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CASE REPORT

Pediatric post-transplant diffuse large B cell lymphoma after cardiac transplantation

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Abstract Post-transplant lymphoproliferative disorders (PTLDs) occur in 3.5–9% of patients after pediatric cardiac transplantation. Caution is needed when treating patients with PTLD because of the risk of allograft rejection frequently caused by withdrawal of immunosuppression. In this report, we describe a 47-month-old boy who developed PTLD as an ileocecal mass 29 months after cardiac transplantation. Immunosuppressive therapy with cyclosporine A (CyA) had been reduced due to an elevation of Epstein-Barr virus (EBV) titer for 8 months before development of PTLD. Histology of the tumor was diffuse large B cell lymphoma. EBV was detected by in situ hybridization assay. Cytogenetic analysis revealed *t*(8;14)(q24;q32) and Southern blot analysis detected a *c-Myc* rearrangement. He was treated with rituximab and combination chemotherapy with excellent response. CyA dose was maintained at reduced levels during chemotherapy and later minimized with introduction of everolimus. The child is free of both PTLD and allograft rejection 41 months after the diagnosis of PTLD.

Keywords Cardiac transplantation · Diffuse large B cell lymphoma · Pediatric · Post-transplant lymphoproliferative disorder

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1 Introduction

Post-transplant lymphoproliferative disorders (PTLDs) are an important cause of morbidity and mortality after pediatric cardiac transplantation. Although its pathological range is quite diverse, from monomorphic to polymorphic proliferation, the majority are B lymphocyte disorders and associated with Epstein-Barr virus (EBV) [1, 2]. Withdrawal of immunosuppression for management of PTLD can often lead to allograft rejection and transplant coronary artery disease [1]. We report a child with EBV-associated post-transplant diffuse large B cell lymphoma (DLBL) after cardiac transplantation who was successfully treated with rituximab and combination chemotherapy.

2 Case report

A 17-month-old boy underwent successful cardiac transplantation for dilated cardiomyopathy in October 2002. He received cyclosporine A (CyA), initially with azathioprine and later with mycofenolate mofetil for post-transplant immunosuppression. He was EBV-seronegative pre-transplant (VCA-IgG: <tenfold) and his donor was EBV-seropositive pre-transplant (VCA-IgG: 320-fold). In November 2003, his EBV antibody titer for VCA-IgG increased to 2,560-fold. In August 2004, 21 months

post-transplant, CyA dose was reduced and mycophenolate mofetil was stopped due to an increase in EBV titers (92,813 copies/DNA 1 μg; Fig. 1); no lymph node swelling or fever was observed; and no abnormalities were detected on CT scan or gallium scintigraphy. He also received ganciclovir or valganciclovir, and IV immunoglobulin with high titer of antibodies against EBV to prevent the development of PTLD. In April 2005, at 47 months of age and 29 months post-transplant, he developed abdominal pain. CT scan revealed an ileocecal mass (Fig. 2a). An open biopsy of the tumor established a diagnosis of monomorphic PTLD, DLBL (Fig. 3a). Immunoblasts were $CD20^{+}$ (Fig. 3b), $CD79a^{+}$, and $CD3^{-}$. In situ hybridization for EBV early RNA (EBER) showed reactivity in lymphoid cells (Fig. 3c). Conventional cytogenetic analysis revealed 46, XY, *t*(8;14)(q24;q32). Southern blot analysis detected a *c-Myc* rearrangement in tumor cells in ascites (Fig. 4). At this point, EBV titer was increased to 15,000 copies/10^6 WBCs (normal value <20 copies). EBV antibody titers for VCA-IgG, VCA-IgM, EADR-IgG, and EBNA were 1,280-fold, <10-fold, <10-fold, and 40-fold, respectively. Laboratory studies showed a WBC count of 4,470 μL^{-1}, hemoglobin 9.9 g/dL, lactate dehydrogenase 1,261 IU/L, serum soluble interleukin-2 receptor (sIL-2R) level 1,947 U/mL (normal value 150–505 U/mL), and normal electrolytes and liver function.

The clinical course of this patient is summarized on Fig. 1. Immunosuppressive therapy with oral CyA was continued to maintain trough levels at 50–90 ng/mL. He received six courses of weekly rituximab (375 mg/m^2) with one course of cyclophosphamide (600 mg/m^2), and seven courses of combination chemotherapy. Combination chemotherapy was as follows: regimen A consisted of high-dose methotrexate, vincristine, cyclophosphamide, pirarubicin (THP-adriamycin), and dexamethasone; regimen B, methotrexate and cytosine arabinoside; and regimen C, cytosine arabinoside and etoposide. Excellent response was observed with resolution of tumor mass (Fig. 2b) and reduction of EBV titer (Fig. 1). Serum sIL-2R level decreased to 987 U/mL 2 weeks after the start of chemotherapy. After completion of chemotherapy, oral everolimus was started, followed by a further reduction of oral CyA to maintain trough levels at 30–60 ng/mL. EBV titer was moderately increased up to 1,000 copies/10^6 WBCs and serum sIL-2R level was maintained at <1,000 U/mL. He remains in complete remission 41 months following diagnosis of DLBL without allograft rejection.

3 Discussion

The incidence of PTLD has been reported to range from 3.5 to 9% after pediatric cardiac transplantation [1–4]. PTLD comprises two pathological types, i.e., polymorphic and monomorphic. Early-onset disease (≤3 years post-transplant) is frequently observed with polymorphic localized disease, while late-onset disease (>3 years post-transplant) is more often associated with monomorphic disseminated disease [1, 5]. Most cases were of B cell

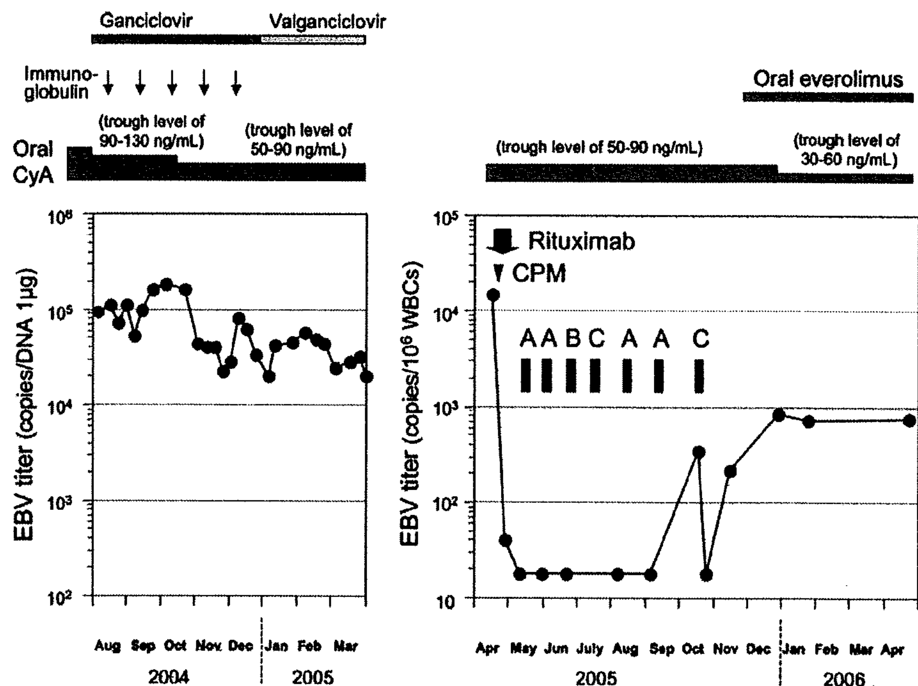

Fig. 1 Clinical course and changes in EBV titer. *CyA* cyclosporine A, *CPM* cyclophosphamide, *EBV* Epstein-Barr virus. **a–c** indicate combination chemotherapy regimens (details are shown in the text)

