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Rituximab as Monotherapy and in Addition to Reduced CHOP in Children With Primary Immunodeficiency and Non-Hodgkin Lymphoma

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Children with primary immunodeficiency or chromosomal breakage syndromes are at increased risk of developing non-Hodgkin lymphomas; they cannot tolerate standard chemotherapy regimens. We report two children with diffuse, large, B-cell lymphoma; one had ataxia telangiectasia and one had common variable immunodeficiency. Both were given rituximab, 1 as monotherapy and 1 in combination with a reduced CHOP regimen. Complete remission was obtained in each patient. Use of rituximab as a first-line monotherapy or in conjunction with reduced chemotherapy should be considered to reduce cytotoxic effects. Pediatr Blood Cancer 2009;52:664–666. © 2009 Wiley-Liss, Inc.

Key words: ataxia-telangiectasia; CHOP; CVID; NHL; rituximab

INTRODUCTION

Children with congenital immunodeficiencies such as common variable immunodeficiency or chromosomal breakage syndromes (e.g., ataxia-telangiectasia and Nijmegen breakage syndrome) are at high risk of developing non-Hodgkin lymphoma [1–4]. Children with a primary immunodeficiency have a reduced tolerance to chemotherapy and an increased risk for life-threatening infections. Furthermore, children with chromosomal breakage syndromes are particularly susceptible to ionizing radiation as well as to chemotherapeutic agents that induce double-stranded breaks in DNA [3–5]. Treatment of non-Hodgkin lymphoma in these children is therefore particularly challenging.

Recently, therapy in children with non-Hodgkin lymphoma has shown excellent results with chemotherapy regimens such as Lymphomes Malins B (LMB) and Berlin-Frankfurt-Münster (BFM) [6–8]. These regimens, however, may be too toxic for children with an immunodeficiency. Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is a less-intensive regimen, and, when combined with rituximab, has shown promising results in adults with non-Hodgkin lymphoma [8–10]. Rituximab is a monoclonal antibody against CD-20 B lymphocytes [11] with only a mild-to-moderate adverse-effect profile [12]. It thus has been suggested that use of rituximab against non-Hodgkin lymphoma with reduced CHOP, or as a monotherapy, may be an appropriate therapy for children with primary immunodeficiency. Here, we report the use of rituximab as a monotherapy, and in combination with reduced CHOP, against non-Hodgkin lymphoma in two children with an underlying immunodeficiency.

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CASE REPORTS

Case 1

The first patient was a 4-year-old child with common variable immunodeficiency who was admitted with respiratory distress. A chest radiograph and computed tomography scan demonstrated a left lung mass. Cytologic, histologic, and immunologic analyses of an open lung biopsy resulted in a diagnosis of stage II non-Hodgkin lymphoma, with CD20 positive B lymphocytes. There was no evidence of a tumor in the patient's bone marrow. Treatment with intravenous rituximab at a dosage of 375 mg/m^2 once weekly for 4 weeks was initiated; subsequent treatment was four more monthly infusions. The patient's immunoglobulin levels were kept at their normal limits by administering intravenous immunoglobulins on a regular basis. Owing to the underlying immunodeficiency and high risk of infection, no chemotherapeutic agents were added.

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ORIGINAL ARTICLE

Natural history of transfusion-independent non-severe aplastic anemia in children

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Abstract Although the known clinical courses of non-severe aplastic anemia (NSAA) in children comprise spontaneous resolution, persistent NSAA, or progression to severe aplastic anemia (SAA), only a few published reports have indicated the outcome of transfusion-independent NSAA. We retrospectively evaluated the incidence and time of progression from transfusion-independent to transfusion-dependent NSAA or SAA. We reviewed the records of 70 children with acquired AA who were referred to our hospital between 1986 and 2006, and among them we found 22 patients who had transfusion-independent NSAA at diagnosis and were treated with supportive care alone until progression to transfusion-dependent AA. 22 patients were followed up for a median of 86 months (range, 11–198 months). The Kaplan-Meier estimates for progression-free survival were 62 ± 12 and 22 ± 13% at 60 and 120 months after diagnosis, respectively. None of the patients treated with supportive care alone improved hematologically. In conclusion, because the incidence of disease progression was high in patients with NSAA, a prospective randomized trial of early intervention with IST or observation alone until disease progression to SAA, followed by IST when the patients become transfusion-dependent is warranted.

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Keywords Aplastic anemia · Transfusion-independence · Natural history · Children

1 Introduction

Aplastic anemia (AA) is a rare disorder presenting with hypocellular marrow and peripheral blood pancytopenia. The severity of AA is classified into severe or non-severe type, which is critical for the prediction of outcome and the choice of therapies [1]. Bone marrow transplantation (BMT) from a human leukocyte antigen (HLA)-identical sibling is the first-line therapy for children with severe AA (SAA), if such a donor is available [2, 3]. Immunosuppressive therapy (IST) using anti-thymocyte globulin (ATG) and cyclosporine (CsA) is currently recommended for patients without HLA-identical sibling donors [4, 5]. Over the past 20 years, several large prospective studies have produced clinical evidence [6–8], and therapeutic guidelines indicating the standard management for patients with SAA [9–11].

In contrast, very few clinical trials have been conducted for patients, including adults, with non-severe AA (NSAA) [12, 13]. European group conducted a prospective study of NSAA patients who required regular transfusions and demonstrated that the response rate to ATG plus CsA was better than that to CsA alone [12]. One of the eligibility criteria in their study was red blood cell and/or platelet transfusion-dependence. The British guideline for treatment of AA recommends observation alone for transfusion-independent NSAA, whereas IST with ATG and CsA is indicated if the disease progress to transfusion-dependent [10]. The clinical course of transfusion-independent NSAA can be variable; it can resolve spontaneously, persist independently of transfusion, or progress to transfusion-dependent

NSAA or SAA. However, no published reports have clarified the natural history of transfusion-independent NSAA. We performed a retrospective analysis of transfusion-independent NSAA to evaluate the incidence and time of progression to transfusion-dependent NSAA or SAA and clinical outcomes.

2 Patients and methods

We reviewed the records of children with acquired AA who were referred to Nagoya University Hospital between 1986 and 2006. The inclusion criteria for the study were as follows: age < 18 years, no specific previous treatment for the disease, red blood cell and/or platelet transfusion independence at the time of diagnosis, and NSAA. We defined AA as pancytopenia with hypocellular bone marrow (BM) and a diagnosis required the presence of at least two of the following: hematocrit < 38%, platelet count $< 120 \times 10^9$/l, neutrophil count $< 2.5 \times 10^9$/l. The severity of the disease was graded according to international criteria and was considered severe if at least two of the following were present: neutrophil count $<0.5 \times 10^9$/l, platelet count $<20 \times 10^9$/l, reticulocyte count $<20 \times 10^9$/l with BM cellularity <25% [1]. AA that did not fulfill the criteria for SAA was defined as NSAA. Patients with constitutional AA were excluded. Bone marrow from all included patients was studied cytogenetically and specific treatment was not administered to any patients until disease progression. If the disease progressed to transfusion-dependent AA, patients with HLA-identical sibling donors underwent BMT, whereas those with no HLA-identical donors received IST or danazol. Non-responders to IST received BMT from an HLA-matched unrelated donor when suitable donors were available. Methods of BMT and IST have been previously reported [2]. We evaluated the response to IST according to the published criteria [4]. We statistically analyzed risk factors associated with the progression to transfusion-dependence. Predictive factors for progression including age at diagnosis, sex, absolute neutrophil count (ANC), platelet, absolute reticulocyte count (ARC), hemoglobin, mean corpuscular volume (MCV), and diagnosis of idiopathic thrombocytopenia (ITP) at first were evaluated by univariate analysis using the Cox regression model. Values of $p < 0.05$ were considered statistically significant. The absence of progression to transfusion-dependent AA was defined as progression-free survival. Death due to any cause and progression to transfusion-dependent AA were both considered as "events". The probability of progression-free survival was estimated using Kaplan–Meier methods [14]. All analyses were performed using SPSS 15.0 (SPSS, Chicago, IL, USA).

3 Results

As of October 2007, we analyzed data from 70 patients with acquired AA who were referred to our hospital during the study period. Of these patients, 22 (age, 3–15 years; median, 8 years; 14 female, 8 male) fulfilled the inclusion criteria. The etiology of AA was unknown in all of the patients and none had a history of hepatitis or drug exposure. At diagnosis, median ANC, hemoglobin level, platelet counts, ARC and red cell MCV were 1.15×10^9/l (range, 0.31–3.2 $\times 10^9$/l), 10.6 g/dl (range, 6.2–13.5 g/dl), 4.4×10^9/l(range, 2.0–12.6 $\times 10^9$/l), 60×10^9/l(range, 10–120 $\times 10^9$/l) and 97.0 fL (range, 90.3–109.6 fL), respectively. Clonal cytogenetic abnormalities were undetectable in any of the patients at diagnosis.

None of them experienced spontaneous resolution of the disease during a median follow-up of 86 months (range, 11–198 months). Ten patients (45%) had persistent transfusion-independent NSAA at a median follow-up period of 35.5 months (range, 11–105 months) after diagnosis. Twelve patients (55%) progressed to transfusion-dependent AA at a median of 51.5 months (range, 9–175 months) after diagnosis and required specific treatment. Two patients fulfilled the criteria for SAA. Progression-free survival was 62 ± 12 and 22 ± 13% at 60 and 120 months after diagnosis, respectively (Fig. 1). None of the potential risk factors including age at diagnosis, sex, ANC, platelet, ARC, hemoglobin, MCV, and diagnosis of ITP at first were significantly associated with disease progression.

