An eight-year-old girl with autoimmune polyglandular syndrome type 3A that developed during the course of primary Epstein–Barr virus (EBV) infection: clinical implication of EBV in autoimmune thyroid disease

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ABSTRACT
An eight-year-old girl was admitted for prolonged fever and general fatigue. Bilateral red dened and swollen tonsils covered with white fur and increased numbers of atypical lymphocytes in blood led to a diagnosis of infectious mononucleosis (IM) due to primary Epstein–Barr virus (EBV) infection, which was confirmed by a positive anti-EBV viral capsid antigen IgM antibody reaction. She had a swollen thyroid gland and glycosuria at admission, which persisted after IM resolved. Undetectable thyroid-stimulating hormone (TSH), increased thyroid hormone and elevated HbA1c levels led to a diagnosis of autoimmune polyglandular syndrome type 3A, based on the presence of antibodies for TSH receptor and glutamic acid decarboxylase. The clinical significance of EBV infection in the development of autoimmune endocrine disorders has been discussed.

1. Introduction
Type I diabetes mellitus (T1DM) is frequently complicated by autoimmune thyroid disease (AITD) [1]. This clinical condition, with both T1DM and AITD is known as autoimmune polyglandular syndrome type 3A (APS3A). Viral infection has been implicated in the onset of various autoimmune disease, and Epstein–Barr virus (EBV) infection has been reported to be associated with the onset of SLE, multiple sclerosis, Sjogren’s syndrome, and rheumatoid arthritis [2,3]. We report an eight-year-old girl diagnosed with T1DM and Graves’ disease during the course of primary EBV infection.

2. Case report
An eight-year-old girl was referred and hospitalized because of prolonged fever (six days) and general malaise. She was 125.5 cm tall and weighed 22.5 kg. Her blood pressure was 105/76 mmHg, heart rate was 156 bpm, and body temperature was 36.6°C under antipyretics. She presented right cervical lymphadenopathy and bilateral red swollen tonsils covered with white fur. Hepatosplenomegaly was not observed. The thyroid gland was soft and diffusely enlarged. Routine laboratory test results are summarized in Table 1. Blood testing showed markedly increased atypical lymphocytes (43.0%) and slightly elevated aspartate aminotransferase (AST) activity (160 IU/L) and alanine aminotransferase (ALT) activity (78 IU/L). On the fourth day of admission, her white blood cell count increased to 13,400/μL, with 61.0% atypical lymphocytes. Tests for IgM and IgG antibodies against EBV viral capsid antigen (VCA) were positive, while tests for anti-EBV nuclear antigen antibody were negative, indicating infectious mononucleosis due to primary EBV infection.

HbA1c was elevated to 7.7% and a urine glucose test was positive, although her blood glucose was 76 mg/dL at admission. Thyroid function tests showed that free triiodothyronine (FT3) and free thyroxine (FT4) were increased to >20.00 pg/mL and 4.61 ng/dL, respectively, while thyroid-stimulating hormone (TSH) was undetectable. Antibodies against TSH-receptors (TRAb) and glutamic acid decarboxylase (GAD) were present at 13.9 IU/L and 247 U/mL, respectively. Thyroid scintigraphy (99mTc) showed diffuse accumulation, with 4.15% uptake (normal range, 0.4–3.0%). Based on these results, she was diagnosed with T1DM and Graves’ disease. Her family history included Hashimoto’s thyroiditis in the maternal grandmother, diabetes mellitus in the maternal great-grandmother, and...
fulminant hepatitis in the paternal grandmother. With occasional administration of antipyretics, her body temperature decreased to normal levels on the ninth of hospitalization. She was treated for hyperthyroidism with propranolol (0.5 mg/kg/day) and methylmercaptimidazole (0.5 mg/kg/day) and became euthyroid on the 49th day after starting treatment. Under diet therapy, she was discharged on the 12th day after admission, without insulin treatment. Insulin therapy was started 1 month later.

3. Discussion

There are many reports on the association between T1DM and AITD, most of which were focused on cases that were positive for anti-thyroid peroxidase (TPO) and anti-thyroglobulin (Tg) antibodies. The association of T1DM with Hashimoto's disease, including latent disease, is well known. However, there is little information about T1DM complicated by Graves' disease. The frequency of this clinical condition was reported to be 0.53% in Italy [4], 0.46% in Germany and Austria [5], and 0.7% in Poland [1] during childhood and adolescence; and was 3% in Italy [6] and 6.3% in Japan [7] during adulthood. In adults, T1DM developed in older patients, with a female predominance, after the onset of Graves' disease [6–8]. In contrast, it was reported that T1DM preceded the onset of Graves' disease in children and adolescents [4]. It was also reported that T1DM and Graves' disease developed simultaneously in one out of seven cases during childhood and adolescence [4], and in four out of 14 cases [6], and three out of 30 cases [8] in studies that included adult cases.

Due to the prevalence of PAS3A among blood relatives, genetic contributions have also been suggested. An association with HLA class II genes has also been reported for T1DM and Graves' disease [9,10]. However, multiple genetic and environmental factors are involved in the pathogenesis of T1DM and Graves' disease, and the pathogenic mechanism is not yet clear. Suspected environmental factors include iodine intake, drugs, radiotherapy, smoking, stress, and virus infection for Graves' disease [11] and virus infection, early exposure to cow's milk proteins, inactivation of vitamin D, and the enteral flora for T1DM [12].