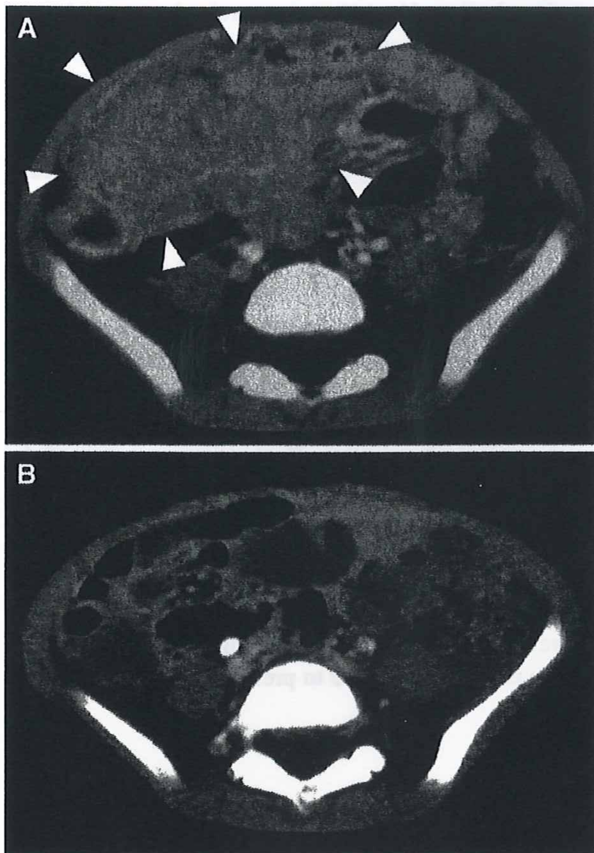

Fig. 2 CT scan images. Abdominal enhanced CT scan showing an ileocecal mass (*arrowheads*) at diagnosis (**a**) and dramatic disappearance after the first course of combination chemotherapy (**b**)

origin and contained EBV in lesions [1, 2]. Polymorphic disease is manifested as reactive B cell hyperplasia or B cell lymphoma, while monomorphic diseases as diffuse large B cell lymphoma or Burkitt lymphoma [2, 4].

Our patient developed monomorphic PTLD, DLBL with *t*(8;14)(q24;q32) possessing *c-Myc* rearrangement, which strongly suggests the existence of immunoglobulin heavy chain (IgH)/*c-Myc* fusion. *t*(8;14)(q24;q32) has been detected not only in patients with Burkitt lymphoma but also in 5–15% of patients with de novo DLBL [6, 7]. This translocation results in overexpression of *c-Myc*, driving cell growth and proliferation, and expression of other genes involved in cell growth [8]. Extranodal lymphomas, particularly gastrointestinal lymphomas, as observed in our case, are more likely to carry *c-Myc* rearrangement than nodal lymphoma [7]. The role of chronic infection with EBV in the pathogenesis of a variety of tumors including Burkitt lymphoma is well documented. However, the mechanisms involved have not been completely defined. EBV might have an initiating role in which growth-transforming B cell infections

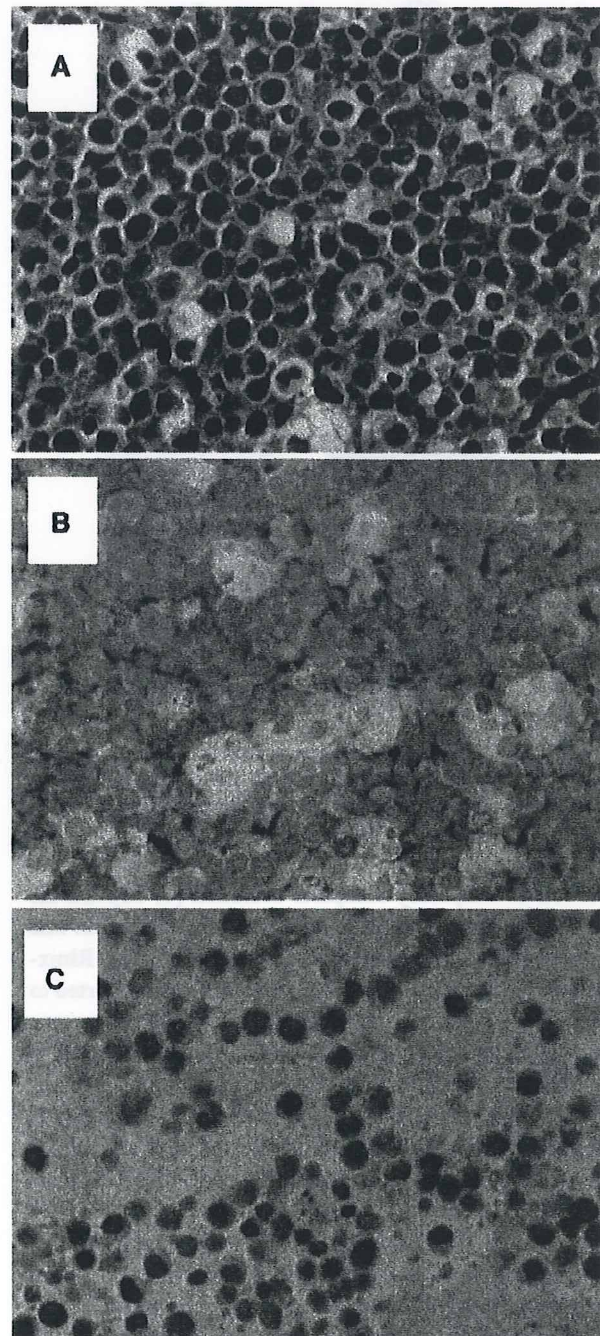

Fig. 3 Pathological studies. **a** Diffuse infiltration of large lymphocytes with clear nucleoli (H&E stain, ×400). **b** Tumor cells showing strong CD20 immunoreactivity stained brown (CD20 stain, ×400). **c** Tumor cells showing reactivity stained brown with the probe specific for EBER-RNA (EBER in situ hybridization, ×400)

establish a pool of target cells that are at risk of a subsequent *c-Myc* translocation [9].

