All patients in our cohort had received initial treatment according to Protocols A of Japan Langerhans Cell Histiocytosis Study Group (JLSG)-02 [6]. Use of Protocol A involves administration of vincristine (VCR) and an intermediate dose of cytarabine (AraC) and prednisolone (PSL), followed by 48 weeks of maintenance therapy with a combination of VCR/AraC/PSL, methotrexate (MTX) and vinblastine (VBL)/PSL. Patients who showed NR to Protocol A or reactivation during maintenance therapy were switched to the intensive salvage regimen, Protocol B1, which involves administration of a combination of doxorubicin (DOX), cyclophosphamide (CPA), VCR and PSL. For extremely high-risk patients who showed PD after induction A, Protocol B2 which includes continuous infusion of cyclosporine A in addition to Protocol B1 was employed. Four patients with MS+ (UPN 4181, 6251, 1031 and 7041) were refractory to both Protocol A and B1, and two patients (UPN 4181 and 6251) were refractory also to Protocol B2. One patient with MS+ (UPN 7151), who had showed PR to Protocol B1, developed PD during maintenance therapy. Three further patients with MS disease (UPN 3451, 6221 and 5301) developed reactivation of disease during maintenance chemotherapy. One of them (UPN 6221) had been resistant to Protocol A. The remaining 5 patients with MS disease developed reactivation following the completion of chemotherapy (UPN 4271, 3601, 5332, 5382 and 6111). Two of them (UPN 4271 and 3601) had been resistant to Protocol A. One patient with MFB disease and a soft tissue mass (UPN 6222) showed no response to Protocol A or B2. One patients with MFB disease developed reactivation during maintenance therapy (UPN 1061). The 2 remaining patients with MFB disease developed reactivation of disease following the completion of chemotherapy (UPN 6052 and 5331). One of them (UPN 6052) was resistant to Protocol A (Table 1).

3.3 Treatment with 2-CdA

A dose of 4–9 mg/m^2/day of 2-CdA was administered daily for 2–5 consecutive days, and this was repeated every 3–4 weeks for a total period that ranged from 2 months to 1 year. The most frequently administered dose of 2-CdA (in 7 out of 17 patients) was 5 mg/m^2 given daily for 5 consecutive days. Four patients with MS disease (UPN 4181, 6251, 1031 and 7151) were administered high dose cytarabine (HD-AraC) in addition to 2-CdA (Table 1; Fig. 1).

3.4 Response to 2-CdA

Four MS+ patients were treated with HD-AraC in addition to 2-CdA. Two showed response (NAD/PR:1/1) and 2 did not (NR/PD:1/1). Two patients who did not respond to 2-CdA with HD-AraC underwent hematopoietic stem cell transplantation (HSCT) and one of them survived (UPN 1031).

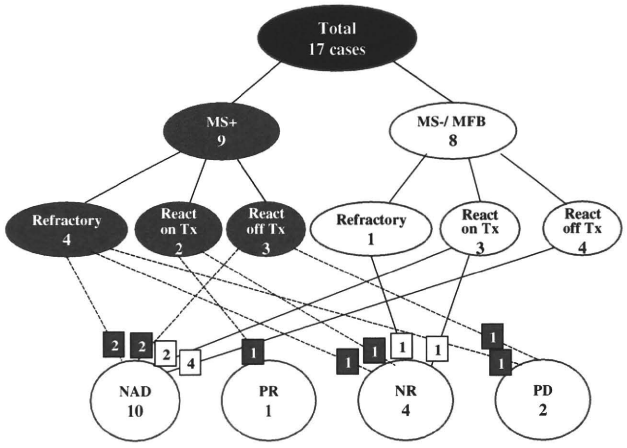

Fig. 1 Schematic representation of outcome of 2-CdA treatment in 17 cases based on disease type and time of reactivation. *MS+* multisystem disease with risk organ involvement, *MS−* multisystem disease without risk organ involvement, *MFB* multifocal bone, *React* reactivation, *NAD* no active disease, *PR* partial response, *NR* no response, *PD* progressive disease. # Both disease type and time of reactivation are described at the time of initiating 2-CdA