The initial treatment for these 12 patients comprised BMT from an HLA-identical sibling donor ($n = 4$), IST with ATG and CsA ($n = 4$), CsA alone ($n = 1$) and

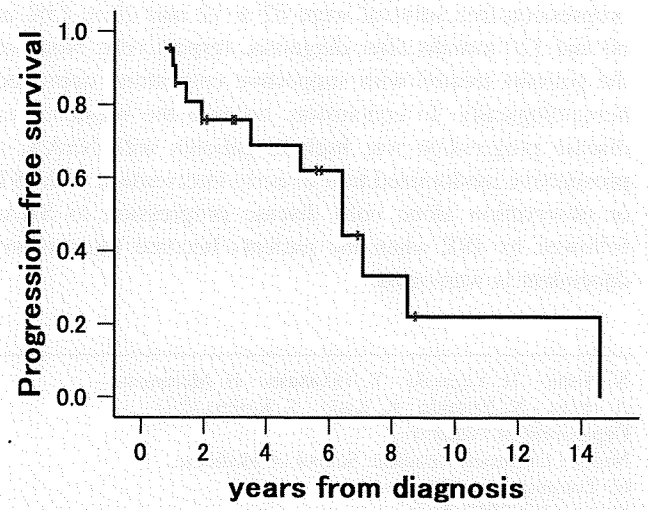

Fig. 1 Progression-free survival of 22 patients with transfusion-independent non-severe aplastic anemia. The absence of progression to transfusion-dependent AA was defined as progression-free survival. Death due to any cause and progression to transfusion-dependent AA were both considered as "events"

danazol ($n = 3$). All four patients treated with ATG and CsA, 1 of the three treated with danazol and the one patient treated with CsA alone did not respond and required salvage therapy with BMT. Marrow donors included HLA-matched unrelated donors ($n = 5$) and an HLA-identical sibling donor ($n = 1$). All ten recipients remained alive with a median follow-up of 39.5 months (range, 3–52 months) from the time of BMT. Malignant disease, clonal cytogenetic abnormalities, and symptoms of paroxysmal nocturnal hemoglobinuria did not develop in any of the patients during the follow-up period and all remained alive at the time of the last follow-up. Table 1.

4 Discussion

Although urgent treatment is recommended for SAA patients, when treatment for NSAA patients should begin is controversial. Transfusion-dependent NSAA has a better chance of response to ATG and CsA than to CsA alone [12]. However, few clinical studies have addressed the issue of how transfusion-independent NSAA patients should be managed. According to the British guideline for treatment of AA, these patients should only be observed, without definitive treatment, until they require regular transfusions [10].

Only two studies from St. Jude Children's Research Hospital have investigated the natural history of NSAA. Khatib et al. [15] reported the outcome of 12 children with NSAA who were treated at St. Jude Children's Research Hospital between 1978 and 1991. Five of them progressed to SAA at a median interval of 18 months, and the other seven did not require therapy. They concluded that patients with NSAA might not need treatment unless the disease becomes severe. Only one of four patients who received ATG after disease progression showed a partial response in that study. Howard et al. [16] reported the natural history of an additional 11 patients with NSAA who were referred to St. Jude Children's Research Hospital between 1992 and 2002. They demonstrated that 16 of 23 patients (67%) with NSAA progressed to SAA at a median of 9.5 months (range, 2–290 months) after diagnosis and that the 3-year

Table 1 Characteristics and clinical outcome of 22 patients with transfusion-independent non-severe aplastic anemia

Age at diagnosis (year)	Sex	Hematological values at diagnosis						Months from diagnosis to transfusion-dependence	Treatment after progression of the disease	Survival time from diagnosis (months)
		WBC ($\times 10^9$/l)	ANC ($\times 10^9$/l)	Hb (g/dl)	Plt ($\times 10^9$/l)	ARC ($\times 10^9$/l)	MCV (fL)			
3.0	Female	3.1	0.31	6.2	45	22	106.7	175	Danazol	198
6.0	Male	4.4	3.0	9.0	66	59	97.0	102	IST, uBMT	182
4.4	Female	3.1	0.80	8.1	26	26	106.9	42	IST, uBMT	151
5.0	Female	6.7	2.7	11.4	105	79	94.0	85	Danazol	146
5.8	Female	4.0	0.50	8.0	32	61	97.8	12	Danazol	132
9.1	Female	5.1	3.0	11.5	72	43	95.4	17	IST, sib BMT	130
5.8	Male	5.1	1.3	10.0	23	55	97.0	23	IST, uBMT	125
4.9	Female	4.25	2.1	10.1	34	55	96.1	61	Sib BMT	120
11.9	Female	4.5	2.3	13.5	60	22	100.8	NE	None	105
8.0	Male	3.0	1.5	11.0	30	23	93.5	77	CsA, uBMT	95
5.9	Male	2.0	0.38	9.5	126	83	99.0	77	Sib BMT	89
7.2	Female	3.0	0.39	11.0	20	61	94.2	NE	None	83
15.2	Female	2.6	0.91	11.3	41	63	109.6	NE	None	67
9.1	Female	3.4	1.0	13.0	64	70	96.8	NE	None	69
3.0	Male	7.8	2.0	11.3	69	69	90.3	13	Sib BMT	65
14.0	Female	2.2	0.8	9.0	30	64	108.5	NE	None	36
12.3	Male	4.6	3.2	11.9	36	10	102.7	NE	None	35
13.2	Female	3.8	1.2	12.8	53	55	94.5	NE	None	25
5.2	Male	5.2	1.0	9.9	61	52	96.0	9	Sib BMT	32
8.2	Male	2.9	1.3	9.6	85	120	97.6	NE	None	23
7.7	Female	2.5	0.60	10.8	36	95	95.0	NE	None	13
9.1	Female	3.6	1.1	10.3	42	63	100.6	NE	None	11

WBC white blood cell, *ANC* absolute neutrophil count, *Hb* hemoglobin, *Plt* platelet, *ARC* absolute reticulocyte count, *MCV* mean corpuscular volume, *NE* not evaluated, *IST* immunosuppressive therapy, *sib BMT* bone marrow transplantation from sibling donor, *uBMT* bone marrow transplantation from unrelated donor, *CsA* cyclosporine

progression-free survival rate was 43.5 ± 24.3%. In the present study, we found that 12 patients (55%) with transfusion-independent NSAA progressed to transfusion-dependent AA at a median of 51.5 months (range, 9–175 months) after diagnosis. The progression-free survival rate was 62 ± 12 and 22 ± 13% at 60 and 120 months after diagnosis, respectively. The interval from diagnosis to disease progression was much longer in our study than in that of Howard (51.5 vs. 9.5 months). They did not specify the requirement for transfusion at diagnosis and the definition of disease progression was progression from NSAA to SAA. Because the start of definitive treatment for AA is determined based on whether the patient becomes transfusion-dependent, we decided to adopt requirement of transfusion as the endpoint in our study.

Non-severe aplastic anemia is sometimes sub-classified as mild AA or moderate AA (MAA) [16]. Moderate disease is defined by the presence of at least two of the following hematological values: neutrophil count $<1.0 \times 10^9$/l, platelet count $< 50 \times 10^9$/l and reticulocyte count $< 60 \times 10^9$/l with hypocellular marrow and non-fulfillment of the criteria for SAA. According to this definition, 19 of our 22 patients were classified as having mild AA and only 3 were classified as having MAA.

Because a Japanese multicenter clinical trial of ATG ± CsA has been conducted for children newly diagnosed with MAA since 1977 [17], recently newly diagnosed MAA children were not included in our analysis since 1997. This explains why fewer patients with MAA are included in our study compared with that of Howard. The difference in endpoints and disease severity of the study populations might account for the difference in the interval from diagnosis to disease progression between the two studies.

The response rates to IST among AA patients with long-standing disease are low [18]. None of the four patients in our study, who received IST at a median of 32.5 months (range, 17–102) after diagnosis, responded. The European Group for Blood and Marrow Transplantation (EBMT) recently analyzed prognostic factors in 912 patients with AA treated with IST [18]. Multivariate analysis showed that a longer interval between diagnosis and treatment is a statistically significant predictor of worse survival. They compared survival rates between the periods from 1991 to 1996 and from 1997 to 2002 and found that the survival rates were comparable among patients with SAA. On the other hand, the survival rate has significantly decreased more recently among patients with NSAA. The decreased survival of patients with NSAA given IST is associated with delayed treatment. The EBMT study indicated the importance of prompt IST therapy for patients with AA.

Another European prospective study showed a response rate of 74% for patients with MAA, which is comparable with that for patients with SAA [10]. A Japanese prospective study of 60 children with MAA showed that 32 of them (53%) responded to IST [17]. The median interval between diagnosis and the start of therapy was 21 days (range, 1–179 days) in that study.

A high ratio of children with NSAA treated only with supportive therapy progress to SAA and are mostly unresponsive to IST after disease progression [16]. On the other hand, BMT, including that from an alternative donor, may restore bone marrow function in NSAA patients even after progression to SAA long after diagnosis [2, 19]. The survival rate of patients given BMT has steadily improved over the past decade, especially among children [18]. However, the morbidity and mortality rates are still considerable among patients who undergo BMT. One therapeutic option for patients with transfusion-independent NSAA is early intervention with IST, and another is observation alone until progression to SAA, followed by IST when the patients become transfusion-dependent. A second-line therapy for non-responders might be BMT from related or unrelated donors. A prospective randomized trial is warranted to clarify the risks and benefits of these two options.