Most patients with polymorphic disease are treated with lowered immunosuppression in most institutes: minor

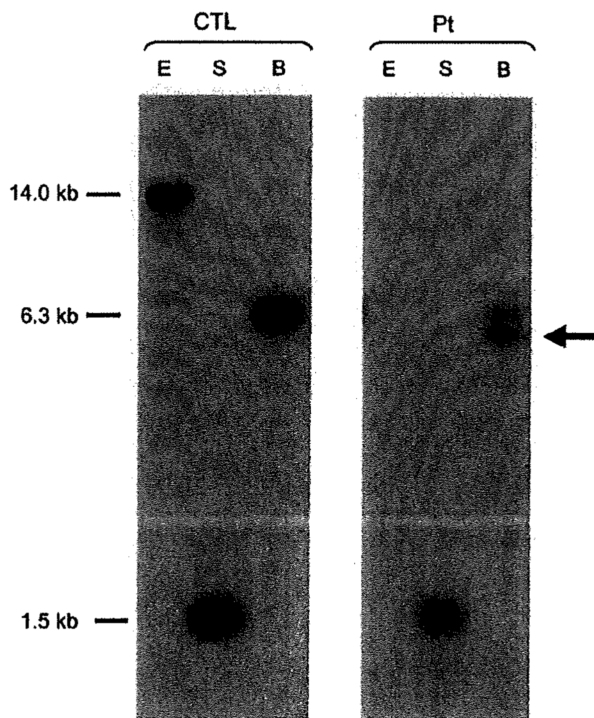

Fig. 4 Southern blot analysis. Rearrangement of the *c-Myc* gene in tumor cells in ascites by Southern blotting with *Eco*RI (E), Sac I(S) and Bgl II (B). The *arrow* indicates a rearrangement band of the *c-Myc* gene. *CTL* control, *Pt* patient

reduction or temporary complete cessation [1, 10]. On the other hand, chemotherapy is commonly used for first-line therapy against monomorphic lymphoma [1, 10]. Rituximab has been widely used for B cell PTLD and reported to be effective [2, 11]. In our case, rituximab and one course of cyclophosphamide were effective for reduction of EBV titer by more than two logs. Subsequent courses of combination chemotherapy were sufficient for inducing and maintaining remission, indicating that block-type chemotherapy containing methotrexate, cyclophosphamide, pirarubicin and dexamethasone, or cytosine arabinoside and etoposide, designed for B cell malignancy, is also effective for post-transplant DLBL after solid organ transplantation, which is in line with other reports [1, 10].

Webber et al. [1] reported 42 cases with pediatric PTLD after cardiac transplantation, of which 16 patients died from progressive PTLD ($n = 7$), acute rejection ($n = 3$), coronary artery disease ($n = 3$), PTLD with acute rejection ($n = 1$), PTLD with sudden death ($n = 1$), and graft failure ($n = 1$). This report also indicated that death from graft loss is a serious issue during PTLD treatment. A fine balance between management against PTLD and preserving allograft from rejection is therefore highly important. With respect to this standpoint, chemotherapy may be useful for maintaining an immunosuppressed state to prevent allograft rejection [1, 10]. Lower rejection rates have been reported when chemotherapy was used as primary therapy [1].

In our case, CyA was continued without further reduction during the treatment of PTLD to protect the allograft from rejection. Following cessation of chemotherapy, oral everolimus was initiated with a further reduction of CyA dose to maintain trough levels at 30–60 ng/mL. Everolimus, an immunosuppressive mammalian target of rapamycin (mTOR) kinase inhibitor, inhibits growth of human EBV-transformed B lymphocytes in vitro and in vivo [12]. Thus, it is promising agent in that it may be effective in both the prevention of PTLD and allograft rejection. In our case it might have contributed to protection against PTLD relapse as well as allograft rejection. Further studies are needed with this agent in this clinical setting.

In summary, we present a child who was successfully treated for post-transplant DLBL after cardiac transplant. The disease was associated with *c-Myc* rearrangement and EBV. Rituximab and combination chemotherapy were effective in inducing and maintaining remission. PTLD should be carefully managed to prevent allograft rejection.

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WT1 (WILMS TUMOR 1) PEPTIDE IMMUNOTHERAPY FOR CHILDHOOD RHABDOMYOSARCOMA: A Case Report

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□ *Immunotherapy using a Wilms tumor (WT1) peptide has been undergoing clinical trials for adulthood leukemia and solid cancer with promising results. In this study, the authors used WT1 peptide vaccination to treat a 6-year-old girl with metastatic alveolar rhabdomyosarcoma. She received weekly intradermal injection with HLA-A*2404-restricted, 9-mer WT1 peptide against residual bone disease. After 3 months her bone disease disappeared, concurrent with an increase in the frequency of WT1-specific cytotoxic T lymphocytes (CTLs). A high proportion of WT1-specific CTLs with effector or effector memory phenotype were detected in peripheral blood of this patient. She is currently still on continued WT1 peptide immunotherapy in a disease-free condition for 22 months. WT1 peptide-based immunotherapy should be a promising option forhigh-risk rhabdomyosarcoma in childhood.*

Keywords childhood, rhabdomyosarcoma, WT1 peptide immunotherapy

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The Wilms tumor gene WT1 was first identified as a gene responsible for Wilms tumor, a childhood renal cancer. This gene encodes a zinc finger transcription factor and plays an important role in cell proliferation, differentiation, apoptosis, and organ development by the positive or negative regulation of the expression of various kinds of genes [1]. Although the WT1 gene was first categorized as a tumor suppressor gene, recent studies showed the overexpression of WT1 mRNA in various kinds of solid tumors [2], the growth inhibition of WT1-expressing cells by WT1 antisense oligomers [2, 3], and a correlation between a high level of WT1 and a poor prognosis [15] in patients with certain kinds of tumors [4, 5], suggesting that WT1 plays an oncogenic role in human cancers. Furthermore, a sequencing study revealed the absence of mutations in the WT1 gene in tumors [2], indicating wild-type WT1 could be oncogenic.

WT1 is often overexpressed in leukemias and various types of solid tumors. Nakatsuka et al. examined overexpression of WT1 in 494 cases of human cancers and found overexpression in 30–70% of tumors of the gastrointestinal and pancreatobiliary system, urinary tract, male and female genital organs, breast, lung, brain, skin, and bone [6]. They also showed WT1 expression in 3 of 7 patients with PNET/Ewing sarcoma and in all 6 patients with rhabdomyosarcoma.

WT1 is now regarded as a molecular target for immunotherapy in various malignant tumor types. Clinical trials of WT1 peptide-based cancer immunotherapy are ongoing: WT1 peptide vaccination has been shown to be safe and clearly effective against several kinds of malignancies [7–10]. The trial for pediatric cancer is currently limited. Here, we describe the case report of a 6-year-old girl with rhabdomyosarcoma who was successfully treated with WT1 peptide-based immunotherapy.

PATIENT AND METHODS

Clinical Study

The WT1 peptide-based phase I/II clinical study was approved by the Institutional Review Board of Osaka University Hospital. Patients aged

<20 years with pediatric cancer or leukemia were eligible if they were resistant to conventional multimodal therapy. Other inclusion criteria were WT1 protein expression in solid cancer tissues or WT1 mRNA expression in leukemic cells determined by immunohistochemistry and RT-PCR, respectively; HLA-A*2402-positive; and performance status 0 to II (Eastern Cooperative Oncology Group). Patients were excluded if they had severely impaired organ function or had received chemotherapy or radiotherapy between the confirmation of residual disease and WT1 peptide vaccination.

