Thymus transplantation, a critical factor for correction of autoimmune disease in aging MRL/+ mice

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ABSTRACT MRL/MP−/+ (+ MRL/+) mice develop pancreatitis and sialoadenitis after they reach 7 months of age. Conventional bone marrow transplantation has been found to be ineffective in the treatment of these forms of apparent autoimmune disease. Old MRL/+ mice show a dramatic thymic involution with age. Hematolymphoid reconstitution is incomplete when fetal liver cells (as a source of hematopoietic stem cells) plus fetal bone (FB; which is used to recruit stromal cells) are transplanted from immunologically normal C57BL/6 donor mice to MRL/+ female recipients. Embryonic thymus from allogeneic C57BL/6 donors was therefore engrafted along with either bone marrow or fetal hematopoietic cells (FHCs) plus fragments of adult or fetal bone. More than seventy percent of old MRL/+ mice (>7 months) that had been given a fetal thymus (FT) transplant plus either bone marrow or FHCs and also bone fragments survived more than 100 days after treatment. The mice that received FHCs, FB, plus FT from allogeneic donors developed normal T cell and B cell functions. Serum amylase levels decreased in these mice whereas they increased in the mice that received FHCs and FB but not FT. The pancreatitis and sialoadenitis already present at the time of transplantations were fully corrected according to histological analysis by transplants of allogeneic FHCs, FB and FT in the MRL/+ mice. These findings are taken as an experimental indication that perhaps stem cell transplants along with FT grafts might represent a useful strategy for treatment of autoimmune diseases in aged humans.

During the past twenty years, remarkable advances have been made in bone marrow transplantation (BMT) as a form of stem cell transplantation. Indeed, BMT has already become a powerful strategy for the treatment of leukemias, lymphomas, other cancers, aplastic anemias, congenital immunodeficiencies, liposomal storage diseases, enzyme deficiencies, and genetically determined hematopoietic abnormalities. However, the success of BMT in older patients is low because such patients develop interstitial pneumonitis and acute or chronic graft-vs. host disease (GVHD) or show a relapse of the primary disease for which the original transplant was performed (1–3). Thus far the reasons for these inadequacies of BMT in elderly patients have not been fully clarified.

We have previously found (4–8) that BMT can be used to prevent or treat many spontaneous autoimmune diseases such as organ-specific autoimmunities like diabetes in NOD mice and systemic autoimmunities in (NZB × NZW)F1, BXSB, and (NZW × BXSB)F1 mice. We have recently shown that transplantation of both bone marrow cells (BMCs) and bones, which exhibit the capacity to recruit stromal cells from the same normal bone marrow donor, leads to complete prevention of certain otherwise highly resistant autoimmune diseases such as those that occur in MRL/pr mice and the autoimmune polyarthritis of NZB/KN mice (9, 10). Old female MRL/+ mice (>7 months) frequently develop apparent autoimmune pancreatitis and sialoadenitis (11). In the present study, we have successfully treated this form of late-onset autoimmune diseases in mice using a combination of FT grafts, fetal liver cell (FHC) transplants plus transplantation of fragments of fetal bones (FBs). We show herein that allogeneic FT grafts given in conjunction with FB grafts and FHCs regularly result in dramatic correction of the manifestations of the apparent autoimmune pancreatitis and sialoadenitis in older MRL/+ mice.

MATERIAL AND METHODS

Mice and Antibodies. Female MRL/+ mice were bred and maintained in the animal facilities at the 1st Department of Pathology, Tohoku University, Sendai, Japan, or obtained from CLEA Japan, Osaka. Pregnant C57BL/6 mice were used as a source of donor cells, bone marrow, fetal liver, and fetal cranial bones for transplantation. Fluorescein isothiocyanate (FITC)-coupled anti-H-2Kd or anti-H-2d antibodies were purchased from the Meiji Institute of Health Science, Odawara, Kanagawa, Japan.

Transplantation. Female MRL/+ mice (>7 months) were lethally (9.5 Gy) irradiated and divided into four groups. In group 1 the mice were injected intravenously (i.v.) with 3–4 × 107 T cell-depleted BMCs from female C57BL/6 mice (2–3 months). These recipient mice were also engrafted with 2 to 3 fragments (2 × 2 × 3 mm) of bone from a femur from the same donor mice. These bone grafts were placed under the renal capsule of one kidney. In group 2, in addition to the transplants of BMCs plus bone, the mice were transplanted with two thymic lobes from fetal (18–20 days of gestation) C57BL/6 mice. The grafts were all placed under the same renal capsule. In group 3, the mice were injected i.v. with 3–4 × 107 FHCs as a source of hematopoietic stem cells and also engrafted with 2 to 3 fragments (2 × 2 × 3 mm) of cranial bone (from the same fetus) placed under the renal capsule (one side). In group 4, two fetal thymic lobes from the same fetal donor were also engrafted under the same renal capsule along with transplants of FB fragments and the i.v. injection of 3–4 × 107 FHCs. Since it proved difficult to obtain large numbers of old MRL/+ mice at any one time, several sets of experiments using relatively small groups of mice were performed; each group included at least 3 mice, and each experiment was repeated three or more times. Highly reproducible observa-

Abbreviations: FB, fetal bone; FT, fetal thymus; FHCs, fetal hematopoietic cells (fetal liver); dGuo, deoxyguanosine; LPS, lipopolysaccharide; BMC, bone marrow cell; BMT, bone marrow transplantation; GVHD, graft vs. host disease; Con A, concanavalin A; FITC, fluorescein isothiocyanate.