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Plasmacytoid Dendritic Cell Leukemia in Children

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Summary: $CD4^+/CD56^+$ malignancies are rare hematologic neoplasms, which have recently been shown to represent the malignant counterpart of plasmacytoid dendritic cells (pDC). A 5-year-old boy initially presented with multiple subcutaneous lesions on his upper and lower extremities. Skin biopsy results showed large atypical lymphoid cells in the dermis. The blast cells were stained with CD4 and CD56. In the bone marrow aspirate, 20% of the blast cells were found. The patient was diagnosed as acute unclassified leukemia and received chemotherapy designed for the treatment of acute myeloid leukemia. He achieved a complete remission that lasted for 8 months. However, multiple subcutaneous lesions recurred 1 month after the end of the therapy, with increasing blast cells in his blood. Immunophenotypically, the blast cells were positive for CD2, CD4, CD7, and CD56, and negative for CD3, CD13, CD19, CD33, and CD34 antigens. The blast cells were positive for CD123 (interleukin-3 receptor α chain) and blood dendritic cell antigen-2, which are expressed on pDC. The patient was diagnosed as acute leukemia derived from pDC. The $CD4^+$, $CD56^+$, $CD3^-$, $CD13^-$, $CD19^-$, $CD33^-$ profile is highly suggestive of this disease, and the CD123 and blood dendritic cell antigen-2 markers are useful in helping to diagnose pDC leukemia.

Key Words: acute leukemia, plasmacytoid dendritic cell, $CD4^+/CD56^+$ hematodermic neoplasm, children

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Several reports in the literature have described unusual and rare hematopoietic tumors that express CD4 and CD56, without expressing CD3, CD13, CD19, and CD33 conventional lineage markers.[1–7] Clinically, these cases are characterized by a rapid aggressive course, extranodal and notable skin involvement, and a frequent evolution toward overt leukemia. In such cases, even if they had been classified as $CD4^+/CD56^+$ agranular cutaneous lymphoma1,[3,6] natural killer cell leukemia/lymphoma,[2,4,7] or myelomonocytic lineage,[5] the data collected from these patients suggested the possibility that this might in fact be a new form of leukemia. In 2002, Feuillard et al[8] reported an extensive study of 23 cases of $CD4^+/CD56^+$ leukemia and proposed a new name for this type of leukemia, type 2 dendritic cell (DC2)/plasmacytoid dendritic cell (pDC) leukemia. The recent World Health Organization (WHO)/European Organization for Research and Treatment of Cancer classification for cutaneous lymphomas decided to classify this entity as the $CD4^+/CD56^+$ hematodermic neoplasm or early pDC leukemia/lymphoma.[9] Even though this disease mainly affects the elderly, with the average age of the patients noted to be 67 years, there are a few pediatric cases with a less aggressive clinical course that also have been reported.[8,10] We describe the clinical course of a 5-year-old boy with pDC leukemia that was confirmed by immunophenotyping.

PATIENTS, MATERIALS, AND METHODS

Case Report

A 5-year-old boy initially presented with multiple subcutaneous lesions on his upper and lower extremities. The lesions were red to purple with elastic hard nodules that were 20 to 30 mm in size and fixed to the skin (Fig. 1A). The results of a complete blood count were normal and blast cells were not found. The skin biopsy showed large atypical lymphoid cells in the dermis (Fig. 1B). Typical of a leukemic infiltrate, these cells infiltrated around the blood vessels. Histopathologically, the blast cells were stained with CD4, CD56, CD68, and lysozyme, whereas they were negative for CD1a, CD3, CD8, CD34, and S-100. In the bone marrow (BM) aspirate, 20% of the blast cells had fine chromatin and nucleoli (Fig. 1C) that were negative for myeloperoxidase (MPO), butyrate esterase, and chloroacetate esterase stains. Chromosome analysis of the blast cells showed a normal karyotype. The patient was diagnosed as acute unclassified leukemia and received chemotherapy in accordance with the guidelines of the national cooperative studies for acute myeloid leukemia (AML99),[11] which included a high dose of cytosine arabinoside, etoposide and mitoxantrone, and central nervous system prophylaxis using intrathecal methotrexate, cytosine arabinoside, and hydrocortisone. At 7 days after starting the induction therapy, the subcutaneous lesions became undetectable, although they did appear once again at day 40. After starting consolidation therapy, the lesions finally disappeared. The patient achieved a complete remission (CR) that lasted for 8 months. However, multiple subcutaneous lesions appeared on his body 1 month after his therapy was discontinued. Skin biopsy results indicated a leukemic infiltration of blast cells that had the same characteristics noted at the initial presentation. At 5 months after the relapse, there was an increase in the blast cells within the blood and the analysis of the BM aspirate

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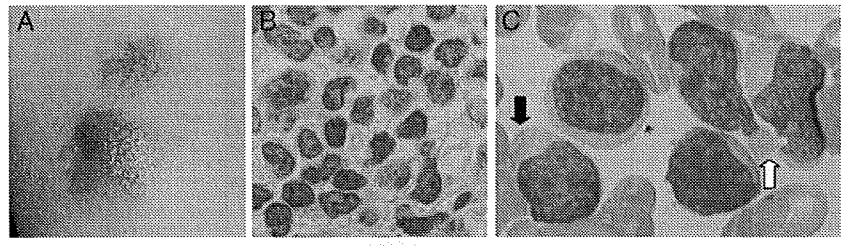

FIGURE 1. Clinical and pathologic findings of the skin lesions and bone marrow findings. A, Nodular erythematous lesions are seen in the left upper extremity; B, Hematoxylin-eosin staining of the skin biopsy. Large atypical lymphoid cells are infiltrated in the dermis; C, May-Giemsa staining of the blast cells in the bone marrow. A peculiar arrangement of the vacuoles along the cytoplasmic outline is observed (pearl necklace appearance, closed arrow). Another feature noted is the presence of pseudopodia-shaped cytoplasmic expansions (open arrow). Original magnification ×1000 for panel (B, C).

revealed that 62% of the cells were blast cells. Immunophenotypically, the blast cells were positive for CD2, CD4, CD7, CD56, and human leukocyte antigen (HLA)-DR, whereas they were negative for CD3 (both surface and intracytoplasmic), CD5, CD10, CD13, CD14, CD19, CD33, and CD34 (Fig. 2). Upon further characterization of the blast cells, we found that they expressed CD123 (interleukin-3 receptor α chain, IL-3Rα) and blood dendritic cell antigen-2 (BDCA-2), both of which are indicative of a pDC origin (Fig. 3). Therefore, we diagnosed the patient as acute leukemia derived from pDC. BM transplantation (BMT) was performed in this patient using cells from a HLA-matched unrelated donor. The conditioning regimen included administration of melphalan and total body irradiation (12 Gy). Prophylaxis against graft-versus-host disease consisted of administration of tacrolimus and short-term methotrexate therapy. After achieving engraftment, the patient developed acute graft-versus-host disease grade 1 and thrombocytic microangiopathy of the intestine, which was controlled by supportive care. The patient achieved a second remission state that was maintained; unfortunately, he developed idiopathic interstitial pneumonia at 164 days after BMT. The patient did not respond to steroid pulse therapy and died of interstitial pneumonia at 193 days after the BMT.

Immunophenotypic Profile

For immunophenotyping of the leukemic blasts, mononuclear cells (MNCs) were isolated by Ficoll-Hypaque density gradient centrifugation from the BM. The cells were analyzed by flow cytometry (Becton Dickinson, San Jose, CA) with a panel of monoclonal antibodies. The antibodies used were CD2, CD3, CD4, CD5, CD7, CD10, CD11c, CD13, CD14, CD16, CD19, CD33, CD34, CD45, CD56, HLA-DR, CD123 (Pharmingen, Heidelberg, Germany), and BDCA-2 (Myltenyi Biotec, Gladbach, Germany). Intracytoplasmic flow cytometric staining of CD3 was performed after paraformaldehyde fixation (2%) and permeabilization of the cells (saponin 0.3%). Samples were scored as being positive for immunologic markers if there was staining of more than 20% of the cells.

Naive T-lymphocyte Stimulation

The proliferative response of naive T lymphocytes was evaluated in response to stimulated and nonstimulated

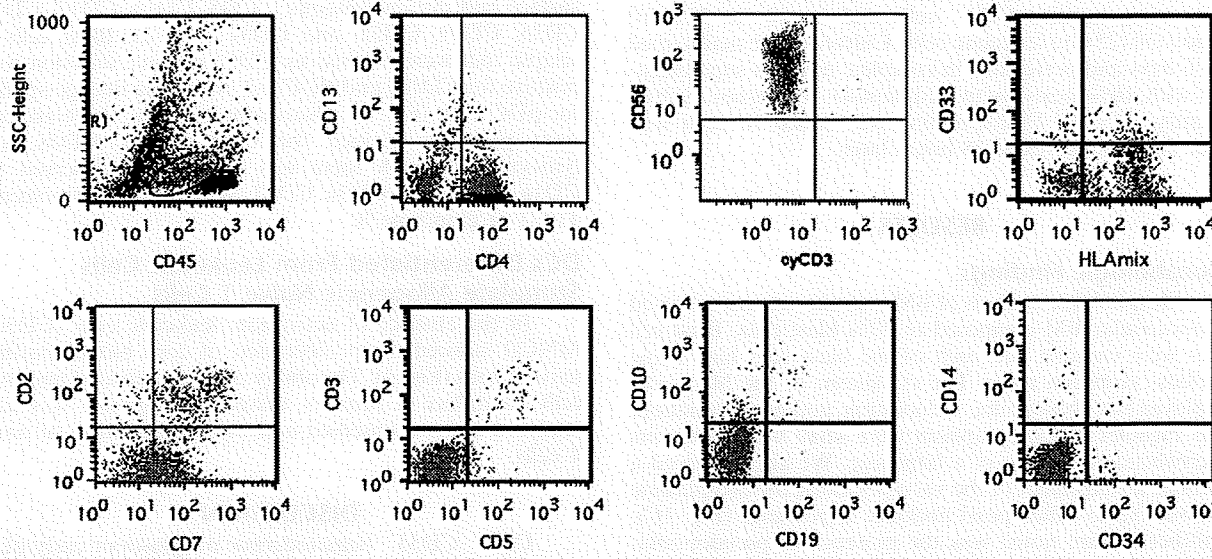

FIGURE 2. Flow cytometry analysis of the blast cells. Bone marrow aspirate shows an abnormal blast population with low CD45 staining and low side scatter (SSC). The blast cells are positive for CD2, CD4, CD7, CD56, and human leukocyte antigen (HLA)-DR, and negative for CD3 (both surface and intracytoplasmic), CD5, CD10, CD13, CD14, CD19, CD33, and CD34.