Of the remaining 13 patients, all but one patient (UPN 5301) were treated with 2-CdA alone (Table 1). In the 5 MS+ patients, 3 showed response (NAD/PR:3/0) and 2 did not (NR/PD:1/1). One of the 2 patients with MS+ who did not respond to 2-CdA therapy underwent HSCT and survived with NAD (UPN 3451). In the 8 MS− or MFB patients, 6 showed response (NAD/PR:6/0) and 2 did not (NR/PD:2/0). However, the 2 patients who did not respond to 2-CdA therapy survived with the disease (UPN 6221 and 6222). Collectively, 8 of 12 patients attained NAD following treatment with 2-CdA monotherapy. Of these 8 patients, six survived with NAD for a median of 20 months (range 7–25 months) from the initiation of 2-CdA therapy without any further treatment. In total, the rate of attainment of NAD was relatively high in patients who had developed reactivated disease while off therapy (6/7 vs. 4/10; $P = 0.13$).

In terms of association with the response to initial therapy, 7 of 13 patients who had been treated with induction B at onset responded to 2-CdA, including the cases treated with a combination of HD-AraC and 2-CdA (UPN 4181, 7151). On the other hand, all 4 patients who had responded to induction A initially responded to 2-CdA (UPN 1061, 5331, 5332, 6111).

3.5 Patients with CNS involvement

Four patients in our cohort had CNS involvement. Of these, 2 attained NAD: 1 patient with a meningeal lesion (UPN

3601) and 1 patient with a hypothalamic-pituitary lesion (UPN 6111). Two patients showed NR: 1 patient with a hypothalamic-pituitary lesion (UPN 6251) and 1 patient with cerebellar and meningeal lesions (UPN 3451).

3.6 Adverse events

Thirteen of the 17 patients experienced a grade 4 hematological adverse event: 10 developed neutropenia, 7 developed thrombocytopenia and 4 developed anemia. Prolonged cytopenia over 4 weeks of duration occurred in 3 of 8 patients who received more than 150 mg/m^2 of 2-CdA (UPN 7151, 6111 and 1061) (Table 1). Four of the 17 patients developed a grade 3 infection (including 1 case of pulmonary aspergillosis), 3 of whom had been treated with a combination of 2-CdA and HD-AraC. One patient with severe lymphopenia developed fatal idiopathic interstitial pneumonia following 9 courses of 2-CdA therapy (UPN 7151), although the patient underwent pneumocystis prophylaxis. Even though extensive search for clarifying pathogens including pneumocystis and fungus was conducted, none of the pathogens was identified. Two patients developed eosinophilia, with absolute eosinophil counts of more than 1,000/μl.

4 Discussion

Administration of 2-CdA is considered to be a potential therapeutic strategy in LCH, since it has been shown to cause monocytopenia and to be effective in the treatment of indolent lymphoma [7, 8]. Saven et al. [9] reported the first adult LCH patient to be successfully treated with 2-CdA, and there have been several subsequent reports of 2-CdA treatment in LCH patients [3–5, 10, 11]. The International Histiocyte Society conducted a retrospective analysis that demonstrated that 2-CdA was an effective salvage therapy in more than 50% of LCH patients who had not responded to intensive first-line therapy [10]. However, they were unable to determine the precise factors that were associated with the effectiveness of 2-CdA in the treatment of LCH. Although those retrospective analyses or case reports described a high degree of effectiveness of 2-CdA as salvage therapy for refractory/reactivated LCH, the prospective study was expected to clarify the effectiveness of this agent. The results of the LCH-S-98 study, which was a prospective phase II Histiocyte Society Study to evaluate the efficacy of 2-CdA monotherapy as salvage therapy in refractory or reactivated LCH, was published in 2009 [12]. They demonstrated that 22% of MS(+) patients had a good response (NAD and PR) while 44% progressed; 62% of patients with MS(−) or MFB disease responded and 11% progressed. These findings suggest that this agent produces higher response rate in patients with MS(−) or MFB than those with MS(+).

