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Appearance of Thyroid Stimulating and Blocking Immunoglobulins after Bone Marrow Transplantation: Presentation of Two Contrasting Cases

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Abstract. Two acute leukemia cases who presented autoimmune thyroid diseases after bone marrow transplantation (BMT) are described with reference to the pathogenesis of their autoimmune clones. A 37-year old Japanese woman developed Graves' hyperthyroidism 39 months after allogeneic BMT for acute myeloid leukemia (AML) donated from her sister. Although both donor and recipient were euthyroid and negative for thyroid autoimmunity before BMT, the donor was positive for anti-nuclear and anti-single strand DNA autoantibodies. Studies on polymorphism for variable number of tandem repeat region of T-cell receptor gene suggested that the lymphocytes responsible for the hyperthyroidism were of donor origin. The second case was a 12-year-old Japanese schoolboy who presented nongoitrous hypothyroidism 2 years after autologous BMT for acute lymphoblastic leukemia (ALL). He had been clinically euthyroid before transplantation. Family history revealed that his mother and sister had a history of Graves' disease. His serum was positive for thyroid-stimulation blocking antibody. It is highly likely that the autoimmune process was activated after transient immune suppression during peri-BMT period in this patient. Pathogenesis, incidence, and observed time lag between BMT and development of autoimmune thyroid diseases were discussed.

Key words: bone marrow transplantation, hyperthyroidism, Graves' disease, hypothyroidism, primary myxedema
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BONE marrow transplantation (BMT) is now a therapeutic option for many kinds of diseases including acute leukemia [1–3]. Transmission of immune cells during the course of BMT may ameliorate certain kinds of autoimmune diseases like rheumatoid arthritis [4, 5]. However, there have been reports on the adoptive transmission of auto- or host-reactive clones to recipients causing pathological conditions in the recipients. In thyroid autoimmunity, there are several case reports of Graves' disease [6–11] and of autoimmune thyroiditis with destructive thyrotoxicosis [12–16], which developed after BMT. In contrast, there are only two well-documented cases of chronic thyroiditis without thyrotoxicosis following BMT [17, 18]. We experienced two contrasting cases, which developed autoimmune thyroid diseases after BMT for acute leukemia. This article presents our illustrative cases and discusses the pathogenesis of thyroid autoimmunity after BMT. To the best of our knowledge, this is the first case report in which thyroid-stimulation blocking antibody (TSBAb) is documented in the post-BMT period.

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Case Report

Case 1

A 34-year-old Japanese woman received allogeneic

BMT for AML (M5a) in September 1995. Family history was unremarkable except that her parents were hypertensive. Before transplantation, both she and her donor sister were euthyroid and negative for thyroid autoimmunity. However, the donor was positive for anti-nuclear autoantibody (× 160) and for anti-single strand DNA autoantibody (5.8 U/ml). HLA typing confirmed mismatch at one HLA-DR antigen [the donor: A33(19)/A2, B61(40)/B13, Cw3, DR6/DR12 (5), DRB1 1405/1202, DPB1 0601/0501, DQB1 05031/0301; and the recipient: A33(19)/A2, B61(40)/ B13, Cw3, DR2/DR12(5), DRB1 1502/1202, DPB1 0901/0501, DQB1 0601/0301]. Preparative regimen consisted of busulfan, cyclophosphamide, total body irradiation and anti-thymocyte globulin. Graft-versus-host disease (GVHD) prophylaxis comprised cyclosporine with short course methotrexate. Prednisolone was administered at a maximum dose of 60 mg daily to ameliorate acute GVHD after BMT, and she had been given cyclosporine (60 mg daily at the onset of hyperthyroidism) against chronic GVHD. In December 1998 (39 months after BMT), she noticed palpitation, tremor, and easy fatiguability. Moderately enlarged diffuse goiter was noted in January 1999, and she was diagnosed as Graves' hyperthyroidism. Serum thyroid hormone levels were elevated (free T_4 at 7.3 ng/dl, normal range: 0.8–1.5; free T_3 at 25.3 pg/ml, normal range: 2.1–4.1) and serum thyrotropin was undetectable (<0.1 mU/l, normal range: 0.44–3.78). Both serum thyrotropin-binding inhibitory immunoglobulin (TBII) and thyroid stimulating antibody (TSAb) were positive (42.9% and 2,110%, respectively). Serum hemoagglutination reaction against thyroid microsomal fraction was positive at × 100, while that against thyroglobulin was negative. She was also marginally positive for anti-nuclear autoantibody (× 20). Anti-double- or single-strand DNA, ENA, RNP, or Sm antibodies were negative both before and after BMT. Ultrasonic thyroid scan revealed accelerated intrathyroidal blood flow. She was treated with thiamazole and her clinical course was uneventful. The donor remains euthyroid, seronegative for both anti-thyroglobulin and anti-thyroid peroxidase autoantibodies, and shows no clinical signs of thyroid pathology at present. Clonal origin of the thyroid-responding autoimmunity was evaluated further. To determine whether the immune clone which brought about hyperthyroidism in this case was adoptively transmitted from the donor or originated from the recipient's own