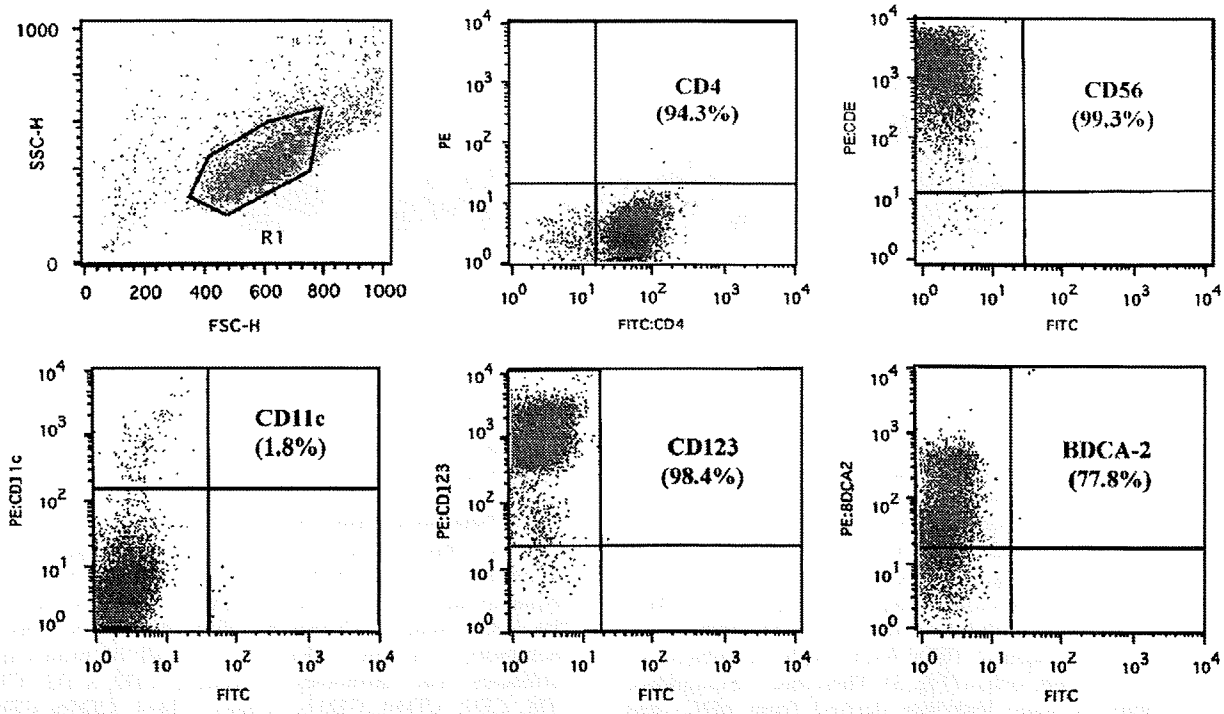

FIGURE 3. Flow cytometry analysis of the blast cells purified by CD56 positive selection. The blast cells purified by CD56 positive selection are positive for CD4, CD123, and BDCA-2 in the absence of CD11c expression. BDCA-2 indicates blood dendritic cell antigen-2; FITC, fluorescein isothiocyanate; FSC, forward scatter; SSC, side scatter.

leukemic cells. To obtain naive T lymphocytes, $CD3^+$ lymphocytes were isolated from cord blood. To obtain leukemic cells, $CD56^+$ MNCs were isolated from the BM of the patient by positive immunomagnetic selection (auto MACS, Miltenyi Biotec), which resulted in more than 97% purity. For activation of leukemic cells, $CD56^+$ MNCs were incubated with IL-3 for 2 weeks. Mixed lymphocyte cultures were conducted in triplicate in 200 μL 96-well U-bottom plates (Applied Biosystems) by mixing 25×10^3 responding purified $CD3^+$ cells and 5×10^3 to 50×10^3 irradiated (25 Gy) leukemic cells. Cultures were maintained for 6 days and performed in complete medium supplemented with 10% heat-inactivated human AB serum. Subsequently, 37×10^3 [^{3}H]thymidine was added to each well with the cells harvested 18 hours later.

RESULTS

Morphologic Findings

Morphologic analysis of BM aspirates at onset showed normocellular BM composed of 20% blast cells. Blast cell sizes were variable and each of the blast cells had a low nucleus-cytoplasm ratio. The cytoplasm of the blast cells displayed faint basophilia, vacuolation, and no granulation. The vacuoles of the cytoplasm were small with numerous microvacuoles or were of a large size. We also observed a peculiar arrangement of the vacuoles along the cytoplasmic outline, the so-called pearl necklace appearance (Fig. 1C). Another feature noted was the presence of pseudopodia-shaped cytoplasmic expansions. The shapes of the nuclei were round or irregular, with each nucleus exhibiting fine chromatin with clear nucleoli. The MPO, butyrate esterase and chloroacetate esterase reactions were all negative. Dysplasia of the trilineage hematopoietic cells was not observed.

Immunophenotype

BM aspirate at relapse shows an abnormal blast population with low CD45 staining and low side scatter. The blast cells were positive for CD2, CD4, CD7, CD56, and HLA-DR, whereas they were negative for CD3 (both surface and intracytoplasmic), CD5, CD10, CD13, CD14, CD16, CD19, CD33, and CD34 (Fig. 2). Blast cells at relapse that were purified by CD56 positive selection were positive for CD123 and BDCA-2, which are lineage-specific for pDC cells (Fig. 3). Histopathologically, the blast cells were positively stained for CD4, CD56, CD68, and lysozyme, although there was no staining observed for CD1a, CD3, CD8, CD34, CD57, S-100, cytotoxic molecule, TIA-1 or granzyme B.

DCs Differentiated From Leukemic Cells Stimulate Allogeneic Naive T Cells

In the absence of activation, leukemic cells ($CD56^+$ MNCs) induced the proliferation of cord blood -purified naive T cells (Fig. 4). The ability of IL-3-activated leukemic cells to induce a primary allogeneic response was also analyzed and we observed a strong proliferation of naive T cells.

DISCUSSION

$CD4^+/CD56^+$ hematodermic neoplasm or early pDC leukemia/lymphoma has been described as a disease entity in the WHO/European Organization for Research and Treatment of Cancer classification for cutaneous

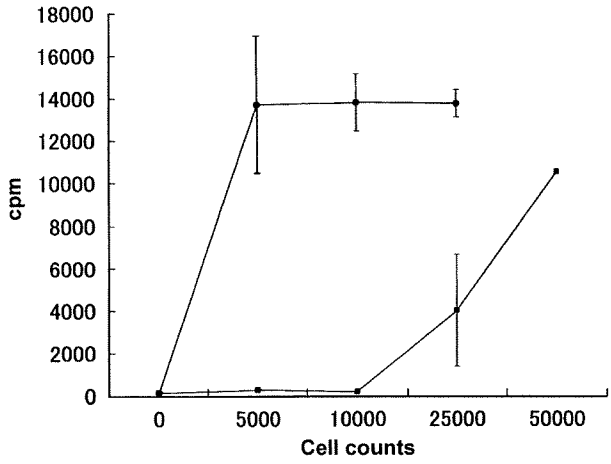

FIGURE 4. Leukemic cells stimulate naive T cells. $CD3^+$ allogeneic naive T-lymphocyte response to increasing numbers of irradiated leukemic cells was measured after a 6-day mixed lymphocyte cultures by 18-hour [^{3}H]thymidine incorporation. Both the fresh isolated blast cells purified by CD56 positive selection (closed squares) and interleukin-3-activated blast cells (closed circles) were capable of stimulating naive $CD3^+$ T-lymphocyte proliferation at different concentrations.

lymphomas.[9] These pDC leukemia/lymphomas coexpress CD4 and CD56 without any other lineage-specific markers and have been identified as arising from pDC.[8,12] The pDC leukemia is rare, representing less than 1% of acute leukemia[13] and 0.7% of cutaneous lymphomas.[14]

Clinical presentation at the time of diagnosis usually consists of finding an isolated cutaneous lesion that rapidly evolves into multiple sites, and then proliferates into the blood, BM, lymph nodes, and other areas. The most common of the clinical features are the skin lesions, which are present in 83% of patients.[8] In our patient, although there was an initial presentation of multiple skin lesions, there was 20% of BM infiltration with leukemic blasts. Although the condition of most patients is generally good at the initial presentation, the clinical course is usually aggressive and rapidly fatal.[8,15]

Feuillard et al[8] described the blast morphology of the pDC leukemia in detail. This blast morphology is pleomorphic and has cell sizes that vary from small to large with regular-shaped round or oval nuclei. There is an average abundance of cytoplasm that is slightly basophilic and nongranular, although it displays a heterogeneous structure with a ring or "pearl necklace" of microvacuoles that are located beneath the cytoplasmic membrane. The cytoplasmic membrane often exhibits pseudopods. Cytochemical reactions for MPO and the butyrate and chloroacetate esterases are negative. In our patient, the morphology of the blast cells was consistent with these descriptions (Fig. 1C).

