Pediatric intestinal Behçet disease complicated by myeloid malignancies

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Abstract Behçet disease (BD) is rarely seen in children. Its clinical manifestations are believed to differ between pediatric and adult patients. The characteristics of BD complicated by myelodysplastic syndrome (MDS) are well established for adult patients; however, because only a few cases of pediatric-onset BD complicated by MDS have been reported, its clinical characteristics remain unknown. We here retrospectively review pediatric-onset BD complicated by myeloid malignancies in Japan, having identified five such patients. All patients were female and had gastrointestinal involvements, but lacked both major features of BD, i.e., uveitis and association with HLA-B51. All patients had advanced MDS or acute myeloid leukemia and received chemotherapy followed by hematopoietic stem cell transplantation. These five cases suggest that intestinal BD and myeloid malignancies have one or more pathophysiological mechanisms in common.

Keywords Pediatric-onset Behçet disease · Myeloid malignancies · Gastrointestinal involvements

Abbreviations
5-ASA 5-Aminosalicylic acid
AML Acute myeloblastic leukemia
BD Behçet disease
BMT Bone marrow transplantation
GVHD Graft-versus-host disease
HSCT Hematopoietic stem cell transplantation
MDS Myelodysplastic syndrome
RAEB-t Refractory anemia with excess of blasts in transformation
TBI Total body irradiation

Introduction
Behçet disease (BD) is a type of systemic vasculitis of unknown cause [1]. BD is characterized by recurrent oral aphthae and other systemic manifestations including genital aphthae, skin lesions, and ocular disease. The prevalence of BD is high along the ancient Silk Road, which extends from East Asia to the Mediterranean Sea. BD is common in adults aged 20–40 years and has a similar prevalence in men and women; however, it rarely occurs in children [2, 3].

Myelodysplastic syndrome (MDS) comprises a heterogeneous group of hematopoietic stem cell disorders characterized by dysplastic and ineffective blood cell production. MDS is uncommon in individuals younger than 50 years and its incidence in children is estimated to be 10% or less.
of that in adults [4]. Associations between intestinal BD, MDS and the chromosomal abnormality of trisomy 8 have been well described. Cells with trisomy 8 are considered to play pivotal roles in both auto-inflammatory processes and increased rate of apoptosis of bone marrow cells [5–7]. However, an association between pediatric-onset BD and myeloid malignancies has rarely been reported. In the present series, we review five cases of pediatric intestinal BD presenting with MDS/acute myeloid leukemia (AML). These five patients all lacked major BD symptoms, had gastrointestinal involvement, were negative for HLA-B51, and were all female. Furthermore, all five underwent hematopoietic stem cell transplantation (HSCT). The symptoms of BD resolved in those who achieved hematological remission. We here discuss the typical clinical phenotype of pediatric-onset BD with myeloid malignancies and the pathological associations between these two distinct disease entities.

### Materials and methods

We retrospectively searched for and reviewed reports of pediatric-onset BD in Japan from 1995 to 2013 and identified five patients aged under 16 years who had presented with intestinal BD and myeloid malignancies. We were unable to find any similar case reports of non-Japanese origin. Case 1, Case 2 and Case 3 were treated in our institution, respectively. Case 4 and Case 5 had previously been published [8, 9]. We here describe the clinical characteristics of the three unpublished cases and summarize the clinical courses of the two previously published cases. The clinical characteristics of all five patients are summarized in Table 1. This study was approved by the Institutional Review Board of Okayama University Hospital.

