD mice were treated with CFA at an early age. Similar histological changes were also seen in this study, showing that transplanted syngeneic islets in CFA-treated diabetic recipients developed mononuclear infiltration around the islets (Fig. 1, *C* and *D*); however, the islets remained intact long term. The histological similarity suggests that the mechanism of CFA-mediated protection of islets from autoimmune destruction may be similar in these two cases.

CFA therapy protects the syngeneic graft from autoimmunity but has no effect on allogeneic islet graft rejection in the NOD mice. This supports the assumption that there may be different pathways for autoimmune destruction of islets from that of allograft immunity as suggested by Wang et al. (7).

The immunomodulating effect of CFA led to an extended acceptance of a second syngeneic islet graft without further treatment. This suggests that the diabetic process (autoimmunity) was reversed or that a state of self-tolerance to islet antigens was established in these mice that previously had active autoimmunity against islets. It is unclear whether the induction and maintenance of this tolerant state after immunomodulation with adjuvant requires the presence of islet antigens.

This study could have important clinical relevance, because clinical islet transplantation has been successful in IDDM patients (3,4). Although CFA therapy would not be applicable clinically, BCG vaccine could be used as an immunomodulating agent in humans. BCG contains attenuated *Mycobacterium bovis*, an organism related to *M. tuberculosis*, which is present in CFA. BCG vaccine is also effective in preventing development of diabetes in NOD mice (15). In studies of allogeneic islet transplantation, the islets have been immunomodulated before transplantation to prevent graft rejection (16,17). However, when immunomodulated allogeneic islets were transplanted into diabetic NOD mice, they were destroyed by the autoreactive T lymphocytes (7). Therefore, our studies suggest that combining adjuvant therapy of the recipients with immunomodulation of the allogeneic islet graft, such as low-temperature culture (16,17), could be applied clinically and may protect the recipient from recurrent IDDM after transplantation. This combined approach could be used to treat IDDM patients before the secondary complications of the disease arise. In addition, this approach of islet transplantation in diabetic NOD mice may help us understand the basic mechanisms underlying autoimmunity and allorejection.

ACKNOWLEDGMENTS

This work was supported by grants from the Edmonton Civic Employees' Charitable Assistance Fund, the Alberta Heritage Foundation for Medical Research, the Muttart Diabetes Research and Training Centre, the Medical Council of Canada, and the Canadian Diabetes Association.

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