Currently, there are several other groups that have been searching for specific validated markers. A series of monoclonal antibodies from blood dendritic cells (BDC) have been produced that make it possible to identify subsets of the DCs in human peripheral blood.[16] BDCA-2 and BDCA-4 are expressed on $CD11c^-CD123^{bright}$ pDCs, whereas BDCA-3 is expressed on a small population of $CD11c^+CD123^-$ DCs. CD123 is also positive for pDCs. The blast cells of our patient highly expressed BDCA-2 and CD123 antigens. So far, the use of BDCA-2 has been restricted to just a few studies. Chaperot et al[17] showed that there was BDCA-2 expression in 6 out of 7 patients with functionally characterized pDC leukemia and in addition, these patients were found to express BDCA-4 in all cases. These findings suggest that BDCA-2 and BDCA-4 antibodies are potential markers for the diagnosis of pDC leukemia.

In the literature, we found that immunophenotyping has been used to definitively diagnose 6 children with pDC leukemia (Tables 1, 2). In a report from the French Groupe d'Etude Immunologique des Leucémies, 3 children examined achieved CR through the use of AML-oriented (n = 1) or acute lymphoblastic leukemia (ALL)-oriented induction therapies (n = 2).[8] However, in the patient treated with AML-oriented therapy, there was a relapse at 12 months after the initial diagnosis and the patient subsequently died. In contrast, no relapse was noted in the 2 patients treated with ALL-oriented therapies and these patients are still alive at 10 and 98 months after diagnosis, respectively. The latter patient received an allogeneic BMT in the first CR. In 2 of these 3 patients, cutaneous lesions typical of pDC leukemia were found.

Rossi et al[10] found 3 patients with pDC leukemia among 1363 new patients with acute leukemia by using an extensive panel of monoclonal antibodies that included BDCA-2 and BDCA-4. These blast cells expressed CD4,

TABLE 1. Clinical Presentation and Outcome of Plasmacytoid Dendritic Cell Leukemia in Children

Patient No.	Age/ Sex	Extramedullary Sites of Involvement: Skin	Lymph Node	Liver	Spleen	Leukocyte ($\times 10^9$/L)	Blasts (BM) (%)	Initial Therapy	CR	Relapse	Survival	Reference
1	5/M	+	—	—	—	5	20	CA, Mit, VP16	+	9 mo (Skin)	20 mo +	Current case
2	8/M	+	+	+	+	2.8	70	CA, Mit	+	12 mo (BM, Skin)	37 mo +	8
3	14/M	+	—	—	—	65	96	PSL, VCR, Rubi, Lasp	+	—	10 mo	8
4	6/F	—	+	+	—	9.3	90	PSL, VCR, DNR, Lasp	+	—	98 mo	8
5	15/M	—	—	+	+	5.6	82	PSL, VCR, DNR, Lasp	+	60 mo (BM)	129 mo	10
6	8/F	—	—	—	—	8.1	96	PSL, VCR, DNR, Lasp	+	—	67 mo	10
7	14/M	—	—	—	—	108	92	PSL, VCR, DNR, Lasp	+	—	54 mo	10

BM indicates bone marrow; CA, cytarabine; CR, complete remission; DNR, daunorubicin; F, female; Lasp, L-asparaginase; M, male; Mit, mitoxantrone; PSL, prednisone; Rubi, rubidomycine; VCR, vincristine; VP16, etoposide; +, death.

TABLE 2. Immunophenotypes of Plasmacytoid Dendritic Cell Leukemia in Children

Patient No.	CD2	CD3*	CD4	CD7	CD10	CD13	CD14	CD19	CD33	CD34	CD56	HLA-DR	CD123	BDCA-2	BDCA-4	Reference
1	+	—	+	+	—	—	—	—	—	—	+	+	+	+	NA	Current case
2	—	—	+	—	—	—	NA	—	—	—	+	+	+	NA	NA	8
3	—	—	+	+	—	—	NA	—	—	—	+	+	NA	NA	NA	8
4	—	—	+	+	—	—	NA	—	—	—	+	+	NA	NA	NA	8
5	+	—	+	—	—	—	—	—	+	—	+	+	+	+	+	10
6	—	—	+	—	—	—	—	—	—	—	+	+	+	+	+	10
7	—	—	+	+	—	—	—	—	+	—	+	+	+	+	+	10

*Both surface and intracytoplasmic.
BDCA indicates blood dendritic cell antigen; HLA-DR, human leukocyte antigen-DR; NA, not available.

CD56, HLA-DR, CD123, BDCA-2, and BDCA-4, whereas specific markers for myeloid, T-lineage, B-lineage, and CD34 were absent. Although the immunophenotypes of the blast cells were clearly that of pDC leukemia, the clinical presentation and the course of the disease was different from that which has been reported in other studies. None of the 3 patients presented with any skin involvement. The morphology of the blast cells was classified as French-American-British L2, and none of the cases showed any characteristic findings of pDC leukemia, such as cytoplasmic vacuolations or pseudopodia-shaped cytoplasmic expansions. The patients were all treated according to the BFM-based ALL protocol[18] and all exhibited a good response to prednisone. Although 1 patient relapsed at 60 months, the patient is now in CR after an allogeneic BMT, and 69 months later, is still leukemia-free. The other 2 patients have remained in CR for 67 and 54 months, respectively. The disease courses were typical of those in children with ALL and seemed less aggressive, as compared with that seen in adult patients with pDC leukemia.

The patient in this study initially presented with cutaneous lesions and the morphology of his blast cells was characterized by numerous microvacuoles and pseudopodia-shaped cytoplasmic expansions. After the initial AML-oriented therapy, he developed an early relapse. Childhood cases may be classified into 2 groups: aggressive type with skin involvement and a less aggressive type without skin involvement.

In conclusion, in this study we report a child with pDC leukemia. The $CD4^+$, $CD56^+$, $CD3^-$, $CD13^-$, $CD19^-$, $CD33^-$ phenotype was highly suggestive of this disease. An additional analysis that used CD123 and BDCA-2 antibodies helped to confirm the initial diagnosis. As an optimal therapy for pDC leukemia in children is still unknown, a cooperative study needs to undertaken to further investigate possible therapies that can be used to treat this disease.

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Treatment of Children With Refractory Anemia: The Japanese Childhood MDS Study Group Trial (MDS99)

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Background. Although hematopoietic stem cell transplantation (HSCT) is offered as a curative therapy for pediatric myelodysplastic syndrome (MDS), it may cause severe complications and mortality. Several reports have shown the efficacy of immunosuppressive therapy (IST) in adult patients with refractory anemia (RA), but its safety and efficacy remains to be fully elucidated in childhood RA. ***Procedure.*** Eleven children diagnosed with RA and enrolled on a prospective multicenter trial conducted by the Japanese Childhood MDS Study Group were eligible for analysis. If patients showed transfusion dependent or suffered from infection due to neutropenia, they received IST consisting of antithymocyte globulin (ATG), cyclosporine (CyA), and methylprednisolone (mPSL). ***Results.*** Eight children received IST, 2 received only supportive therapy, and one underwent HSCT without IST. Five (63%) of eight children who received IST showed hematological response. Of note, one patient showed the disappearance of monosomy 7 after IST. Responders were significantly younger than non-responders (29 months vs. 140 months; $P=0.03$). No severe adverse events related to IST were reported in this study. Of 6 children with chromosomal abnormalities who received IST, four showed hematological response. The probability of failure-free and overall survival at 5 years was $63 \pm 17\%$ and $90 \pm 9\%$ respectively. ***Conclusion.*** IST is likely to be a safe and effective modality for childhood RA. Pediatr Blood Cancer 2009;53:1011–1015. © 2009 Wiley-Liss, Inc.

Key words: myelodysplastic syndrome; refractory anemia; children; immunosuppressive therapy

INTRODUCTION

Myelodysplastic syndrome (MDS) is a hematopoietic stem cell disorder and rarely occurs in childhood [1,2]. Refractory anemia (RA) is a subgroup of MDS with less than 5% of blasts in the bone marrow (BM) and little is known about childhood RA because of its rarity. European Working Group of MDS in Childhood (EWOG-MDS) retrospectively analyzed the clinical characteristics of children with RA [3]. They found that neutropenia and thrombocytopenia were more prominent than anemia [3,4] and karyotype had a strong impact on prognosis in children with RA [3]. Children with monosomy 7 were significantly more likely to progress to advanced disease and they recommended hematopoietic stem cell transplantation (HSCT) for this unfavorable group as early as possible, whereas, appropriate treatment for children with chromosomal abnormalities other than monosomy 7 and those with normal karyotypes remained to be determined.

Disturbance of the immune system may play a role in pathogenesis in some adults and children with RA [5–7]. Several reports have shown positive effects of immunosuppressive therapy (IST) in adult patients with RA [8–12]. The hematological response rate of IST was reported as 30–80% but IST could not restore the cytogenetic abnormalities or dysplastic features. Recently, EWOG-MDS reported the results of IST consisting of antithymocyte globulin (ATG) and cyclosporine A (CyA) in children with hypoplastic refractory cytopenia (RC) and normal karyotype or trisomy 8 who were thought as being at low risk of progression to advanced MDS [13]. However, the role of IST in children with RA has not been fully elucidated because the above study selected children with favorable predictive factors for a positive response to IST.