### Case 1

A Japanese girl had experienced recurrent oral aphthae and anal ulcers from the age of 4 years (Fig. 1a). She had undergone colonoscopy at age 6 years because of exacerbation of her gastrointestinal symptoms. Round, punched-out ulcers were observed between the ileocecal region and sigmoid colon (Fig. 1b). Pathological examination of biopsies of the intestinal lesions demonstrated non-specific inflammation with neutrophil infiltration, leading to a diagnosis of intestinal BD without ocular or genital lesions. Glucocorticoid therapy was initiated, but was ineffective. Accordingly, thalidomide therapy was substituted and her intestinal BD symptoms resolved. However, she developed pancytopenia 6 months after initiation of thalidomide. Because bone marrow suppression by thalidomide was initially suspected, her thalidomide dosage was tapered down and discontinued, her BD remaining in remission after its cessation. However, her peripheral blood began to show blasts 18 months after the diagnosis of intestinal BD. Bone marrow examination revealed a high percentage of blast cells (22%) and dysplastic changes.
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characterized by megaloblasts and micro-megakaryocytes (Fig. 1c, d). The karyotype of her bone marrow cells was 46, XX, −7, +8[20/20]. She was diagnosed as having MDS refractory anemia with excess of blasts in transformation (RAEB-t) according to the FAB classification and eventually developed AML for which she underwent bone marrow transplantation (BMT) from her HLA 2-loci-mismatched mother. Her conditioning regimen included total body irradiation (TBI) 12 Gy and melphalan 180 mg/m². Graft-versus-host disease (GVHD) prophylaxis comprised tacrolimus and a short course of methotrexate. One month after BMT no blast cells were detected in her bone marrow. However, four months after BMT, 10% of bone marrow cells were found to be positive for monosomy 7 by fluorescent in situ hybridization (FISH). The patient achieved transient complete remission by donor lymphocyte infusion (DLI); however, she had a second bone marrow relapse after the fifth course of DLI. After administration of a sixth course of DLI, the percentage of blast cells was greater than 40%. Accordingly, she underwent BMT from her haploidentical father with a reduced-intensity conditioning regimen consisting of fludarabine 150 mg/m², melphalan 140 mg/m², low-dose TBI 2 Gy and anti-thymocyte globulin (rabbit) 4 mg/kg. GVHD prophylaxis comprised cyclosporine and a short course of methotrexate. Bone marrow examination 36 days after the second BMT revealed mixed chimerism with XX 44.2% and XY 55.8% and a high percentage of blast cells (20.4%). Karyotype analysis showed positivity for trisomy 8 and monosomy 7. The patient finally died of pneumonia. She had no BD symptoms after the first BMT.

Case 2

An 8-year-old girl with genital ulcers and abdominal symptoms was diagnosed as having intestinal BD, for which she received prednisolone and 5-aminosalicylic acid (5-ASA). Two years after the diagnosis of BD, she was found to have leukocytopenia with a white blood cell count between 2000 and 3000/µL and anemia with Hb 8 g/dL and was admitted because of exacerbation of abdominal symptoms. Bone marrow examination revealed marked dysplasia of erythroid, granulocytes, and eosinophils. The karyotype of bone marrow cells was 46, XX, +1, der(1;7)(q10;p10). She was diagnosed as having MDS refractory anemia. After having had persistent pancytopenia for 1 year, she developed MDS RAEB. Bone marrow examination revealed a nucleated cell count of 89,000/µL, and a high percentage of blasts (28.3%). She underwent allogeneic BMT from a male sibling with one mismatched locus at HLA-B in the

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**Fig. 1** Case 1. a Oral aphthae. b Colonoscopy demonstrating round punched-out ulcers throughout the colon. c, d Smear of bone marrow obtained after blasts had appeared in the peripheral blood, showing dysplasia characterized by megaloblastic erythroid precursor (arrow) and poorly lobulated micro-megakaryocyte (red arrow). The blast cells have a high nuclear to cytoplasmic ratio (arrowheads).
direction of graft-versus-host and two mismatched loci in the direction of host-versus-graft at HLA-A-B and HLA-DR. The conditioning regimen consisted of oral busulfan 16 mg/kg, cytarabine 8 g/m², and cyclophosphamide 120 mg/kg. GVHD prophylaxis comprised cyclosporine and a short course of methotrexate. Bone marrow examination performed 50 days after BMT showed no blast cells and chimerism analysis by short tandem repeat revealed 100% donor type. However, she developed increasingly severe infection, bone marrow failure, grade IV GVHD, hemorrhagic cystitis, sepsis, disseminated intravascular coagulation, and renal failure and finally died of these complications.

