Treatment of type 1 diabetes mellitus in non-obese diabetic mice by transplantation of allogeneic bone marrow and pancreatic tissue

(immunologic tolerance/autoimmunity)

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ABSTRACT Non-obese diabetic (NOD) mice provide a model for type 1 diabetes mellitus. We previously showed that allogeneic bone marrow transplantation (ABMT) can prevent and treat insulin open diabetes in NOD mice. However, ABMT alone could not be used to treat overt diabetes in NOD mice whose islets had been completely destroyed. To provide insulin-producing cells, pancreatic tissue from newborn mice was grafted under the renal capsules in combination with ABMT. The aims of concomitant ABMT are as follows. (i) It induces immunological tolerance to the donor-type major histocompatibility complex determinants and permits the host to accept subsequent pancreatic allografts from the bone marrow donor. (ii) ABMT replaces abnormal stem cells with normal stem cells. After transplantation of bone marrow plus newborn pancreas, NOD mice showed reduction of the glycemia and a normal response in the glucose-tolerance test. Immunohistological study revealed the presence of clustered insulin-containing beta cells in the grafted pancreatic transplants. ABMT may become a viable treatment of established type 1 diabetes mellitus in humans.

It is thought that autoimmune mechanisms are involved in the etiopathogenesis of type 1 diabetes mellitus: humoral and cellular autoimmune responses specific for insulin-producing beta cells in both humans and animal models have been well documented (1–3).

Experiments using BB rats, which spontaneously develop autoimmune insults resulting in type 1 diabetes mellitus, have contributed to the elucidation of the etiopathogenesis of diabetes and also to identification of rational therapy (4). It has been reported that diabetes in BB rats can be adoptively transferred to both young BB rats and diabetes-resistant rats when concanavalin A-activated spleen cells of BB rats with overt diabetes are introduced into these rats (5, 6). In reverse, allogeneic bone marrow transplantation (ABMT) from normal rats into BB rats has prevented diabetes (7). These observations suggest that abnormal immunocompetent cells are responsible for the development of type 1 diabetes, and that ABMT, which replaces these abnormal stem cells with stem cells from normal bone marrow, has prophylactic and curative effects in BB rats.

Another animal model for type 1 diabetes, the non-obese diabetic (NOD) mouse, was established by Makino et al. (8). More than 90% of both male and female NOD mice develop insulin open diabetes by the age of 200 days. This is followed by overt diabetes that has been shown to be due to the destruction of beta cells in the pancreatic islets in 80% of the females and 20% of the males by the age of 210 days. Non-treated NOD mice die within 1 month of the development of glycosuria.

In a previous report (9), we demonstrated that ABMT could be used to treat autoimmune insults and prevent overt diabetes in NOD mice but that ABMT alone could not cure established diabetes. This is because the beta cells of the islets are already destroyed by the time the disease is established. More recently, we found that mice which underwent liver allografts combined with ABMT accept donor-type as well as host-type liver tissue (10), prompting us to treat type 1 diabetes mellitus in NOD mice by ABMT followed by pancreatic allografts. In the present study, we provide evidence that bone marrow plus pancreas transplantation can be used to treat type 1 diabetes mellitus in NOD mice.

MATERIALS AND METHODS

Mice. The NOD mice were bred and maintained under specific pathogen-free conditions in our animal facilities. Female NOD mice were used for the following experiments.

Bone Marrow Plus Pancreas Transplantation. NOD mice with overt diabetes were irradiated (9.0 Gy) from a 60Co source 1 day before ABMT. Bone marrow cells were collected from the femurs and tibias of BALB/c nu/nu mice, and 2–3 × 107 cells were injected intravenously into the NOD mice. One to three weeks following ABMT, pancreatic tissue from newborn BALB/c mice was grafted under the renal capsules. NOD mice with overt diabetes were injected subcutaneously with insulin (20–50 units/kg of body weight) every day until 1 week after pancreatic transplants.

Glucose-Tolerance Test. Food was removed for 14 hr before the glucose-tolerance test was started. Each mouse was injected intraperitoneally with 1 mg of glucose per g of body weight. Blood samples were obtained at 0, 30, 60, and 120 min by orbital sinus puncture.

Radioimmunoassays for Insulin in Sera. Rat standard insulin (NOVO Research Institute, Copenhagen) was used as standard insulin. Radioimmunoassays were performed according to the method of Desbuquois and Aurbach (11).

Histological Study. Major organs were obtained at autopsy, and sections were stained with hematoxylin/eosin.