This study reports the outcome of 11 children with RA enrolled on a prospective multicenter trial (MDS99) conducted by the Japanese Childhood MDS Study Group, which applied IST with ATG and CyA to unselected patients who needed intervention.

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PATIENTS AND METHODS

Patients

Eleven children younger than 16 years of age were enrolled onto MDS99 from September 1999 to March 2004. They were diagnosed as having RA according to the French-American-British (FAB) classification [14] and diagnosis was confirmed by the central review of morphology by two independent investigators [15]. Cytogenetic analysis of the bone marrow cells was performed in each institution. There were no patients who had undergone previous chemotherapy or radiotherapy, nor patients with a history of congenital bone marrow failure syndrome or aplastic anemia in the analysis. The study was approved by the Steering Committee of the Japanese Childhood MDS Study Group and the institutional review boards of the participating institutions or the equivalent organization. Informed consent was obtained from the guardians of the patients.

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The authors report no potential conflicts of interest.

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Treatment Protocol

Each patient with RA required repetitive bone marrow aspiration at 6–8 weeks intervals in order to confirm the diagnosis. If the disease was stable and blood transfusion was not required, patients were observed closely without any therapy. If patients showed transfusion dependent or suffered from infection due to neutropenia, IST was administered as follows: horse ATG (15 mg/kg/day) for 5 days as a slow intravenous infusion over 12 hr, CyA (6 mg/kg/day given orally as an initial dose, and the dose was adjusted to achieve a whole blood trough level of 100–200 ng/ml) was started on day 1 and continued until day 180, and methylprednisolone (mPSL; 2 mg/kg/day) was administered intravenously on days 1–7, then mPSL was administered orally and slowly tapered from day 8 to end on day 29. In this study, the use of G-CSF was not restricted. HSCT was recommended when a patient showed no response to IST and required further intervention because of cytopenia or progression to more advanced disease.

Evaluation and Statistical Analysis

Response to IST was evaluated at 6 months. Complete response (CR) was defined as a neutrophil count $>1.5 \times 10^9$/L, platelet count $>100 \times 10^9$/L, and hemoglobin (Hb) level of >11.0 g/dl. Partial response (PR) was defined as a neutrophil count $>0.5 \times 10^9$/L, platelet count $>20 \times 10^9$/L, and Hb level of >8.0 g/dl. When neither the CR nor the PR criteria were met, a patient was considered as no response (NR) to IST.

Mann–Whitney test and Fisher's exact test were applied to evaluate the differences between patients that responded to IST and those who did not. Failure-free survival (FFS) was calculated from the date of initiating IST to the date of treatment failure as follows; death, no response to IST at 6 months, HSCT, a second course of IST, acquisition of chromosomal abnormality, progression to advanced disease, or relapse. Overall survival (OS) was calculated from the date of diagnosis to the date of death or last follow-up. Both FFS and OS were estimated by the Kaplan–Meier method.

RESULTS

Patient Characteristics

Eleven children, 6 males and 5 females, were analyzed in this study (Table I). The median age at diagnosis was 67 months (range, 9 months to 15 years). Eight of 11 children had neutrophil counts of less than 1.5×10^9/L. All except 1 patient had Hb levels below 10 g/dl. Eight patients had platelet counts below 50×10^9/L. In total, one patient had anemia only, five had bi-cytopenia (anemia and neutropenia 2, anemia and thrombocytopenia 2, and neutropenia and thrombocytopenia 1), and five had pancytopenia at diagnosis. Since bone marrow biopsy specimen was available in only 6 of 11 cases, we determined cellularity by central pathological review from bone marrow smear rather than biopsy specimens and used a more suitable term, cell content, instead of cellularity in this report. Overall, there were only three patients in whom BM cell content was low. All patients showed dysplasia in multilineage series, which was compatible with the definition of refractory cytopenias with multilineage dysplasia (RCMD) in the World Health Organization (WHO) classification [16]. Data on the cytogenetic analyses at diagnosis were available for all patients. Karyotype was normal in three patients, and of the remaining eight patients, two had monosomy 7, two had trisomy 8, and four had other abnormalities; del (7)(q11), i(8)(q10), 20q-, and +der(1;19)(q10;q10). Of four patients in whom presence of paroxysmal nocturnal hemoglobinuria (PNH) cells was assessed by flow cytometry, none showed an expansion of PNH clone. Of five patients in whom data on HLA-DR was available, only one patient showed DR2 antigen, which is a broad antigen of DR15 and DR16.

TABLE I. Patients Characteristics

	Median (range)
Age	5y7m (9m to 15y5m)
Gender	M/F = 6:5
WBC ($\times 10^9$/L)	3.8 (1.1–12.5)
Neutrophil ($\times 10^9$/L)	0.94 (0.16–8.1)
PB blast (%)	0 (0)
Hb (g/dl)	6.2 (3.6–11.7)
Reticulocyte (%)	2 (1–44)
Reticulocyte ($\times 10^9$/L)	41.7 (12.3–572.0)
MCV (fl)	104 (84–123)
Plt ($\times 10^9$/L)	23.0 (3.0–117.0)
BM blast (%)	1.0 (0–4.8)
BM cell content	Low 3, normal 5, high 3
Chromosome	Normal/abnormal = 3:8
Cytopenia[a]	Anemia only 1, bi-cytopenia 5, pancytopenia 5

[a]Cut-off; neutrophils <1,500/μl, Hb < 10.0 g/dl, Plt < 50,000/μl.

Observation Without Intervention

Figure 1 shows the outcome of the 11 patients analyzed. Of 3 patients who initially received only supportive therapy, one with normal karyotype was still stable without therapy, one with trisomy 8 showed spontaneous improvement of anemia but the chromosomal abnormality remained. One with 20q- (UPN 046) showed stable disease for 2 years, but cytopenia deteriorated and IST was initiated at 968 days after diagnosis.

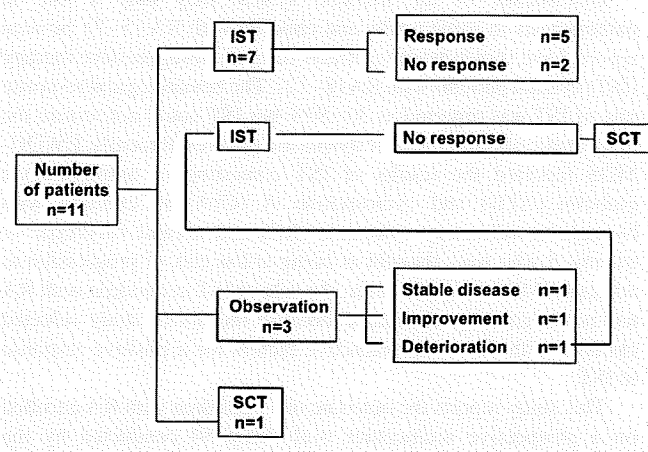

Fig. 1. Outcome of 11 patients with refractory anemia. SCT, stem cell transplantation; IST, immunosuppressive therapy.

Immunosuppressive Therapy

Seven patients received IST as the first-line treatment and one (UPN 046) received IST because of recurrence of cytopenia after 2-year observation. IST was given at a median of 42 (range 0–968) days after the diagnosis of RA. Five of eight patients showed response to IST at 6 months after the initiation of treatment (response rate was 63%; CR 2, PR3). Of five responders, three were able to successfully discontinue IST and remained disease-free, and the remaining two patients have been continuing therapy. Of note, the disappearance of a monosomy 7 clone after IST was observed in UPN 035 [17] and the patient is still in remission after 63 months. Of three non-responders, one was lost to follow up, one responded to a second course of IST, and one (UPN 046) underwent HSCT 3 months after initiating IST.

To address predictive factors for response to IST, the characteristics were compared between children who responded to IST and those who did not (Table II). The age at diagnosis was significantly younger in responders than in non-responders (median 29 months vs. 140 months; $P = 0.03$), whereas there was no statistically significant associations between response to IST and sex, neutrophil count, Hb level, platelet count, interval from diagnosis to IST, chromosomal abnormality, BM cell content, or number of cytopenia. Serious adverse events related to IST were not observed, including the progression to advanced disease. The most frequent adverse event in this study was pyrexia.

Hematopoietic Stem Cell Transplantation

Two children underwent HSCT in this series. One patient with 20q- (UPN 046) received bone marrow transplantation (BMT) from her human leukocyte antigen (HLA) 1-locus-mismatched father at 1,088 days after diagnosis because of non-response to IST. This patient suffered from adenoviral colitis, salmonella colitis, herpes zoster, and grade III acute GVHD of the skin, however, she is still alive without disease 23 months after BMT. One other patient with monosomy 7 (UPN 053) received BMT from a matched unrelated donor on 537 days after diagnosis without IST by physician's decision. His post-transplant course was uneventful, but disease relapsed 151 days after transplantation. A BM specimen at relapse showed severe fibrosis and progression to overt leukemia, and this patient died of disease at 656 days after transplantation.