Case 3

An 11-year-old girl had recurrent fever with oral aphthae, tonsillitis, and painful lymphadenopathy for 1 year. Subsequently, erythema nodosum and bloody diarrhea developed in addition to exacerbation of her oral aphthae. Colonoscopy demonstrated mucosal erosion with infiltration of lymphocytes and plasma cells. A diagnosis of intestinal BD was made. Administration of prednisolone and 5-ASA improved her intestinal BD symptoms. Blood tests showed a high proportion of mature monocytes and bone marrow examination a high proportion of monoblasts (35%). Flow cytometric analysis of bone marrow cells demonstrated positive expression of myeloid markers (CD13, CD14, CD33). The karyotype of her bone marrow cells was 46, XX, t(11;19) (q23;p13.1); furthermore, the \textit{MLL-ELL} fusion gene was identified by reverse transcription polymerase chain reaction (RT-PCR). A diagnosis of AML M5b was made and chemotherapy administered according to the protocol of the Japanese Pediatric Leukemia/Lymphoma Study Group AML05 study induced complete remission. She was well for 8 months after finishing chemotherapy, but then developed bone marrow relapse. Bone marrow examination revealed a high proportion of monoblasts (64%). RT-PCR and FISH analysis were both positive for \textit{MLL-ELL}. Karyotype analysis revealed cells with 46, XX, t(11;19) (q23;p13.1). She received allogenic BMT from an HLA-matched unrelated donor. The conditioning regimen included cyclophosphamide, cytarabine, and TBI. She developed acute graft versus donor disease, with trilineage dysplasia and type 1 blast cells, leading to overt leukemia and induction chemotherapy for AML was administered. She received cord blood transplantation from a HLA-1-locus-mismatched unrelated donor. The conditioning regimen included cyclophosphamide, cytarabine, and TBI. She developed acute graft versus donor disease, which was successfully treated with pulsed glucocorticoids. This patient remained free of intestinal BD symptoms after cord blood transplantation.

Case 4 [8]

A 10-year-old girl who had been diagnosed as neurofibromatosis type 1 was admitted because of persistent fever and significant weight loss. On admission, she was found to have oral mucosal ulcers and hepatosplenomegaly and after admission she developed erythema nodosum. No other BD symptoms such as genital ulcers, uveitis or neurological abnormalities were found. Laboratory tests revealed mild anemia with Hb 10.3 g/day and thrombocytopenia with platelet count 64,000/µL. There was evidence of slight inflammation with C-reactive protein concentration 1.5 mg/dL and erythrocyte sedimentation rate 154 mm/h. Her stools were positive for occult blood. Gastrointestinal endoscopy revealed multiple punched-out ulcers and aphthae throughout the upper and lower gastrointestinal tract. A diagnosis of intestinal BD was made.

During hospitalization, neutrophils with dysplastic changes were noted in the peripheral blood. Bone marrow examination revealed megakaryocytes and erythroblasts with dysplastic changes and type 1 blast cells, leading to a diagnosis of MDS (RAEB-t in the FAB classification). The chromosomal abnormality of Ring chromosome 6 was identified, but neither monosomy 7 nor trisomy 8 was found. Her intestinal BD symptoms improved with glucocorticoid therapy. However, the MDS progressed to overt leukemia and induction chemotherapy for AML was administered. She received cord blood transplantation from a HLA-1-locus-mismatched unrelated donor. The conditioning regimen included cyclophosphamide, cytarabine, and TBI. She developed acute graft versus donor disease, which was successfully treated with pulsed glucocorticoids. This patient remained free of intestinal BD symptoms after cord blood transplantation.

Case 5 [9]

A 4-year-old girl was admitted because of recurrent symptoms, including oral ulcers, erythema nodosum, and fever. She then developed swelling of the ankles and abdominal pain accompanied by exacerbation of the erythema nodosum. Neither neurological involvement nor uveitis was found. Laboratory examination showed thrombocytopenia with a platelet count 42,000/µL and slight increase in C-reactive protein concentration (0.56 mg/dL). A pathergy test was weakly positive but HLA-B51 antigen was negative. Colonoscopy showed multiple ulcerations without specific features of inflammatory bowel disease and a diagnosis of intestinal BD was made.

Bone marrow examination revealed hypercellularity with trilineage dysplasia. Blast cells comprised more than 20% of non-erythroid cells and erythroblasts more than 50% of nucleated cells. Trisomy 8 cells were identified by both the G-banding method and FISH analysis. She was diagnosed as having AML M6 transformed from MDS (RAEB-t). Genetic examination revealed mutation of the \textit{PTPN11} gene.

BMT from an HLA-full-matched related donor was performed. The conditioning regimen included busulfan,
cytarabine, and cyclophosphamide. Since the BMT, both intestinal BD and MDS have remained in remission.