This is the first nationwide survey to have assessed the outcome of 2-CdA treatment in LCH patients in Japan. In this survey, we identified 17 pediatric LCH patients who had received second-line treatment with 2-CdA following ineffective initial treatment according to the JLSG-02 protocol. NAD attained in the present study was 64.7% (11/17), which is similar to that reported in previous retrospective studies [10]. In terms of biological and clinical characteristics that may predict a favorable outcome with 2-CdA therapy, reactivation of disease that occurs while off therapy may be a factor that appears to be associated with a favorable response to treatment, although it is not statistically significant because of the small number of the current cohort. The time of reactivation is considered to be deeply associated with the sensitivity for the chemotherapeutic agents in each patient. In other words, the disease that reactivates while off therapy might maintain sensitivity even for the agents used initially, indicating that resuming the original treatment might be also effective. In this cohort, 2-CdA was used for the patients who were resistant to chemotherapeutic agents, including not only VCR, but also Ara-C, DOX and CPA, suggesting that these patients had highly chemo-resistant disease. Thus, 2-CdA is not suitable to treat such cases, if used as a single agent.

Neutropenia and/or thrombocytopenia were the most frequently observed adverse events occurring during 2 CdA treatment in our cohort. These adverse events were observed more frequently in patients who had received combination therapy with AraC. 2-CdA is known to induce prolonged cytopenia [13], although this was not a cause for discontinuation of therapy in our cohort. One patient in our cohort died of interstitial pneumonia with lymphopenia. Transient neutropenia and thrombocytopenia are relatively common adverse events. However, in our cohort, prolonged cytopenia occurred in 3 of 8 patients who received more than 150 mg/m^2 of 2-CdA. These findings suggest that further studies are indicated to determine the total amount of 2-CdA that can be administered without the development of prolonged hematological toxicity.

The treatment of patients with refractory/reactivated MS+ disease is challenging. A combination of AraC and 2-CdA has been shown to exert synergistic cytotoxicity, even in resistant cells [14]. Bernard et al. [15] have reported chemotherapy with 2-CdA and HD-AraC to be a promising treatment for patients with refractory LCH. They administered a combination of 2-CdA (9 mg/m^2/day) and AraC (1,000 mg/m^2/day) for 5 days and control of disease was achieved in all ten patients in their study. However, all patients suffered grade 4 hematological toxicity and two patients died of infection. In our cohort, treatment with 2-CdA, even when combined with HD-AraC, only induced

remission in 1 of 4 patients with refractory MS+ disease. The low response rate in our refractory cases might be attributable to the higher resistance to treatment of our patients, who had not responded to the high intensity Protocol B of the JLSG [6].

HSCT is considered to be a curative treatment for patients with refractory/reactivated MS+ disease [16, 17]. However, this procedure has a high mortality rate [16]. Before performing HSCT, it is important to control disease activity with minimum toxicity. In one patient in this survey (UPN 3451), who developed reactivated MS+ disease while maintenance therapy, partial control of the disease with minimal adverse events was achieved using 2-CdA, and the patient subsequently underwent successful HSCT.

Optimal treatment for LCH involving the central nervous system has not yet been established, and the response of LCH patients with parenchymal brain lesions to chemotherapy has been disappointing [18, 19]. Our cohort included four patients with CNS-LCH (Table 1). 2-CdA crosses the blood–brain barrier, and 2-CdA levels in the cerebrospinal fluid are 25% of those found in plasma [20]. 2-CdA would therefore be expected to be effective in LCH patients with CNS lesions, with the exception of CNS degeneration (CNS-D). Dhall et al. [21] reported that 8 of 12 patients with CNS-LCH who were treated with 2-CdA showed a complete response radiographically. Treatment with 2-CdA was also shown to be effective for CNS-LCH in our cohort. These findings suggest that 2-CdA may be the most effective agent for a CNS lesion in LCH. Further studies of larger patient samples are required to establish the efficacy of 2-CdA in CNS-LCH.