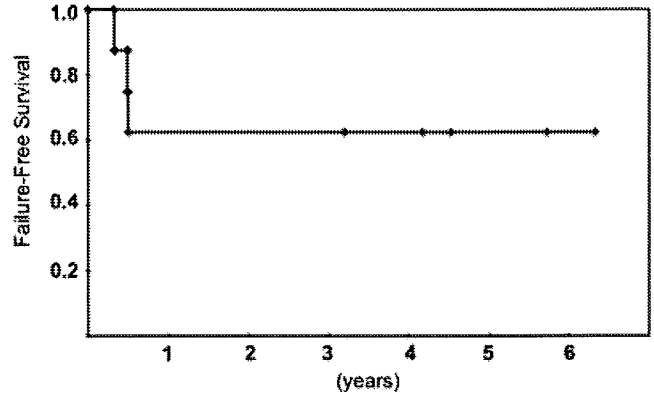

Fig. 2. Kaplan–Meier estimate of failure-free survival of patients who received immunosuppressive therapy. Failure-free survival was calculated from the date of initiating IST to the date of treatment failure as follows; death, no response to IST at 6 months, HSCT, a second course of IST, acquisition of additional chromosomal abnormality, progression to advanced disease, or relapse. The 5-year failure-free survival was $63 \pm 17\%$ (n = 8). Median follow-up was 1,346 days.

Chromosomal Abnormality

There were eight children with chromosomal abnormality in this study. Of those, six received IST and four showed responses to IST, including one with cytogenetic response (UPN 35).

Survival

Of eight children who received IST, three non-responders were considered as treatment failure. No patient died with IST after a median follow-up of 1,346 days; the 5-year FFS was $63 \pm 17\%$ (Fig. 2). Of total, 10 patients are alive after a median follow-up of 1,685 days; the 5-year OS was $90 \pm 9\%$ (Fig. 3).

DISCUSSION

Although HSCT is the curative modality for children with MDS, it may cause severe complications, mortality, and late sequelae. Several reports have shown encouraging results from the use of IST in adults with RA, and the hematological response rate to IST was 30–80% [8–12]. Yoshimi et al. [13] reported on 31 children with hypoplastic RC and normal karyotype or trisomy 8 treated with IST, which resulted in a response rate at 6 months of 71%, 3-year OS of

TABLE II. Comparison of Characteristics Between Responders and Non-Responders to IST

	Responder (n = 5)	Non-responder (n = 3)	*P*-value
Age[a]	2y5m	11y8m	0.03
Gender (male/female)	3:2	1:2	n.s.
Neutrophils[a] ($\times 10^9$/L)	1.27	0.63	n.s.
Hb[a] (g/dl)	8.0	6.2	n.s.
Plt[a] ($\times 10^9$/L)	31.0	20.0	n.s.
No. of cytopenia (tri-/bi-/anemia only)	2:2:1	2:1:0	n.s.
Decreased BM cell content	1/5	2/3	n.s.[b]
Time to IST[a] (day)	42	42	n.s.[b]
Chromosomal abnormality	4/5	2/3	n.s.[b]

[a]Median; [b]Evaluated by Mann–Whitney test and Chi-square test.

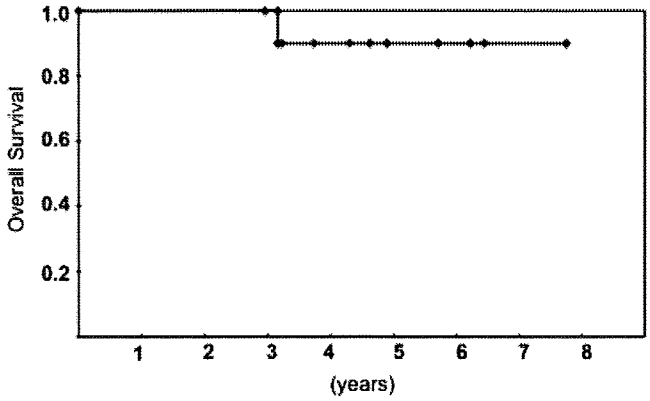

Fig. 3. Kaplan–Meier estimate of overall survival of all evaluable patients. Overall survival was calculated from the date of diagnosis to the date of death or last follow-up. The 5-year overall survival was $90 \pm 9\%$ (n = 11). Median follow-up was 1,685 days.

88%, and 3-year FFS of 57%. In contrast to the larger series by Yoshimi et al. children with RA and karyotypic abnormalities or non-hypoplastic marrow were also enrolled in this study. Overall, 5 of 8 patients (63%) responded to IST, and similar responses were observed in two-thirds of patients with chromosomal abnormalities. Patients whose BM cell content was not low also responded to IST (responder 4, non-responder 1); however, the significance of cellularity in pediatric RA still needs further study. No severe adverse events, disease progression, or death due to any cause after IST was reported. Only one death in this study was due to disease progression after HSCT, which was not related with IST. As a whole, the 5-year OS and FFS were 90% and 63%, which were comparable with the previous study in adult MDS and superior to our previous retrospective analysis of children with RA (4-year OS was 79%) [2]. Therefore, although the number of subjects was limited, we infer from these results that the IST is effective and safe for children with MDS.

The rationale for IST used as treatment of RA is based on previous studies, which suggested that alterations in the immune system might contribute to the pathogenesis in some subgroups of RA [5–7]. Dysregulated T cells are thought to destroy normal hematopoietic cells as bystanders as well as MDS clones [6]. IST can reduce MDS clone-specific T cells and improve normal hematopoiesis, but cytogenetic abnormalities and dysplastic features often persist [9,11,12]. However, in this study one patient showed the disappearance of karyotypic abnormalities. In addition, three of the responders were able to successfully discontinue IST. These results might be explained by the findings that the residual healthy stem cells can compensate for the loss of stem cells after the immune-mediated destruction is interrupted by IST in the setting of aplastic anemia [18,19]. Recovery of healthy hematopoiesis might outstrip MDS clones in these patients. In the patient with monosomy 7 who experienced cytogenetic response another mechanism could be speculated. The investigators from the EWOG-MDS reported that almost half of children with RA had monosomy 7 and they were likely to experience disease progression [3]. In contrast, anecdotal case reports described a decline or disappearance of a monosomy 7 clone [20]. Sloand et al. [21] reported paradoxical responses of monosomy 7 cells to G-CSF. Namely, high concentrations of G-CSF induced significant proliferation of monosomy 7 cells, but survival and proliferation of monosomy 7 cells were inferior to those of diploid cells at lower G-CSF levels. Thus, there is a possibility that the recovery of normal hematopoiesis after the administration of IST might affect the intrinsic level of G-CSF and survival of monosomy 7 cells. However, the interpretation of the present results still needs caution because most patients with RA and monosomy 7, including another case in this study, showed poor prognosis.

Previous studies on IST in adult RA found some factors that could predict good responders to IST, such as younger age, shorter duration of transfusion dependence, HLA-DR15, and presence of an expanded clone of PNH cells [8,10–12]. In this study, age was the only factor that showed a statistically significant difference between responders and non-responders to IST. The European study published by Yoshimi et al. [13] also contained older patients, but the proportion and treatment responses of older patients were not shown. Therefore, the effects of patient age on pathophysiology of pediatric RA and treatment response remain to be elucidated. Of the limited cases who were examined, no patient showed an expansion of PNH clone and only one patient had HLA-DR2 antigen, who responded to IST well. We did not systemically examine the immunological status such as TCR Vbeta repertoire [7] in this study. Clinical trials, including systematic studies on immunological status, are required to investigate prognostic factors more precisely in childhood RA because the sample size in this study was small.

Thus, a significant drawback of our study was small size of registered patients. We assumed that considerable number of patients with RA did not enter this study and might have received HSCT without IST. In fact, retrospective analysis of pediatric MDS in Japan showed that 52 patients with RA were diagnosed by the central morphological review between 1999 and 2006 [22]. Consecutive enrollment on both diagnostic and therapeutic trials would be essential for a future trial. It might allow the determination of biologic parameters that correlated with clinical characteristics.

In conclusion, the present results suggest the efficacy and safety of IST for children with RA. Disease-free status might be expected with IST in a subset of patients. Chromosomal aberration was not an absolute contraindication for IST, whereas using this approach for patients with monosomy 7 has not been substantiated. A larger prospective study including biological surrogate markers for therapeutic interventions would be important to elucidate the clinical characteristics of this rare disease as well as the prognostic factors and mechanism of IST.

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CASE REPORT

Successful treatment with rituximab of refractory idiopathic thrombocytopenic purpura in a patient with Kabuki syndrome

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Abstract Kabuki syndrome (KS) is often associated with autoimmune abnormalities, such as idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia, leukoplakia and thyroiditis, as well as congenital anomalies. We herein present a KS patient with refractory ITP who achieved durable and complete remission in response to a total of four once-monthly infusions of rituximab. KS patients are often more susceptible to infection, so splenectomy should be avoided. Therefore, rituximab therapy is an alternative option for KS patients with ITP who fail to respond to first-line therapy.

Keywords Kabuki syndrome · Thrombocytopenia · Rituximab

1 Introduction

Kabuki syndrome (KS) is a rare condition that displays a wide range of congenital anomalies and mental retardation. This syndrome was named after kabuki (Japanese theater) because of the distinctive facial appearance, and was independently reported by both Kuroki and Niikawa in 1981 [1, 2]. The incidence is estimated at 1/32,000 in Japan [3]. Most patients present as sporadic cases, and no causative gene or cytogenetic abnormality has yet been identified. KS is often associated with autoimmune abnormalities, such as idiopathic thrombocytopenic purpura (ITP), autoimmune hemolytic anemia, leukoplakia and thyroiditis, as well as congenital anomalies [4–6]. The reported frequency of ITP ranges from 1.6 to 17%, and its severity varies from moderate to severe [3, 4].

Rituximab is a humanized chimeric monoclonal antibody against CD20 that is cytotoxic for B lymphocytes. Rituximab has been widely used against B-cell malignant lymphoma, and recent reports have also demonstrated its efficacy for autoantibody-mediated hematological disorders, including ITP [7]. We report herein the case of a KS patient with ITP who achieved a rapid and complete response to rituximab.