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were stained with appropriate antibodies. The cells were cultured for 72 hr in 96-well flat-bottomed microculture plates with 50 μg/ml of lipopolysaccharide (LPS, Difco), 5.0 μg/ml of concanavalin A (Con A, Sigma) in RPMI 1640 medium containing 10% FCS, 5 × 10⁻⁵ M 2-mercaptoethanol and 2 mM glutamine. [³H]-thymidine (0.5 Ci/ml; 1 Ci = 3.7 × 10¹² Bq) was introduced to each culture 16 hr prior to termination and proliferation then assessed.

Measurement of Amylase. Mouse sera were diluted in PBS (1:16) and the amylase levels then quantified using an ELISA assay (available at Falco, Osaka). The data are expressed as international units per liter.

Statistical Analyses. Statistical analyses were performed using Student’s paired t test.

RESULTS

Survival Rate. To treat autoimmune pancreatitis and salivary adenitis in old female MRL/+ mice, we carried out BMT plus other tissue transplants using four methods. In group 1, old female MRL/+ mice (>7 months) were lethally irradiated and then injected i.v. with T cell-depleted BMCs. Mice of this group were engrafted with bone fragments (femur) free of BMCs from adult C57BL/6 mice (transplantation of adult BMCs plus bone grafts) as described (9, 10). In group 2, in addition to these transplants, the mice were engrafted with C57BL/6 FT (transplantation of adult T cell-depleted BMCs, adult bone plus FT). In group 3, the mice were injected i.v. with C57BL/6 fetal hematopoietic stem cells (FHCs) obtained from fetal liver at 18–20 days gestation and also engrafted with C57BL/6 cranial FB (transplantation of FHCs plus FB). In group 4, the mice were treated as in group 3 but, in addition, engrafted with the C57BL/6 FT from the same embryo as the donor (transplantation of FHCs, FB, and FT). As shown in Fig. 1, the survival rate of the mice in group 1 at 100 days was 10%. In group 2, survival was ~70% at 100 days. In contrast, approximately 20% of the mice in group 3 survived at 100 days, while the survival rate in group 4 was ~80% after the several transplants. None of the mice in groups 2 or 4 showed clinical signs of GVHD or runting syndrome reminiscent of GVHD.

We compared the mice in group 3 with those in group 4 to analyze the benefits of thymus grafts, since the mice in group 1 died much earlier than those in the other groups.

H-2 Typing. To examine whether the hematopoietic cells of the recipients were derived from donor cells, we carried out
Fig. 3. Serum amylase levels in mice in groups 3 (A) and 4 (B) before and 2–3 months after transplantation. Statistical analyses performed using Student’s paired t test revealed significant differences between the mice of these two groups.

H-2 typing using spleen cells from the chimeric mice in groups 3 and 4. As shown in Fig. 2A, more than 50% of the spleen cells of the mice in group 3 were H-2Kb-positive (donor-derived) and approximately 30% were H-2Kk-positive (host-derived). This finding suggests that the host hematopoietic cells had recovered from the lethal irradiation and differentiated into mature cells within 2 months of BMT. By contrast, in the mice of group 4, almost all of the spleen cells were derived from the donor hemopoietic cells. Only a few cells (3%) appeared to be derived from the cells of the recipient major histocompatibility complex (Fig. 2B).

Serum Amylase Levels. The amylase levels in the sera of the mice in group 3 that were elevated initially did not fall after the fetal liver plus bone transplantations and actually increased. These levels were 3906 ± 289 units/liter before the transplantation and 4339 ± 1115 units/liter after the transplantation (Fig. 3A). By contrast, the amylase levels of the mice in group 4 showed significant reduction following the transplantation; 3792 ± 242 units/liter before transplantation and 3201 ± 488 units/liter following transplantation (P < 0.05) (Fig. 3B).