In conclusion, our results suggest that 2-CdA is a potentially useful agent in the treatment of indolent recurrent LCH or CNS-LCH. Further prospective studies are warranted to firmly establish the role of this agent in the treatment of patients with LCH.

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LETTER TO THE EDITOR
VCR/AraC Chemotherapy and ND-CNS-LCH

To the Editor: We read with great interest the recent article by Allen et al. [1] on the use of vincristine (VCR) and cytosine arabinoside (AraC) combination for neurodegenerative central nervous system Langerhans cell histiocytosis (ND-CNS-LCH). The authors chose to employ this treatment regimen for two reasons: first, AraC can cross the blood–brain barrier, although no significant pharmacokinetic studies were done at the dose used; second, this combination was originally described by Egeler et al. [2] to be effective for patients with LCH at onset or relapse of disease.

Starting in 1996, we have been testing whether induction therapies that consist of VCR/AraC/prednisolone (PSL), called the JLSG-96 and JLSG-02 protocols, are effective for newly diagnosed patients with LCH in Japan [3,4]. By the end of 2009, more than a total of 340 multifocal LCH cases have been treated with these two protocols. Prior to 1996 there was no clinical trial. Even after 1996 the patients not participating on the study were treated with individual non-VCR/AraC regimens. We compared the cases of ND-CNS as defined by the Vienna group [5,6], in the VCR/AraC-treated group and the group who received other treatments for newly diagnosed LCH. Of the 16 cases in total 13 cases developed ND-CNS after systemic treatment for LCH; 8 had MRI abnormalities only and 5 had neurological symptoms. One case already had ND-CNS at the time LCH was diagnosed and the remaining two cases already had ataxia before LCH was diagnosed (Table I).

Of the 13 cases who developed ND-CNS after treatment, 5 had been treated with JLSG-96/02 protocols. The remaining eight had received other regimens (mostly a combination of vinblastine and PSL), of which four were the cases treated before 1996. The ND-CNS incidence for the JLSG-96/02 group was 1.5% but it could not be calculated for the other group because the total number of patients with LCH treated with other regimens was unknown. The incidence of ND-CNS LCH in the JLSG-96/02 protocol looks low compared with other international treatment protocols [7], but we must wait for longer and more comparable follow-up. When we used the EDSS [8] to score the neurological symptoms as minimal (<2.5), moderate (2.5–5), or severe (>5.0), we found that, with the follow-up of a median 6.2 (range; 0.5–18) years, 9 of the 16 patients have moderate to severe ND-CNS disease. One case with ND-CNS at onset has been stable with JLSG-02 treatment as there was no progression of neurological symptoms. The remaining two cases who had presented with ataxia before the diagnosis of LCH and received JLSG-02 protocol, have the worst prognosis as their neurological symptoms are now severe. We are also testing whether high-dose intravenous immunoglobulin (IVIG) regimen can prevent further progression of neurological symptoms particularly in cases with minimal to moderate EDSS scores [9].

It remains unknown whether the initial systemic chemotherapy for multifocal LCH patients can limit the later occurrence of ND-CNS. However, our data suggest that ND-CNS can also develop in LCH patients who were initially treated with the VCR/AraC combination. Thus, while this drug combination can be effective on active non-CNS LCH lesions, it is at present unclear whether it also acts on neurodegenerative lesions with the same mechanism.