EBV infection most frequently occurs during childhood and remains in B lymphocytes in a latent form [13]. EBV has been implicated in autoimmune diseases [14], because it has been reported that various autoantibodies are produced during EBV infection, and SLE and multiple sclerosis have developed after primary EBV infection [2]. In addition, the EBV antibody titer was significantly higher in patients with T1DM than in healthy controls [15], and the EBV antibody titer was significantly higher in children with autoimmune thyroid disease than in normal children [16].

It has been shown that viral infection occasionally induces the production of various autoantibodies, but the titer is not sufficient to lead to clinical manifestations [17]. Nagata et al. reported three women who presented with Graves' disease during the acute phase [18]. TRAb levels were high in all three cases, and a family history of Graves' disease developed in two patients. Miyashita reported three patients, 15-year-old female, 16-year-old female, and 18-year-old male, who developed hyperthyroidism 28–45 days after EBV primary infection [19], and all three presented high TRAb levels. Nagata et al. reported a

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**Table 1. Laboratory data of the patient.**

| Urinalysis | Chemistry | Serological test |
|------------|-----------|-----------------|
| TP (±)     | TP 7.7    | anti-EBV VCA-IgG 80 x |
| Glucose (4+) | Alb 3.6 | anti-EBV VCA-IgM 40 x |
| Ketone (1+) | T-Bil 1.0 | anti-EBV EBNA <10 x |
| Complete Blood Count | ALT 160 IU/L | anti-TPO Ab 184 IU/mL |
| WBC 7500 /µL | LDH 862 IU/L | anti-TsAb 1328 % |
| neutrophil 41 % | ALP 940 IU/L | anti-TRAb 13.9 IU/L |
| basophil 1 % | AMY 49 IU/L | anti-GAD Ab 247 U/mL |
| lymphocyte 14 % | CK 32 IU/L | anti-IA-2 Ab <0.6 U/mL |
| monocyte 1 % | BUN 16.3 mg/dL | anti-nuclear Ab 80 x |
| atypical lymphocytes 43 % | Cre 0.18 mg/dL | |
| RBC 466 × 10^12 /µL | UA 2.9 mg/dL | Endocrine test |
| Hb 11.3 g/dL | Na 134 mEq/l | TSH 0 µIU/mL |
| Ht 33.4 % | K 6.4 mEq/l | FT3 >20.00 pg/mL |
| MCV 77.1 fl | Cl 103 mEq/l | FT4 4.61 ng/mL |
| MCH 24.2 pg | Ca 9.6 mg/dL | HbA1c 7.7 % |
| MCHC 33.8 % | IP 5.0 mg/dL | anti-TPO Ab 184 IU/mL |
| Plt 19.3 × 10^12 /µL | CRP 0.26 mg/dL | anti-TRAb 13.9 IU/L |

TPO: thyroid peroxidase; Tg: thyroglobulin; TSAb: thyroid stimulating antibody; TRAb: TSH receptor antibody; GAD: Glutamate decarboxylase; IA-2: insulinaoma-associated antigen-2; IRI: immunoreactive insulin; CPR: C-peptide immune reactivity.
3-year-old boy who temporarily became TRAb positive during the acute phase of primary EBV infection [20]. In a histological study of the thyroid gland, 21/24 (80.7%) of patients with Hashimoto’s thyroiditis, and 5/8 (62.5%) of patients with Graves’ disease were EBER1 positive [21]. Nagata et al. also reported that TRAb(+)EBV(+)B cells were present in the peripheral blood of patients with Graves’ disease as well as healthy controls [22], and these cells expressed latent membrane protein 1 (LMP1) and TRAb when cultured at low temperature to progress to lytic condition of EB virus [10,23]. Kikutani et al. generated mice in which LMP2A was expressed in germinal center B cells, and found that these autorreactive B cells avoided cell death and differentiated into autoantibody-producing cells [24]. It has been reported that ZEBRA, one of the EBV early gene products interacts with p53 and NF-κB, which are involved in cell death and survival [25,26].

In our case, Graves’ disease and T1DM were simultaneously diagnosed during primary EBV infection. As described previously, Graves’ disease usually develops late after the onset of T1DM in children with APS3A. Considering the high level of HbA1c at admission in our case, we speculated that T1DM developed before EBV infection and that the infection induced Graves’ disease or activated latent Graves’ disease. There has been no report of Graves’ disease developing after EBV infection in younger children. This might be related to the transient, low titer antibody production in younger children. In this case, we speculate that latent TRAb antibody-producing cells were already present and that these cells were induced to produce TRAb by EBV infection, causing symptomatic hyperthyroidism. This seems to be a very interesting and valuable case regarding the association of autoimmune endocrine diseases with EBV infection.

Disclosure statement
No potential conflict of interest was reported by the author.

Author contributions
All authors meet the International Committee of Medical Journal Editors (ICMJE) authorship criteria. S.K. and H.N. contributed to drafting of a paper. A.H., A.S., K.O., M.O., M.I., N.S., and A.O. contributed to data analysis. M.N. contributed to data analysis, writing and final approval of a paper.

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