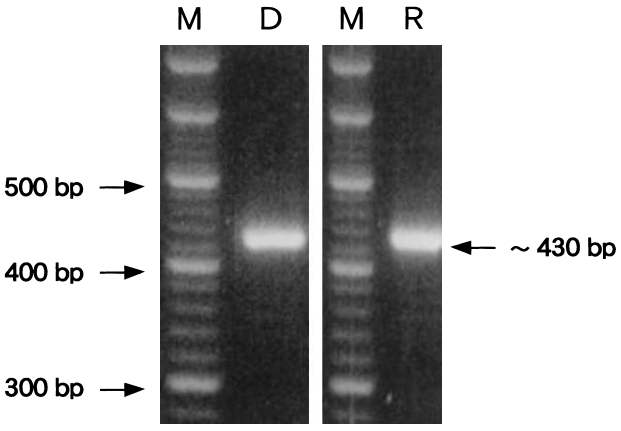

Fig. 1. PCR-RFLP pattern of donor and recipient. A DNA marker for variable number of tandem repeat region of the T-cell receptor gene was amplified. M: size marker, D: donor, R: recipient

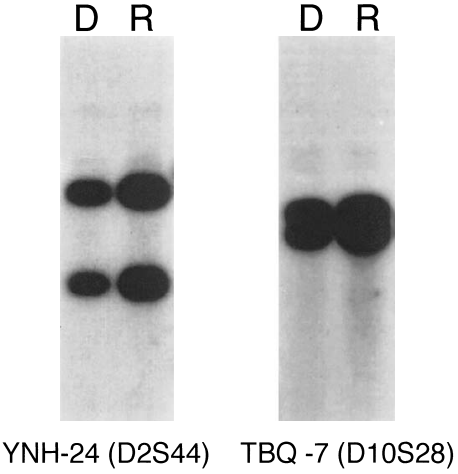

Fig. 2. Southern blot-RFLP pattern of donor and recipient. YNH-24 (D2S44) and TBQ-7 (D10S28) were used as DNA markers. D: donor, R: recipient

marrow, the following experiments were conducted. Genomic DNA was extracted from peripheral lymphocytes of both donor and recipient with informed consent. A DNA marker for variable number of tandem repeat (VNTR) region of T-cell receptor gene (MCT118 [D1S80]) was amplified by polymerase chain reaction (PCR). The resultant DNA fragments were subjected to agarose gel electrophoresis and visualized with ethidium bromide. A single band of about 430 bp was detected for both donor and recipient (Fig. 1). Identity of the hematolymphoid systems of donor and recipient was further determined by Southern blot analysis. Restriction fragment length poly-

morphism (RFLP) pattern was determined using two different probes, YNH-24 (D2S44) and TBQ-7 (D10S28). The RFLP patterns of donor and recipient showed an exact match for the two probes tested (Fig. 2).