Immunohistochemistry Determination of WT1 Expression in Solid Cancer Tissue

Formalin-fixed tissue sections (3-μm thickness) were cut from each paraffin block. After being dewaxed with xylene and rehydrated through a graded series of ethanol, the sections were microwaved for 15 min in 10 mM citrate buffer (pH 6.0) for antigen retrieval. These sections were incubated in phosphate-buffered saline containing goat serum albumin, reacted with anti-WT1 6F-H2 mouse monoclonal antibody (mAb) (Dako Cytomation. Carpinteria, CA, USA) diluted 1:50 at 4°C overnight, and then reacted with EnVision kit (Dako Cytomation) according to the manufacturer's instructions. After treatment with 3% H_2O_2 solution to reduce endogenous peroxidase activity, immunoreactive WT1 protein was visualized with diaminobenzidine tetrahydrochloride. The sections were then counterstained with hematoxylin.

WT1 Peptide Treatment Plan

The 9-mer WT1 peptide (a.a. 235–243, CYTWNQMNL) was used for immunization [11]. GMP grade WT1 peptide was purchased from Multiple Peptide Systems (San Diego, CA) as a lyophilized peptide, which was dissolved just prior to injection. After written informed consent was obtained from the patient and her parents, a skin test for an HLA-A*2402-restricted, 9-mer WT1 peptide was performed and confirmed to be negative. WT1 peptide (1 mg) was emulsified with Montanide ISA51 adjuvant (SEPPIC S.A., Paris, France) [7, 8, 12], and the emulsion was injected intradermally at several different regions, including upper arms and lower abdomen. The WT1 peptide vaccination was scheduled to be performed weekly.

Analysis of WT1-specific Cytotoxic T-lymphocytes

The procedure for cell staining was performed as described elsewhere [13]. Peripheral blood mononuclear cells were stained with phycoerythrin (PE)-conjugated HLA-A*2402-WT1 235–243 tetramer (WT1-Tet) (MBL, Tokyo, Japan). The cells were then stained with fluorescein

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isothiocyanate-labeled anti-CD4, CD14, CD16, CD19, and CD5 mAbs (eBioscience, San Diego, CA), APC-Cy7-labeled anti-CD8 mAb (BD Pharmingen, San Diego, CA), ECD-labeled anti-CD45RA mAb (2H4LDH11LDB9, Beckeman Coulter, Fullerton, CA), and PE-Cy7-labeled CCR7 mAb (3D12, BD Biosciences, San Jose, CA). After this procedure, cells were analyzed with FACS Aria (BD Biosciences). CD4, CD14, CD16, CD19, and CD56-negative WT1-Tet$^+$ CD8$^+$ T cells were considered to be the WT1 peptide-specific CD8$^+$ T cells. We measured the frequency (%) of WT1-Tet$^+$ CD8$^+$ T cells among the CD8$^+$ T cells, and defined it as the WT1-specific cytotoxic T-lymphocyte (CTL) frequency. In addition, we analyzed the phenotype of WT1-Tet$^+$ CD8$^+$T cells according to their expression of CD45RA and CCR7. The WT1-Tet$^+$ CD8$^+$ T cells were phenotypically classified into four differentiation stages: naïve (CD45RA$^+$CCR7$^+$), central memory (CD45R$^-$CCR7$^+$), effector memory (CD45RA$^-$CCR7$^-$), and effector(CD45RA$^+$CCR7$^-$) [13].

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CASE REPORT

A 6-year-old girl presented with a mass on her lower left leg (Figure 1A). A diagnosis of alveolar rhabdomyosarcoma was made by histopathology with presence of left inguinal PAX3-FKHR. Image studies showed a lymph node metastasis from the right axial to para-aortic legion (Figure 1B) and multiple bone metastases located on right parietal, right 4th rib, and thoracic vertebrae (Figure 1C). Bone marrow aspiration revealed aggregation of tumor cells (Figure 1D). The disease status was stage 4 and group IV.

Combination chemotherapy was started. The combination consisted of cyclophosphamide, etoposide, THP-adriamycin, cisplatin, and vincristine (course 1), followed by ifosfamide, etoposide, actinomycin-D, and vincristine (course 2). However, a new bone metastasis lesion was observed on lumbar vertebrae (Figure 2A) after two courses of chemotherapy. At this point, bone marrow aspiration showed no residual tumor cells. She then received two further courses of chemotherapy intensified with nogitecan (2 mg/m^2 × 3–5). She subsequently underwent operation on the primary site and additional two courses of chemotherapy with radiotherapy on the primary site, high-dose chemotherapy consisting of thio-TEPA and L-PAM with autologous bone marrow and peripheral blood stem cell rescue, and radiotherapy on the metastatic lymph node site. The metastatic lesions of the vertebrae and right 4th rib were not irradiated. After all these therapies were completed, no residual disease was observed at the primary site or the metastatic para-aortic site. However, the bone disease on lumbar vertebrae remained (Figure 2B). We did not perform a biopsy of the uptake region.

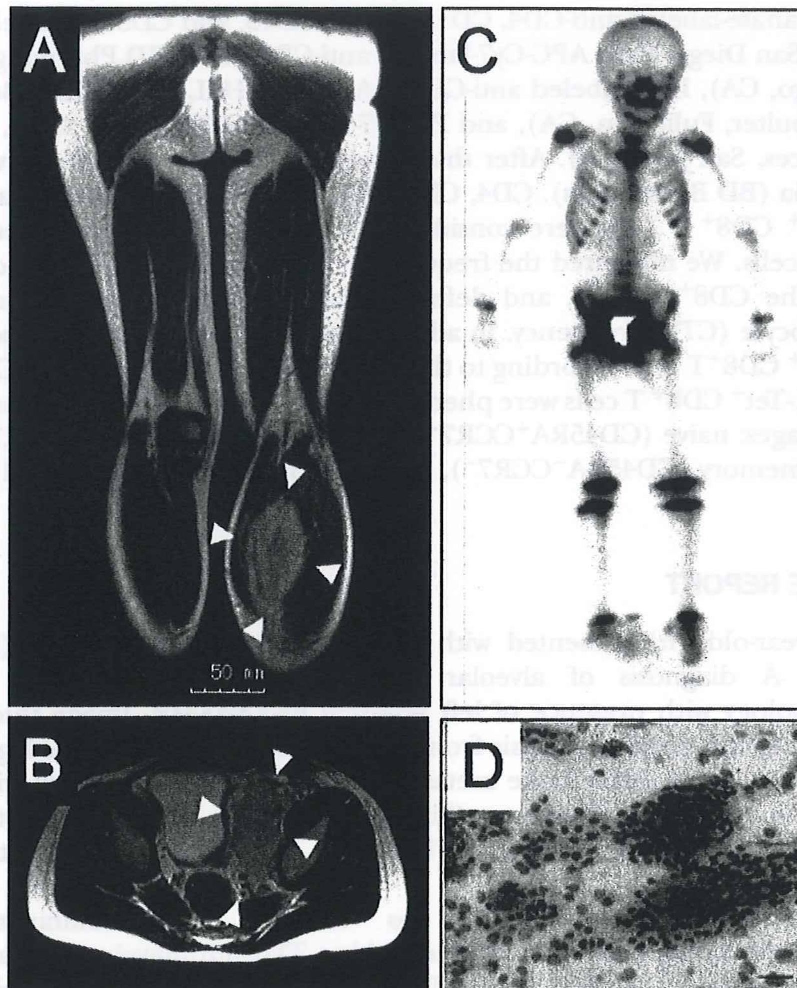

FIGURE 1 Studies at diagnosis. T2-weighted MRI images showing a mass on left leg (A, arrowheads) and metastatic swelling of para-aortic lymph nodes (B, arrowheads). (C) Scintigraphy of bone showed uptakes on right parietal, right 4th rib, and thoracic vertebrae. (D) Bone marrow aspiration showed aggregation of tumor cells.