Histology. Histopathological findings in sections of the pancreas and salivary glands in nontreated female MRL/+ mice (A and B) or mice (15 months) from group 4 (C and D). (×20.)
mice (12 months) are shown in Fig. 4 A and B. Impressive infiltration of lymphoid cells was regularly apparent in the exocrine component of the pancreas of the untreated mice. Only hypertrophy of the islets without insulinitis was noted in these control specimens (Fig. 4A). Focal lymphocyte infiltration was also present around the ducts of the salivary glands of the untreated mice (Fig. 4B). The histological findings of the mice in group 4, 3 months following transplantation, are shown in Fig. 4 C and D. Lymphocyte infiltration had disappeared from both pancreas (C) and salivary glands (D) with irradiation plus transplantation of FHCs, bone fragments and thymus lobes. As shown in Fig. 5, the bone engrafted under the renal capsule in the mice in group 3 contained only a few cells. These findings reflected the incomplete reconstitution of hematopoiesis observed in mice that had not been given a thymus (Fig. 5A). By contrast, the mice from group 4 that received FHCs plus FB and FT showed a normal appearance in the hematopoietic tissue, reflecting the complete hematopoietic reconstitution achieved (Fig. 5B).

Immunologic Functions. T cell and B cell functions were evaluated using responses to mitogens as indicators (Fig. 6). The spleen cells of old MRL/+ mice (13 months) showed a lower response to T cell mitogen (Con A) than young MRL/+ mice. By contrast, the response to the B cell mitogen (LPS) of these MRL/+ mice did not change with age (Fig. 6). The spleen cells of mice from group 3 (FHCs and FB) that had survived showed decreases in both Con A and LPS responses, reflecting the incomplete reconstitution of lymphoid cells in these mice. Since the mice in group 3 showed a mixed chimeric state of the hematolymphoid cells (as shown in Fig. 2), this immunologic deficiency may be attributed either to failed reconstitution or to allogeneic cell–cell interactions. In contrast, the mice in group 4 showed vigorous responses to both Con A and LPS, the levels observed being comparable to those of normal C57BL/6 mice.

DISCUSSION

In the present study we performed allogeneic stem cell-based reconstitution following total body irradiation to determine which of the four methods might be the most advantageous approach to treating autoimmune disease in older MRL/+ mice. Almost all the mice in group 1 (transplantation of adult BMCs and bone) died within 2 wk of transplantation, while the mice in group 3 (transplantation of FHCs and FB), although surviving somewhat longer, did not achieve high survival rates over the 100 days after transplantation. By contrast, the mice in group 2 (transplantation of adult BMCs, bone fragments and FT) and group 4 (transplantation of FHCs, FB, plus FTs) survived much longer (Fig. 1). These findings indicate that (1) mice given either adult BMCs plus bone fragments or FHCs plus FB do not survive long, and that the transplantation of FT is essential to rescue the old mice of this strain after stem cell transplantation. In analyses of the ability to reconstitute donor-type cells, we found that the spleen cells of the mice in group 4 were almost all donor-derived, while a significant number of host-type cells could be found in the spleens of the mice in group 3 (Fig. 2). Furthermore, the thymus showed a normal structure, and the transplanted bones strongly supported hematopoiesis in the mice in group 4, not in those in group 3 (Fig. 5). This finding suggests that FT transplantation facilitates the development and engraftment of donor-type hematopoietic cells, probably by suppressing the differentiation of host-derived cells. It is therefore likely that the donor-derived T cells induced by FT grafts may play a crucial role in the suppression of graft rejection that might otherwise be mediated by host-derived cells such as T cells, natural killer cells, and macrophages.

Concerning the treatment of autoimmune diseases, the chronic pancreatitis and saloadenitis that characterizes the MRL/+ mice appear to have been regularly cured in the mice in groups 2 (data not shown) and 4 (Figs. 3 and 4). The spleen cells in the mice in group 4 showed a vigorous proliferative response to the mitogens (Con A and LPS) at a level comparable to that of nontreated adult C57BL/6 mice (Fig. 6). In humans, it has been reported that the major causes of death after BMT in old recipients (>45 years old) are interstitial pneumonitis, acute GVHD, and relapse of the primary disease for which hematopoietic transplantation was carried out. Except for acute GVHD, these causes of death appear to be due to incomplete reconstitution followed by immunodeficiency or graft rejection.
There have been numerous reports concerning thymus grafts in humans (13–15). Most of these contain negative or unimpressive data that are probably attributable either to rejection of the engrafted thymus by radioresistant host cells or the development of GVHD by T cells contaminating the engrafted thymus (13, 14). However, we have recently examined the cytotoxic effects of irradiation and deoxyguanosine (dGuo) on the thymus and found that FT is sensitive to both irradiation and dGuo (>1.35 mm) (16). In addition, we have found that the transplantation of allogeneic FT without irradiation or dGuo treatment leads to long-term T cell reconstitution in nu/nu mice, and that the nu/nu mice do not develop GVHD even when the allogeneic FT used has contained immature thymocytes at the time of engraftment (16). Based on these findings and those observed in the present report, we would propose that the transplantation of the FT (without using irradiation or dGuo treatment) together with FHCs plus FB might be appropriate as a strategy to treat patients older than 45 yr who suffer from a variety of diseases, including life-threatening autoimmune diseases.

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