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TABLE I. Clinical Characteristics of the 16 Cases of ND-CNS-LCH That Are Registered in JLSG

Onset of ND-CNS	n	Initial treatment for LCH*	Time to ND-CNS from onset (years)	IVIG for ND-CNS (n/total)	Current status of ND-CNS**
After LCH treatment	5	JLSG-96/02	3.5 (2.0–6.9)	4/5	Minimal (n = 3) Moderate (n = 1) Severe (n = 1)
	8***	Other regimens	4.5 (1.5–13.5)	4/8	Minimal (n = 3) Moderate (n = 4) Severe (n = 1)
At diagnosis of LCH	1	JLSG-02	0	1/1	Minimal (n = 1)
Ataxia preceded the diagnosis of LCH	2	JLSG-02	−0.5, −0.8	2/2	Severe (n = 2)

* VCR/AraC was employed in the JLSG-96/02 protocol but not in the other regimens. ** EDSS scores: minimal (<2.5), moderate (2.5–5.0), and severe (>5.0), *** Of which, four had the onset of LCH before 1996.

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ORIGINAL ARTICLE

Improved outcome of refractory Langerhans cell histiocytosis in children with hematopoietic stem cell transplantation in Japan

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Langerhans cell histiocytosis (LCH) that is refractory to conventional chemotherapy has a poor outcome. Hematopoietic stem cell transplanta tion (SCT) is a promising approach for refractory LCH because of its immunomodulatory effect. In this study, the outcomes of children with refractory LCH undergoing SCT in Japan were analyzed. Between November 1995 and March 2007, 15 children younger than 15 years (9 males, 6 females) with refractory LCH underwent SCT. The patients' median age at diagnosis was 8 months (range, 28 days to 28 months), and all had failed conventional chemotherapy. The median age at SCT was 23 months (range, 13–178 months). Nine had risk organ involvement at diagnosis, including liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). For SCT, a myeloablative regimen was used for 10 patients, and a reduced-intensity conditioning regimen (RIC) was used for five. The donor source varied among the patients, but allogeneic cord blood was primarily used ($n=10$). Subsequently, 11 of 15 patients have survived with no evidence of disease, with a 10-year overall survival (OS) rate (median ± standard error) of 73.3 ± 11.4%. The 10-year OS rate of nine patients with risk organ involvement at diagnosis was 55.6 ± 16.6%, whereas six without risk organ involvement have all survived with no evidence of disease ($P=0.07$). These results indicate that SCT is promising as a salvage approach for children with refractory LCH.

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Keywords: Langerhans cell histiocytosis; refractory; stem cell transplantation; reduced intensity conditioning

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Introduction

Langerhans cell histiocytosis (LCH) is a rare disease with a wide variety of clinical presentations, from localized disease to disseminated disease.[1–3] Although the risk factors for LCH have not been fully elucidated, patients younger than 2 years of age at onset, with risk organ involvement, including the hematopoietic system, liver, spleen, or lung, and disease refractory to conventional chemotherapy have a very poor outcome, with survival rates of about 20%.[4–8]

The treatment strategy for these high-risk LCH patients has not yet been established. Recently, it was reported that a combination of 2-chlorodeoxyadenosine (2-CdA) and cytarabine (Ara-C) was effective for refractory LCH.[9] Allogeneic stem cell transplantation (SCT) has also been used because of its strong immunomodulatory effects for LCH.[10,11] This report describes the improved outcomes of 15 children with refractory LCH who underwent SCT in Japan.

Patients and methods

Data collection

The HLH/LCH committee of the Japanese Society of Pediatric Hematology (JSPH) sent the first questionnaires to all hospitals in Japan where pediatric hematologists (JSPH members) worked, asking for the number of children with LCH who underwent SCT between November 1995 and March 2007. The second questionnaires were then sent to 16 hospitals where SCT was done for LCH, asking about the clinical features at onset, treatment before SCT, donor source, conditioning regimen, complications, and outcome. Thirteen hospitals responded to the second questionnaires, with a total of 15 eligible patients. The registration data of the pediatric SCT program, independently managed by the SCT committee of the JSPH, were also available to confirm the profiles of the patients who underwent SCT.