She had HLA-A*2402 and her cancer tissue was determined by immunohistochemistry to express WT1 protein (Figure 3). She met the criteria for entry into the WT1 peptide-based clinical trial. Intradermal injection of the modified 9-mer WT1 peptide (1 mg) emulsified with Montanide ISA51 adjuvant was started from April 2005, 3 months after the last therapy (radiotherapy on the metastatic site) and continued at 1-week intervals.

The newlesions on the lumbar vertebrae remained weakly positive at the start of WT1 peptide vaccination (Figure 2B), but became negative after 3 months (12 courses) of weekly injections (Figure 2C). At 14 and 21 months after starting vaccination, scintigraphic uptake remained negative (Figure 2D, E).

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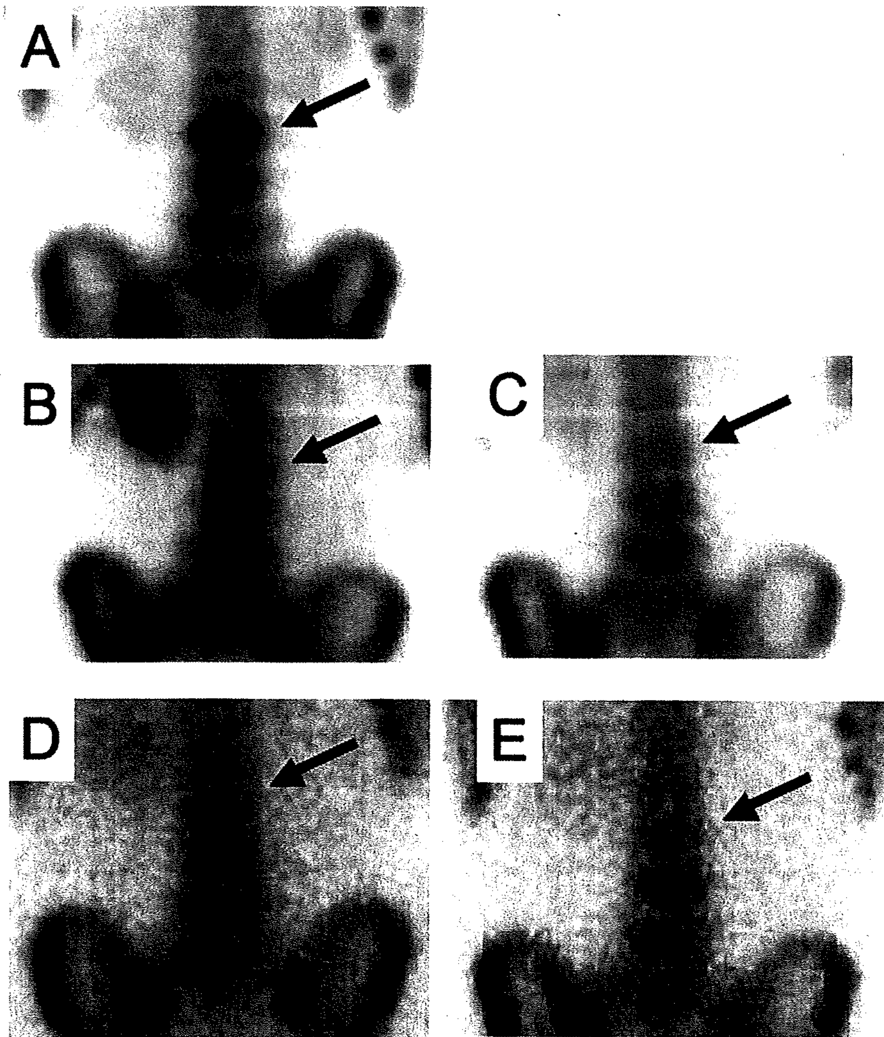

FIGURE 2 Control of new lesions of bone metastasis after the start of WT1 immunotherapy. (A) New lesions (L2, 3, 4) were observed on bone scintigraphy after two courses of combination chemotherapy. Bone scintigraphy before (B) and 3 (C), 14 (D), and 21 (E) months after WT1 vaccination. Scintigraphic uptake disappeared after vaccination. Arrow indicates L2 vertebra.

To evaluate immunological responses to WT1 peptide vaccination, WT1-specific CTL frequencies in peripheral blood and their differentiation state were analyzed by flow cytometry using WT1 tetramer. The frequency of tetramer^{+}CD8^{+} T cells among CD8^{+} T cells was defined as the WT1-specific CTL frequency. The frequency increased from 0.24% before vaccination to 0.37% at 1 month after the start of vaccination (1.54-fold increase). The frequency decreased to the prevaccination level at 4 months, and this was maintained at 13 months. It has recently been shown that these

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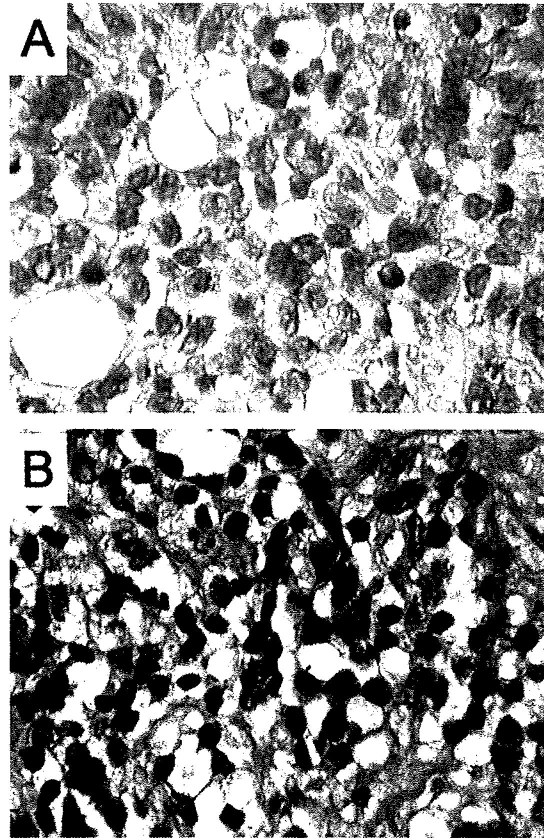

FIGURE 3 Immunohistochemical detection of WT1. Tissues were stained with anti-WT1 antibody 6F-H2 (A). WT1 protein was stained brown. The sections were then counterstained with hematoxylin (B).