2 Case report

At 1.5 years of age, the patient's condition was diagnosed as KS on the basis of the distinctive facial appearance (long palpebral fissures, eversion of the lateral third of the lower eyelid, sparse eyebrows in the lateral one-third, and large ears) and finger pads. Other characteristic findings compatible with KS included short stature, microcephaly, moderate mental retardation, brachydactyly (5th finger) and hearing loss. After 2 years of age, frequent episodes of bleeding due to thrombocytopenia developed. The patient received repeated high-dose gamma globulin therapy and a course of dexamethasone pulse therapy.

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However, the effect was transient and the platelet count remained at $<10 \times 10^9$/L. Although he also had repeated occurrences of otitis media, the results of immunological tests were not available.

The patient was referred to our hospital at 5 years of age. Numerous petechiae were apparent on his legs and arms. The complete blood count showed severe thrombocytopenia (platelet count, 4×10^9/L); other laboratory results were normal. Bone marrow findings showed normocellular marrow and an increased number of immature megakaryocytes, consistent with the diagnosis of ITP. Immunostaining of bone marrow showed increased B lymphocytes. In peripheral blood, the B-cell fraction accounted for 30% (normal, 5–15%). These findings also suggested the possible involvement of B cells in the thrombocytopenia. Because recent reports showed that rituximab had been effective in 31–79% of children with chronic ITP, we decided to treat the patient with rituximab as a salvage therapy [8, 9]. Rituximab therapy was approved by the Institutional Review Board of the Graduate School of Medicine at Nagoya University, and written informed consent was obtained from his parents.

Because an immediate response to rituximab was not expected, a high dose of gamma globulin (2 g/kg) was given a day before the first administration of rituximab in June 2007. The dose of rituximab administered, 375 mg/m^2, is the standard dose for malignant lymphoma in adults. Fever and chills were seen during the infusion.

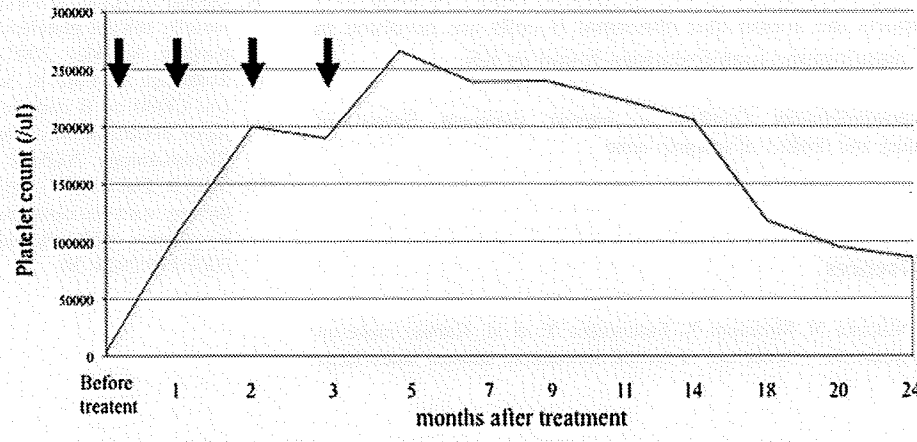
Laboratory test results and the clinical course are shown in Fig. 1. Briefly, the platelet count increased to 109×10^9/L at 1 month and 200×10^9/L at 2 months. The total course of four once-monthly infusions was completed by October 2007. Thereafter, the platelet count ranged from 206×10^9 to 266×10^9/L up until August 2008. No late adverse effects such as hypogammaglobulinemia or infection were observed. Of note, thrombocytopenia has not recurred, despite recovery of the B-cell population in the peripheral blood (19% of total lymphocytes). The last follow-up laboratory data (April 2009) showed a platelet count of 86×10^9/L.

3 Discussion

This report describes the successful treatment of KS-associated ITP with rituximab and raises several key issues regarding rituximab therapy for ITP.

Therapy with rituximab is attractive because splenectomy can be avoided, and rituximab was well tolerated with no significant infusion-related or late events seen in this patient. Although splenectomy has been considered as the second-line choice for refractory ITP, children who undergo splenectomy become especially vulnerable to infection by encapsulated organisms. Because of their immune dysfunction, KS patients are often more susceptible to infection, and splenectomy should be avoided [5].

Many studies have shown the efficacy of rituximab against various autoimmune cytopenic disorders, but the doses and usages have been the same as those used for malignant lymphoma, that is, four once-weekly infusions of 375 mg/m^2. The optimal dose for autoimmune cytopenia may, however, differ from that used for malignant disease.



	before treatment	1 month	2 months	3 months	5 months	7 months	9 months	11 months	14 months	18 months	20 months	24 months
plt cont (/ul)	4000	109000	200000	190000	266000	239000	240000	224000	206000	118000	95000	86000
lymphocyte (/ul)	3100	1900	2300	1700	2100	2200	2400	1700	1800	1790	910	1670
CD3+ Tcell (%)	65	91	94	91	92	90	76	78	71	67	ND	ND
CD19+ B cell (%)	30	1	1	1	1	1	10	16	19	22	ND	ND

Fig. 1 Time course of the platelet and lymphocyte count (CD3+ T-cell and CD19+ B-cell fractions) before and after rituximab therapy. *Arrows* indicate the timing of rituximab therapy

Unlike other reports, our study employed a total of four once-monthly infusions of rituximab, which may have contributed to durable remission in this patient. Recently, high response rates of 75% with low-dose rituximab (100 mg/m^2) for adult chronic ITP have been reported in two series [10, 11].

Our patient responded rapidly to rituximab, as has been observed in other patients with chronic ITP [12]. Plasma cells producing autoantibodies do not express CD20 antigen and are not a target of rituximab. Considering the lifespan of plasma cells, the response we observed was too rapid to be explained simply by the depletion of autoantibodies. The following mechanisms are hypothesized to be involved in the immediate response: (1) rituximab or rituximab-coated B cells bind to Fc receptors of the reticuloendothelial system and thereby prevent platelet phagocytosis (the immune complex decoy hypothesis) [13]; and (2) activation of autoreactive T cells is terminated by depletion of the antigen-presenting capacity of B cells and down-regulation of costimulatory molecules such as CD40 and CD80 on B cells, because direct T-cell-mediated platelet lysis in patients with chronic ITP was demonstrated [14, 15]. On the other hand, the durable response may result from eradicating B cells that will differentiate into plasma cells.

It is interesting that thrombocytopenia did not recur despite B-cell recovery. A recent report demonstrated that rituximab reversed the Th1/Th2 imbalance to regulate the immune system properly in chronic ITP [16]. Rituximab may remove autoreactive B cells and reset the immune response leading to a durable remission.

Our observation suggests that refractory ITP in KS may be a good indication for treatment with rituximab and supports the notion that abnormal B cells are involved in the autoimmune manifestations found in KS.

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MAJOR ARTICLE

Identification of Epstein-Barr Virus (EBV)–Infected Lymphocyte Subtypes by Flow Cytometric In Situ Hybridization in EBV-Associated Lymphoproliferative Diseases

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To diagnose Epstein-Barr virus (EBV)–associated diseases and to explore the pathogenesis of EBV infection, not only must the EBV load be measured, but EBV-infected cells must also be identified. We established a novel flow cytometric in situ hybridization assay to detect EBV^+ suspension cells using a peptide nucleic acid probe specific for EBV-encoded small RNA (EBER). By enhancing fluorescence and photostability, we successfully stained EBER and surface antigens on the same cells. In 3 patients with hydroa vacciniforme–like lymphoproliferative disease, we demonstrated that 1.7%–25.9% of peripheral lymphocytes were infected with EBV and specifically identified these lymphocytes as $CD3^+CD4^-CD8^-$ γδ T cell receptor–positive T cells. The results indicate that this novel and noninvasive assay is a direct and reliable method of characterizing EBV-infected lymphocytes that can be used not only to diagnose EBV infection but also to clarify the pathogenesis of EBV-associated diseases.

Epstein–Barr virus (EBV) is a ubiquitous virus and occasionally causes infectious mononucleosis in primary infection. In rare cases, chronic active EBV infection develops in apparently immunocompetent hosts [1–3]. EBV preferentially infects B cells through CD21 and HLA class II molecules and establishes latent infection in memory B cells [4]. Several types of B cell–origin lymphomas or lymphoproliferative diseases, including Burkitt lymphoma, Hodgkin lymphoma, primary central nervous system lymphoma, and opportunistic B cell lymphoproliferative disorders, are etiologically linked to EBV infection [2, 3, 5]. EBV also infects T cells and natural killer (NK) cells and is associated with T/NK lymphoproliferative diseases and lymphoma or leukemia, such as EBV-related hemophagocytic lymphohistiocytosis, systemic EBV^+ T cell lymphoproliferative disease of childhood, hydroa vacciniforme–like lymphoma, nasal NK cell lymphoma, and aggressive NK cell leukemia [2, 3, 5, 6].

Because EBV is a ubiquitous virus that latently infects various lymphocytes, simply detecting EBV is insufficient to diagnose EBV-associated diseases [7]. To diagnose EBV-associated diseases and to explore the pathogenesis of EBV infection, one must not only measure the EBV load, but one must also identify EBV-infected cells. In situ hybridization (ISH) with the EBV-encoded small RNA (EBER) is widely used to detect EBV-infected cells in tissue specimens [8, 9]. EBER is a good marker for EBV infection because it is detectable in virtually all EBV-infected cells and is expressed at very high levels, reaching 10^7 molecules per cell [5]. Therefore, EBER ISH is a specific and direct method of identifying EBV-infected cells in tissue specimens [9]. How-

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