Case 2

A 7-year-old Japanese schoolboy underwent autologous BMT for ALL in his first remission period in August 1995, because appropriate donor was not available. The HLA haplotypes of his family members were as follows: the patient, A2, B46/B62(15), Cw1, DR9/DR12(5); his father, A11/A2, B39(16)/B62(15), Cw7, DR4/DR12(5); his mother, A2, B46/B51(5), Cw1, DR9/DR14(6); and his sister, A2, B51(5)/B62(15), DR14(6)/DR12(5). His conditioning regimen consisted of busulfan 35 mg/m^2 4 times daily for 4 days and 70 mg/m^2/day of melphalan for 3 days. He had been clinically euthyroid before transplantation, and serum total cholesterol level was well within normal limit (187 mg/dl). His growth had been average for his age before BMT. However, he and his family were aware that his growth had been virtually arrested in the past few years (Fig. 3). He was admitted for evaluation of thyroid status in July 2000. Family history revealed that his mother had a history of Graves' disease in her second decade, and his sister, who was 14 years old, had been suffering from Graves' disease for a few months as well. On examination, puffy face with slow speech and hoarseness were suggestive of hypothyroidism. Serum free T_4 and free T_3 levels were depressed at <0.2 ng/dl and 1.5 pg/ml, respectively, and serum thyrotropin was elevated at 459 mU/l. He was negative for goiter, and ultrasonography showed a small thyroid gland with low echogeneity. His serum was positive for TBII (73.5%). Serum TSAb was negative (167%, normal range: <180%). Serum TSBAb was negative (32.0%, normal range: <45.6%) on the initial examination. However, it appeared positive (75.5%) after he became euthyroid with thyroid hormone replacement therapy. Echo-guided fine needle aspiration biopsy/cytology was not conducted because the parents were reluctant to submit to the procedure. Based on the diagnosis of primary myxedema, he was given oral dose of *levo*-thyroxine (50 μg daily) and catch-up growth was successfully achieved (Fig. 3).

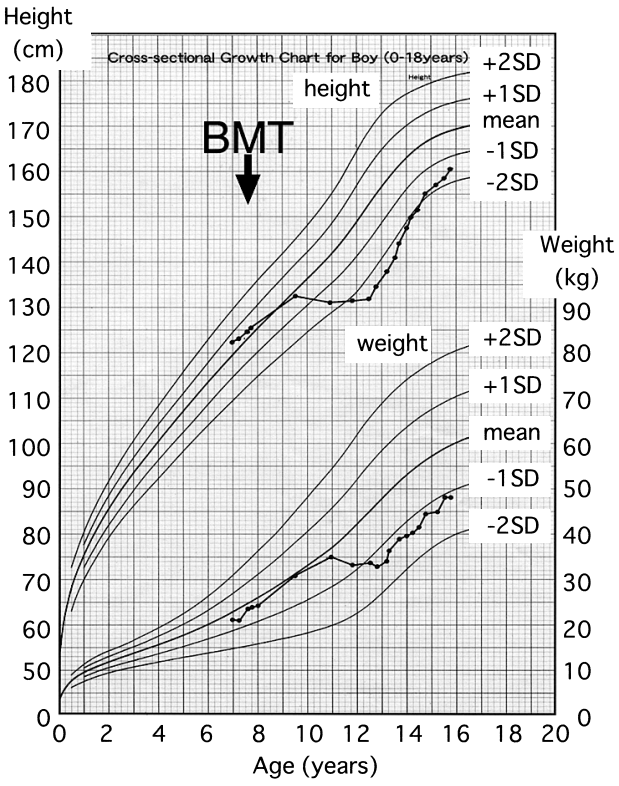

Fig. 3. Growth chart of case 2. Growth record was plotted on cross-sectional growth chart for Japanese boy in 2000, used with permission of Dr. K. Tachibana, Kanagawa Children's Medical Center.

Discussion

We have experienced two cases of autoimmune thyroid diseases following BMT for acute leukemia. Although these two cases share common clinical features of thyroid autoimmunity, their pathogeneses present quite a contrast. The first case suffered from Graves' hyperthyroidism, presumably by adoptive transmission of host-reactive clone from the donor. Because both donor and recipient were female, we could not utilize chromosome analysis to determine the origin of the hematopoietic cells. Yet, we speculated that the clone was of donor origin, because immunity in long-term allogeneic BMT survivors is mostly of donor origin [19]. Identity of the T-cells of donor and recipient was also supported both by VNTR-PCR and by Southern blot-RFLP patterns with two different probes.