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CTLs can be phenotypically classified into 4 differentiation stages according to their expression of CD45RA and CCR7: naïve ($CD45RA^{+}CCR7^{+}$), central memory ($CD45R^{-}CCR7^{+}$), effector memory ($CD45RA^{-}CCR7^{-}$), and effector ($CD45RA^{+}CCR7^{-}$). Before vaccination, approximately half of $tetramer^{+}CD8^{+}$ T cells had an effector memory or effector phenotype, and these cells are considered to attack cancer cells quickly upon antigen-stimulation (Figure 4). This subset composition did not change substantially during vaccination. Compared to peripheral blood of healthy donors, in which the majority (about 80%) of $tetramer^{+}CD8^{+}$ T cells belonged to naïve phenotype [13], a high proportion of WT1-specific CTLs in peripheral blood of our patient were in an activated or differentiated stage.

No adverse effects were observed except for local erythema at the injection sites. The patient's general condition has been good without clinical relapse during WT1 peptide vaccination. The dose of WT1 peptide vaccination was increased to 2 mg from the 64th injection according to her

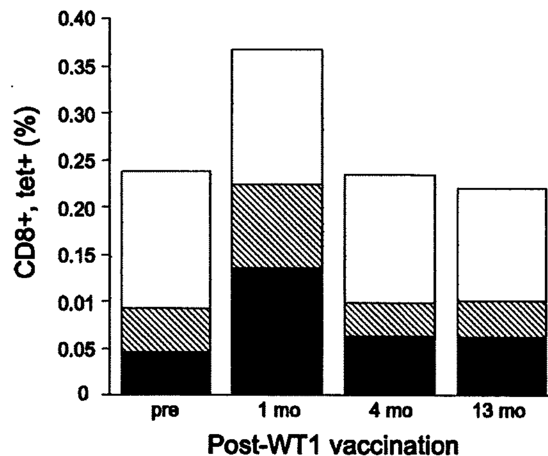

FIGURE 4 WT1-specific CTL frequencies in peripheral blood and CTLs subset composition. WT1-specific CTL frequencies are shown as percentage of WT1-tetramer$^+$CD8$^+$ T cells among CD8$^+$ T cells. CTLs were phenotypically classified into four subsets according to CD45RA and CCR7 expression: naïve (white bars), central memory (not detected), effector memory (black bars), and effector (striped bars).

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weight gain. WT1 peptide vaccination has been continued to date (March 2008) without systemic adverse effects.

DISCUSSION

Rhabdomyosarcoma is the most common malignant soft tissue tumor of childhood. Patients with metastatic disease have a poor prognosis, with 5-year progression-free survival usually less than 30% [14]. Alveolar histology, confirmed by the presence of PAX3-FKHR fusion, is also associated with poor prognosis [15]. Current multidisciplinary treatment has contributed to an improvement of clinical outcomes, but control of disease is often difficult for children with metastatic alveolar rhabdomyosarcoma. Estimated 3-year event-free survival for patients with more than three metastatic sites and non-embryonal histology has been reported to be only 5% [16].

Our patient had primary disease in the lower leg with metastases on distant lymph node, bone, and bone marrow. She also developed a new metastatic bone lesion during the initial two courses of chemotherapy, indicating poor response to chemotherapy. Although she received a total of six courses of combination chemotherapy, high-dose chemotherapy, surgery on the primary site, and radiotherapy on primary and metastatic sites, bone disease remained positive. Considering her poor prognosis, we chose WT1 peptide immunotherapy. After the start of WT1 peptide immunotherapy, uptake disappeared on bone scintigraphy. Despite the resistance to initial chemotherapy, her continuing remission for more than 22 months suggests a positive effect from WT1 peptide vaccination.

The WT1 gene is physiologically expressed in some organs such as kidney, bone marrow, and pleura. Recent studies have shown that WT1-specific CTLs kill WT1-expressing tumor cells, but not normal cells. In mice immunized with MHC class I-restricted 9-mer WT1 peptides or *WT1* cDNA, WT1-specific CTLs induced killing of WT1-expressing tumor cells, but never damaged normal tissues [17, 18]. Several mechanisms have been postulated to account for WT1-specific CTLs ignoring WT1-expressing normal cells: (1) *WT1* expression levels may be different between cancer cells and normal cells; (2) mechanisms for processing of WT1 protein or presentation of WT1 peptide may be different; and (3) susceptibility of the cell membranes to CTL-producing molecules such as perforin may be different [19].

The frequency of WT1-specific CTLs is usually about 0.1% or less in healthy donors [9]. Since the frequency in our case was as high as 0.24% before WT1 peptide vaccination, this indicates that the patient had responded to the WT1 protein derived from the tumor cells and elicited WT1-specific CTLs before WT1 peptide vaccination. The frequency increased from 0.24% before vaccination to 0.37% at 1 month after starting the vaccination (1.54-fold increase). We have previously demonstrated that the emergence of clinical responses is correlated with a greater than 1.5-fold increase in tetramer$^+$ cell frequencies [9]. This finding strongly suggested that WT1 vaccination-driven induction of WT1-specific CTL responses led to a clinical effect in responders. This observation was also in line with the present case in which a greater than 1.5-fold increase in tetramer$^+$ cell frequency was observed with clinical response. Although the frequency decreased to the pre-vaccination level at 4 and 13 months, levels were maintained higher than those in healthy donors. The reason for the decrease in frequencies at later points might be explained by several mechanisms, e.g., activation-induced cell death of WT1-specific CTLs, migration of the CTLs to a tumor site, reduced stimulation of the immune system by WT1 protein owing to reduction in tumor burden (achievement of complete response). We also analyzed phenotype to evaluate the differentiation state of WT1-specific CTLs in our patient. Analysis revealed that many of the tetramer$^+$ cells had the phenotype of effector memory or effector cells, which are considered to be ready for cancer cell attack upon antigen stimulation. Taken together, the high frequencies of WT1-specific CTLs, their increase in frequency after vaccination, and the differentiated (functionally matured) state of the CTLs may contribute to the induction of clinical response.

In conclusion, WT1-peptide immunotherapy was effective with immunological response against residual disease in a child with metastatic alveolar rhabdomyosarcoma. WT1 peptide-based immunotherapy should be considered as a promising option for high-risk rhabdomyosarcoma in childhood.

Declaration of Interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Pediatr Blood Cancer 2009;53:1324–1326

BRIEF REPORT

Cytomegalovirus Infection Mimicking Juvenile Myelomonocytic Leukemia Showing Hypersensitivity to Granulocyte–Macrophage Colony Stimulating Factor

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Sachiyo Kamimura, MD,[1] and Hiroyuki Nunoi, MD, PhD[1]

We describe an infant with cytomegalovirus (CMV) infection presenting as transient myeloproliferation resembling juvenile myelomonocytic leukemia (JMML). The patient fulfilled the international diagnostic criteria of JMML, including hypersensitivity to granulocyte–macrophage colony-stimulating factor (GM-CSF). Viral studies using serologic assays and polymerase chain reaction (PCR) were positive for CMV. Clinical symptoms disappeared and laboratory values returned to normal without specific treatment within 1 year. Follow-up showing a decrease in viral titers suggested CMV infection as an etiologic factor for the development of myeloproliferative features. We conclude that the CMV infection transiently induced abnormal myelopoiesis in this infant. Pediatr Blood Cancer 2009;53:1324–1326. © 2009 Wiley-Liss, Inc.