Discussion

We here present five patients from Japan with pediatric-onset BD complicated by myeloid malignancies. An association between intestinal BD and myeloid malignancies is well recognized in adults. Of 36 patients with BD and MDS presenting between 1998 and 2005 in Japan [7], 23 (63.9%) reportedly developed intestinal ulcers and 28 (77.8%) had the chromosomal abnormality of trisomy 8 in their bone marrow cells. Interestingly, a similar disease entity has rarely been described in children or non-Asian individuals.

The incidence of BD is lower in children than in adults. According to a clinical database of Japanese patients with intractable diseases receiving financial aid in 2003, only 112 of 6704 patients with BD (1.67%) were under 15 years of age [10]. Pediatric-onset BD frequently presents without the major symptoms of adult-onset BD, rather being characterized by frequent gastrointestinal involvement and absence of the HLA-B51 antigen [2, 3]. In a clinical study of 31 patients with pediatric-onset BD in Japan, no associated hematologic disorders were recognized [3].

The present five cases indicate that intestinal BD and myeloid malignancies have one or more pathophysiological mechanisms in common. One such postulated mechanism is sustained abnormalities of inflammation [11–14]. Tumor necrosis factor-α (TNF-α) is a pleiotropic cytokine associated with inflammation and apoptosis. In patients with BD there is reportedly a correlation between production of TNF-α and disease activity, suggesting that TNF-α has a pivotal role in the inflammatory reactions that characterize BD [11, 12]. Moreover, a recent meta-analysis demonstrated that several TNF-α promoter polymorphisms are significantly associated with BD [15]. Additionally, TNF-α is also considered to play an important role in MDS. TNF-α serum concentrations are significantly higher in patients with MDS than in normal controls [13, 14] and activation of the Fas-mediated apoptotic pathway by TNF-α results in increased apoptosis of hematopoietic cells in patients with MDS [16, 17]. The bone marrow cells of patients with BD show morphological dysplasia and higher rates of cell apoptosis than those of normal individuals; however, those findings are more pronounced in patients with MDS [18]. These facts suggest that the inflammatory process alone is insufficient to result in progression to myeloid malignancies in patients with intestinal BD; additional genetic/epigenetic alterations are likely needed.

Somatic chromosomal abnormalities such as monosomy 7 and trisomy 8 are frequently found in adult and pediatric patients with MDS and these cytogenetic abnormalities are considered to have some influence on hematopoiesis [4]. The influence of trisomy 8 on the immune system and programmed cell death has been investigated [19–22]. Comparison of gene expression profiles between CD34 cells with trisomy 8 and monosomy 7 has shown that immune and inflammatory response genes are upregulated and cell growth and maintenance genes are downregulated in CD 34 cells with trisomy 8 compared with monosomy 7.

Of interest, our cases share four distinctive clinical characteristics. First, none of them presented with typical manifestations of BD. Only one patient had genital ulcers and none had uveitis. All five had gastrointestinal involvement, which is observed in only 15.5% of patients with BD in Japan [1]. This lack of major symptoms of BD and frequent gastrointestinal involvement is consistent with adult-onset BD/MDS and Japanese pediatric-onset BD. Second, all our patients were negative for HLA-B*51, the prevalence of which exceeds 50% in patients with adult-onset BD in Japan. Third, they all developed advanced stage myeloid malignancies: three developed RAEB or RAEB-t, and the other two AML. HSCT was performed in four of our patients, two of whom died. In contrast, in one reported series of adults, 25 of 36 patients with BD (69.4%) presented with milder forms of MDS, such as refractory anemia or refractory anemia with ring sideroblasts [7]. Fourth, the present five cases were all female; a female predisposition has not been reported in Japanese patients with BD [1].

In conclusion, we here present five patients with pediatric-onset BD complicated by myeloid malignancies. Considering the rarity of pediatric-onset BD, simultaneous development of myeloid malignancies in all five cases is unlikely to be coincidental. The present five cases may comprise a separate disease entity in which auto-inflammatory processes affect the hematopoietic system.

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Compliance with ethical standards

Conflict of interest This study was supported in part by a Grant-in-Aid for Cancer Research and a grant for Clinical Cancer Research and Research on Children and Families from the Ministry of Health, Labour and Welfare of Japan. The authors declare no conflict of interest associated with this manuscript.

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