Immunohistochemical Study. Specimens were immediately embedded in optimal-cutting-temperature (OCT) compound and frozen in dry ice/acetone. Two-micrometer cryostat sections were used for immunohistochemical study, as described (12, 13).

Abbreviations: NOD, non-obese diabetic; ABMT, allogeneic bone marrow transplantation.
RESULTS

Five of the seven treated NOD mice showed an improvement in glycosuria and survived more than 30 days after pancreatic transplants; four have survived more than 90 days, and one is still alive 365 days (at the time of writing) after pancreatic transplants (Table 1). By contrast, control NOD mice with overt diabetes died within 2 weeks unless they had been treated with insulin.

As shown in Fig. 1, control NOD mice with glycosuria showed an abnormal response in the glucose-tolerance test, whereas the NOD mice that had been operated on showed a

Table 1. Survival of NOD mice with allogeneic bone marrow and pancreatic transplants

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Sex</th>
<th>Age at onset of diabetes, days</th>
<th>Length of survival, days</th>
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<tr>
<td></td>
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<td>From birth</td>
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<tr>
<td>1</td>
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<td>119</td>
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<tr>
<td>7*</td>
<td>M</td>
<td>150</td>
<td>&gt;495</td>
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NOD mice underwent ABMT 4–15 days after onset of diabetes and underwent pancreatic transplantation (PT) 5–22 days after ABMT (9–35 days after onset).

*Still alive.

Fig. 1. Glucose-tolerance test. Glucose (1 mg/g of body weight) was injected intraperitoneally into NOD mice that underwent bone marrow plus pancreas transplantation (○), untreated NOD mice (●), and BALB/c mice (□). Blood samples were serially collected, and the concentration of glucose was determined. Error bars indicate ± SEM for n = 5 mice.

Fig. 2. Histology of engrafted pancreas. Clusters of islet cells were observed under the renal capsule by hematoxylin/eosin staining (Left). These cells were shown to contain insulin by means of immunohistological staining (Right). Paraffin-embedded sections were sequentially incubated with guinea pig IgG anti-rat insulin and peroxidase-conjugated rabbit IgG anti-guinea pig IgG. After incubation, the sections were processed to catabolize diaminobenzidine in the presence of hydrogen peroxide.
such treated NOD mice, the immunoreactive insulin content being 20.3–31.0 µg/ml after a 14-hr fast.

Grafted islets were found under the renal capsules of the NOD mice (Fig. 2 Left). Immunohistochemical study revealed the presence of clustered insulin-producing beta cells in the islets (Fig. 2 Right).

DISCUSSION

The results show that pancreatic tissue grafts combined with ABMT can be used to treat overt diabetes mellitus in NOD mice. The aims of the concomitant ABMT are as follows: (i) ABMT induces immunological tolerance to the donor-type major histocompatibility complex-encoded determinants and permits the host to accept subsequent allogeneic pancreatic grafts from the bone marrow donor and (ii) ABMT replaces abnormal stem cells with normal stem cells following the preparative lethal whole-body irradiation, thus eliminating the danger of a recurrence of diabetes.

Total lymphoid irradiation and immunosuppressive agents such as azathioprine, cyclophosphamide, anti-lymphocyte globulin, and more recently cyclosporine have been used not only to treat autoimmune diseases, including organ-specific autoimmune diseases, but also to prevent the rejection of other organ allografts. However, these approaches exert cytotoxic effects on lymphocytes, especially T cells, and patients undergoing these therapies over long periods often suffer from life-threatening infections and cancers attributable to the immunodeficiency. In contrast to these approaches, pancreatic allografts combined with ABMT have been shown in the present study to have no harmful side effects in mice. Furthermore, we have found, using animal models for systemic autoimmune diseases, that the immunological functions of T cells, B cells, and macrophages can be normalized after ABMT and that the renal damage characteristic of these diseases can also be ameliorated after ABMT (S.I., R.Y., Takao Nakamura, Ken’ichi Sekita, M.I., Shozo Izui, Kyoko Hayakawa, Junko Toki, K.S., H.I., Y.H., and R.A.G., unpublished observation).

These findings suggest that fetal pancreatic allografts in conjunction with transplantation of fetal liver cells as a source of hemopoietic stem cells from the same aborted fetus may become a viable treatment of type 1 diabetes and possibly some forms of type 2 diabetes. However, it remains to be determined what triggers these autoimmune responses and how it is that ABMT can cure insulitis. Further analysis at the genetic and molecular levels will be necessary to clarify the exact mechanism of the development of type 1 diabetes mellitus.

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