Key words: cytomegalovirus; juvenile myelomonocytic leukemia; GM-CSF hypersensitivity

INTRODUCTION

The majority of prenatal and postnatal cytomegalovirus (CMV) infections are asymptomatic in newborn periods; however, clinical features of symptomatic CMV infection often overlap other hematologic diseases. Juvenile myelomonocytic leukemia (JMML) is a myeloproliferative/myelodysplastic disorder that primarily affects children younger than 5 years of age. However, there are several reports describing difficulties in discriminating between JMML and infectious diseases [1,2]. The diagnosis of JMML is based on the presence of defined diagnostic criteria including a characteristic hypersensitivity of myeloid progenitors to granulocyte–macrophage colony-stimulating factor (GM-CSF) [2]. We present a case with CMV infection showing GM-CSF hypersensitivity mimicking JMML.

CASE REPORT

A 2-month-old Japanese female with a 1 month history of failure to thrive and repeated infections was referred to our hospital. Physical examinations revealed hepatosplenomegaly with the liver descended 5 cm below the right costal margin and the spleen 4 cm below the left costal margin. Laboratory data showed a white blood cell count of 12.9×10^9/L; hemoglobin, 10.4 g/dl; and platelets, 265×10^9/L. The differential count showed elevated monocytes and immature granulocytes (23% neutrophils, 49% lymphocytes, 4% eosinophils, 14% monocytes, 7% myelocytes, and 3% metamyelocytes). Hemoglobin F was normal at 30.8% (normal range for age: 25–60%). Serologic tests and/or polymerase chain reaction (PCR) analysis for Epstein–Barr virus (EBV), human herpesvirus (HHV)-6, and parvovirus B19 were negative. IgM titer for CMV by enzyme linked immunosorbent assay was positive and the existence of CMV infection was further confirmed by PCR.

The bone marrow was hypercellular with a myeloid/erythroid ratio of 4.6:1. Blasts and promyelocytes comprised 0.4% of nucleated cells. No myelodysplasia was seen in the bone marrow. Karyotyping of marrow cells revealed 46XX, with no Philadelphia chromosome or monosomy 7. To differentiate JMML from CMV infection, in vitro culture assays of bone marrow and peripheral blood were examined [3]. The results showed spontaneous proliferation of predominantly monocytic/macrophage colonies. In vitro assays showed the patient's cells were hypersensitive to GM-CSF (Table I).

Though the patient fulfilled the criteria of JMML [2], no specific treatment was required because no sign of progressive disease was seen. The patient was closely monitored and her clinical course was unremarkable with gradual resolution of hepatosplenomegaly and blood counts. Peripheral blood monocyte count dropped below

TABLE I. Spontaneous CFU-GM Formation and GM-CSF Dose–Response Analysis From Patient Samples

GM-CSF (ng/ml)	Peripheral blood (normal range)	Bone marrow (normal range)
0	73 (0–8)	78 (0–3)
0.01		103 (1–6)
0.1		118 (3–6)
1		129 (5–14)
10		126 (23–46)

The colony assays were performed as previously described [3]. The depletion of monocytes was employed for bone marrow cells. 1×10^5 cells were used for peripheral blood whereas 2×10^4 cells were cultured for bone marrow cells. CFU-GM, colony forming unit-granulocyte and macrophage; GM-CSF, granulocyte–macrophage colony-stimulating factor.

Additional Supporting Information may be found in the online version of this article.

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TABLE II. Serial Evaluation of Peripheral Blood Counts and Viral Titers for Cytomegalovirus

	1/22/2002	1/29/2002	2/7/2002	2/14/2002	2/26/2002	4/16/2002	7/4/2002
Hemoglobin (g/dl)	10.4	9.8	9.9	10.1	10.1	12.8	14.2
WBC count ($\times 10^3/\mu l$)	12.9	15.6	14.6	15.6	8.9	10.8	8.9
Platelet count ($\times 10^3/\mu l$)	265	384	352	345	244	443	325
Differential leukocyte count (%)							
Neutrophils	23	20	22	32	16	15	15
Lymphocytes	49	62	50	46	69	71	74
Eosinophils	4	0	4	0	5	6	2
Monocytes	14	11	18	12	8	8	8
Basophils	0	0	0	2	1	0	1
Myelocytes	7	3	2	2	0	0	0
Metamyelocyte	3	3	4	6	1	0	0
Blasts	0	0	0	0	0	0	0
Cytomegalovirus IgM	3.94		3.39		2.92	1.01	0.55

1×10^9/L at 3 months of age, and hepatosplenomegaly disappeared at 9 months. A fall in viral titers for CMV was also observed during follow-up (Table II). After a follow-up of 2 years, the patient is well at 26 months of age with no sign of hepatosplenomegaly, and has completely normal blood counts.

DISCUSSION

CMV infection is commonly associated with hematologic abnormalities including leukocytosis with atypical lymphocytes, hemolytic anemia, and thrombocytopenia. JMML is known to be mimicked by a variety of infectious organisms, including EBV [4], HHV-6 [5], parvovirus B19 [6], and CMV. However, there are three reports of progressive cases where infants suffered from JMML in spite of a diagnosis of CMV infection [1,7,8]. Although it is important to differentiate JMML from an infectious disease, definite discrimination is often very difficult. Since half the children with JMML have evidence of some clonal disorder, in cases with no clonal abnormality, caution should be taken in making a diagnosis of JMML. Tartaglia et al. [9] reported that a somatic mutation in PTPN11 is responsible for oncogenesis in JMML. Further exploration of mutations in genes involved in the RAS/MAPK pathway (NRAS, KRAS2, NF1, and PTPN11) may be helpful to confirm a diagnosis of JMML. Our case spontaneously recovered a normal blood count and has survived more than 2 years without any treatment for JMML after the hematologic abnormalities were detected, even though hypersensitivity to GM-CSF, which is believed to be critical for the diagnosis of JMML, was seen. Our patient had three characteristics that were inconsistent with JMML. First, elevation of hemoglobin F above the normal range was not detected. Second, thrombocytopenia was not seen throughout the clinical course. Finally, dysplastic features were not found. These findings may be helpful in distinguishing infection from JMML. Recently, Koetecha et al. [10] published that GM-CSF induced phosphorylated STAT5 could be detected by flow cytometry-based signaling assays in JMML cells, but not from other childhood myeloprolifearative disorders. Furthermore, Gaipa et al. [11] described three JMML case reports using similar method and confirmed that its application in JMML might represent a new integrated diagnostic tool. They suggest the JAK-STAT signaling pathway has a critical role in the biologic mechanism of JMML. We also investigated GM-CSF-induced phosphorylation of STAT5 in our patient's frozen bone marrow sample and compared it with the monocytic cell line U937 that expresses a PTPN11 exon 3 mutation, as well as a frozen bone marrow sample from a normal healthy volunteer. We used phosphospecific flow cytometry after exposure to increasing concentrations of GM-CSF according to their methods. The expression of a phosphorylated STAT5 population in samples from our patient or a healthy volunteer was minimal compared to the reaction seen in U937 cells (Supplemental Fig. 1), consistent with their reports. Elucidation of these molecular mechanisms will contribute to a definitive differentiation between JMML and mimicking cases.