In contrast, Case 2 suffered from non-goitrous hypothyroidism with positive TBII. The mechanism through which this young boy got hypothyroidism is

of pertinent interest. The possibility of radiation injury to the thyroid gland was totally ruled out because he had not been given total body irradiation. Also he received autologous BMT, not an allogeneic one, which implies that the pathological clone was in fact self-reactive. GVHD, which can lead to thyroid destruction, is also unlikely. We speculated that the reactivation of his immune system after immunosuppression during the course of BMT was the trigger for his thyroid pathology. Takasu *et al*. described three cases of autoimmune thyroid dysfunction after unilateral adrenalectomy for Cushing's syndrome [20]. In their cases, reactivation of the immune system following the reversal of glucocorticoid excess seemed to be the trigger for the thyroid pathology. In this context, we have also experienced the case of a female with autoimmune hypothyroidism with positive TSBAb following short-term corticosteroid therapy against drug-induced hepatitis (Yamamori, unpublished observation). However, we cannot rule out the possibility that our present case would have suffered from autoimmune hypothyroidism anyway even without BMT, because of his strong genetic predisposition to thyroid autoimmunity.

Several cases have been reported in which adoptively transferred lymphocytes infiltrating to the thyroid gland brought about hypothyroidism in the recipient [12–16, 18]. Since we could not perform fine needle aspiration biopsy in our case, we could not entirely rule out the possibility that self-reactive lymphocytes had infiltrated to the thyroid gland, and that thyroiditis was the major cause of hypothyroidism. Yet, positive serum TBII was at least partly indicative of B-cell involvement for the thyroid pathology in our case. To the best of our knowledge, this is the second case in which positive TBII was documented in post-BMT period. Marazuella and Steegman described a case with weakly positive TBII who developed hypothyroidism 10 months after allogeneic BMT [21]. However, they did not do further studies on the biological properties of the TBII in their report. Because their patient had been given 12 Gy total body irradiation fractionated in four daily doses of 3 Gy, the recipient's thyroid might have been damaged, and even the stimulating type of TBII (*i.e.* TSAb) might not have caused hyperthyroidism. False negative TSBAb on initial examination in our case may be misleading. We hypothesized that his markedly elevated intrinsic TSH was the interfering factor for this factitious TSBAb value, since TSBAb appeared positive after he became euthyroid with thyroid hormone replacement therapy. We believe that *de novo* production of blocking type immunoglobulin by his own immune system was the major cause of the post-BMT hypothyroidism in this case.

Au *et al*. systematically examined post-BMT thyroid status among 194 allografts and 28 autografts in Hong Kong [18], and reported 4 cases of autoimmune thyroid dysfunction (2 hyperthyroidism and 2 hypothyroidism) after BMT (3 allogeneic and 1 autologous) at a median follow up of 4 years. The onset of hyper- or hypothyroidism was 1, 3, 5, and 5.5 years after BMT in each case. They also found that all cases carried the HLA A2-B46-DR9 haplotype, strongly associated with autoimmune thyroid diseases in the Chinese population. Case 2 in our report also carried HLA A2-B46-DR9 haplotype, which came from his mother who had past history of Graves' disease. Interestingly, his sister who was also suffered from Graves' disease did not carry this allele. Thus, HLA A2-B46-DR9 haplotype may not be essential for the development of thyroid autoimmunity in this family. The onset of clinical hyperthyroidism in case 1 occurred 39 months after BMT. Growth arrest in case 2 occurred at about 2 years post-BMT (Fig. 3). This delay in onset of post-BMT thyroid autoimmunity is compatible with that observed in former case reports (*i.e.* 5 months to 8 years) [6–18, 21]. In our experience of 105 autologous and 425 allogeneic BMT during 1991 and 2001, we observed much lower incidence of post-BMT autoimmune thyroid diseases compared with Au *et al*., although we have not systematically examined thyroid status post-BMT.

In conclusion, thyroid autoimmunity following BMT for acute leukemia may display a wide spectrum, namely hyper- and hypothyroidism. Pathologic clones may be either transmitted or self-originated, and thyroid autoimmunity is likely to occur a few years after BMT. We believe that our two contrasting cases provide clues to better understanding thyroid autoimmunity occurring after BMT against acute leukemia.

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