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緩和支持治療の量と質の充実と普及をめざす先進的な取り組み

社会福祉法人聖隷福祉事業団 総合病院 聖隷三方原病院（874床：静岡県浜松市）

屋上にヘリポートがあるのが聖隷三方原病院．当地域は，聖隷クリストファー大学，聖隷おおぞら療育センター（重症心身障害児者施設），浜名湖エデンの園（老人ホーム），三方原ベテルホーム（介護老人保健施設）など，聖隷福祉事業団の保健・医療・福祉の一大拠点となっている．

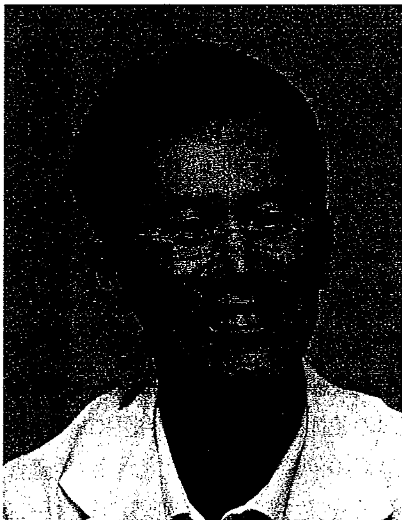
緩和ケアチームは口腔ケアとリハビリが加わる総合的な体制

聖隷三方原病院はキリスト教精神に基づく隣人愛を基本理念に，1942年の開院以来，地域に根差した住民に信頼される病院づくりを進めてきた．1981年には国内初のホスピス病棟「聖隷ホスピス」を開設して終末期医療分野のさきがけとなり，現在その地位は不動のものとなっている．

近年の早期緩和ケアに対するニーズの高まりから，2003年に患者とその家族のQOL向上も視野に入れた緩和支持治療科を開設．標榜科について部長の森田達也先生は，「欧米で使われているpalliative and supportive careは，痛みや不快な症状を和らげることだけでなく，治療の下支えとなる栄養療法や心理療法なども含めた緩和医療を示します．それを治療として取り組んでいきたいと考え，緩和支持治療科と命名しました」と思い入れを話す．

同院の緩和ケアチームの構成は，専任が緩和支持治療科の森田先生と緩和ケア認定看護師の1名で，兼任がホスピス所属の医師1名と精神科医2名，麻酔科医1名，看護師2名，薬剤師3名の合計9名．これに口腔チーム，リハビリテーション部らが加わる．他院と比べ非常に充実した体制で，チーム内の連携についても緩和ケアを熟知したベテランスタッフが多くスムーズだという．特に口腔チームは患者からのニーズが高く，歯科医と歯科衛生士が併診する体制をとっている．

緩和ケアチームの構造には2つの種類がある．緩和治療専門医と看護師をコアに，麻酔科医や精神科医らが支える総合病院向きのチームと，看護師をリエゾン役とし，麻酔科医や精神科医，内科医が協働する専門病院向きのチームである（図1）．同院では総合病院の強みを生かし，緩和ケアチームが効率的に活動できる



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Editorial board of：Journal of Pain and Symptom Management, Journal of Palliative Care, Palliative Medicine, Palliative and Supportive Care, 緩和ケア，緩和医療学

Editor：Japanese Journal of Clinical Oncology

緩和支持治療科ならびに緩和ケア普及のための地域プロジェクトのスタッフ

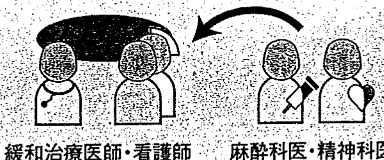

図1　緩和ケアチームの構造

前者の体制を採用した．

また，緩和ケアチーム内の多職種連携だけでなく，主治医や病棟看護師らと緩和ケアチームとの連携も円滑に行われている．国内で初めてホスピスを開設した同院ならではの緩和医療に親和性のある環境が，緩和ケアチームの活動を活発なものにしている．

特に神経ブロックによる疼痛治療では，緩和支持治療科が主にその必要性を判断し，患者からの同意書の取得と処置後の調整まで行う．麻酔科は処置を行うことに専念できるため，迅速な疼痛管理が可能になった．森田先生は「麻酔科との間で，週1回，合同カンファレンスを開き，難治性疼痛に関して症例検討を行っているからこそ，信頼しあえる」と，他科連携の重要性を説く．

症状緩和の専門家として どれだけ緩和技術の「引き出し」を多く持つことができるか

緩和ケアチームの機能が，より一層発揮できるようにするにはどうしたらよいのだろうか．これについて森田先生は，「主治医や病棟看護師が持っていない知識とスキルを身につけること．それに加えてコミュニケーションが大切なポイントです」と強調する．

たとえば主治医から，「…の疼痛で，オキシコドン40mg，エトドラク錠2Tで鎮痛は少しできてきましたが，まだ痛みが強く眠気があります．方法はありますか」と相談を受けたとする．このとき，「フェンタニル貼付剤を加えてみてはどうですか」というようなシンプルなオプション提示だけでは不十分だという．

「眠気の原因として高カルシウム血症がスクリーニングされていないので，次回の採血時に除外してください．CNSの情報は3カ月前のCTではネガティブですが，今回の対応でも眠気が続く場合は，一度見ておいたほうがいいと思います」と，前提となる診察情報をレビューした上で，「身体的に眠気をきたす要因がないことが確認できたら，鎮痛治療薬として次のような選択があります」と，具体的な選択肢を示す．

①NSAIDsを強化（エトドラク錠2T→4T，ロキソプロフェン3T，ジクロフェナクナトリウムSR2C）
②エトドラク錠にアセトアミノフェン1.5～2.0gを追加
③オキシコドン40mgをフェンタニルパッチMT4.2mgに変更した後，眠気のない範囲で4.2mg→6.3mg→8.4mgと増量
④オキシコドンのまま，あるいはフェンタニルパッチにペモリンを併用．投与量は0.5T頓用で開始し1日2Tまでとする．10%で不眠・不安・不穏，1%以下で重篤な肝障害のリスクがある．

コンサルト結果を「…してください」と言うのではなく，「①，②，③…の選択肢があり，その順で推奨します」と複数の適切な選択肢を提示する．このようなオプション提示のできる緩和ケアチームは，症状緩和に悩む主治医にとっては心強い存在だろう．多くの症例を扱って経験を蓄積することで主治医にはない知識とスキルをもつことができる．複数の選択肢を提示して専門家として応えることが緩和ケアチームの信頼感につながるのだ．貴重な知識とスキルの一部は整理・マニュアル化され，同院のホームページ上で閲覧可能である（図2）．

また，もうひとつの方法としては，主治医に慣れている方法をあげてもらい，緩和ケアチームがその方法の工夫を提示する方法もある．治療全体のイニシアチブは主治医にあり，主治医の治療法を尊重しながら最適な結果を出すことが求められるケースもあるからだ．森田先生は「主治医チームを支えるのが緩和ケアチームです．知識とスキルに加え，主治医チームからの信頼を得るコミュニケーション能力も求められます」と繰り返しアピールする．

また，緩和ケアチームは外部から見て1つの集団であることも肝要で，主治医が緩和ケアチームの誰にたずねても同じ内容を伝えられることが，信頼の秘訣だという．