Diagnostic criteria and definition of disease state

All patients were diagnosed as having LCH by histopathological examination of the affected organs, which were positive for either CD1a or S100 staining. Each patient was divided into one of three subsets at diagnosis: single system single-site (SS-s), single system multi-site (SS-m), and multi-system (MS). In the MS subset, patients with one of the following factors were classified as the high-risk group: younger than 2 years of age at onset; risk organ involvement, including the hematopoietic system (bone marrow, BM), liver, spleen, or lung; or disease refractory to conventional chemotherapy. Patients without these factors in the MS, SS-m, and SS-s subsets were classified as the low-risk group. The characteristics of these 15 patients are shown in Table 1. The disease state was evaluated at three time-points in all patients: (1) within 6 weeks after initial diagnosis, (2) within 12 or 14 weeks after diagnosis, and (3) before SCT. A good response (GR) was defined as the disappearance of signs or symptoms of disease; a partial response (PR) was defined as regression $>50\%$ of signs or symptoms of disease with no organ dysfunction and no new lesions; a non-response (NR) was defined as regression $<50\%$ of signs or symptoms of disease with or without organ dysfunction and no new lesions; and progressive disease (PD) was defined as progressive signs or symptoms of disease and/or the appearance of new lesions.[8]

Statistical analyses

Continuous variables were compared using the Mann–Whitney *U*-test. The overall survival (OS) rate with standard error (s.e.) was estimated using the Kaplan–Meier method and compared using the log-rank test.[12] The OS was calculated for the period from the day of diagnosis until the day of death from any cause. The outcome data were updated in December 2008.

Results

Clinical course of 15 patients before SCT

Between November 1995 and March 2007, 15 children (9 males, 6 females) with LCH refractory to conventional chemotherapy underwent SCT at 13 institutions. The characteristics of all 15 patients are summarized in Table 1, and the details of each patient are shown in Table 2. The median age at diagnosis was 8 months (range, 28 days to 28 months). At initial diagnosis, 12 patients had MS type LCH, and nine had risk organ involvement, including the liver ($n=6$), spleen ($n=5$), lung ($n=5$), and/or hematopoietic system ($n=4$). One patient had diabetes insipidus (DI) and pituitary gland involvement at diagnosis. No CNS disorders other than DI were found in any of the patients. Two patients had SS-m type LCH at diagnosis, with multiple bone lesions. One patient had SS-s type LCH, with thymus involvement and respiratory dysfunction at initial diagnosis.

Eleven patients had received conventional chemotherapy according to the study protocol JLSG-96 ($n=4$) or JLSG-02 ($n=4$) of the Japan LCH study group,[8] and DAL-HX 83 ($n=3$) of the Deutsche Arbeitsgemeinschaft fur Leukaemieforschung Histiocytosis X-83 study group.[4] Remaining four patients had received multi-drug chemotherapy following their institutional protocol. Two patients received the combination of 2-CdA and high dose Ara-C before SCT as salvage therapy and failed to achieve complete remission (patients 6 and 8). Six weeks after initial diagnosis, the disease state was PD ($n=6$), NR ($n=2$), PR ($n=5$), and GR ($n=2$). Twelve or 14 weeks after chemotherapy, the disease state was PD ($n=6$), NR ($n=0$), PR ($n=6$), and GR ($n=3$). Eleven patients with MS type, two with SS-m type, and one with SS-s type at diagnosis had risk organ involvement before SCT.

Table 1 Summary of the clinical characteristics of the LCH patients who underwent SCT

No. of patients	15
Age, median (range)	8 months (28 days to 28 months)
Sex, male/female	9 males, 6 females
Stage at diagnosis	
MS	12
SS-m	2
SS-s	1
Age at SCT, median (range)	23 months (13–178 months)
Allo-SCT/Auto-SCT	13 allo, 2 auto
Time from diagnosis to SCT	
Median (range)	12 months (7–164 months)
Observation time	
From SCT ($n=11$), median (range)	100 months (20–158 months)
From Dx ($n=11$), median (range)	110 months (27–277 months)

Abbreviations: allo = allogeneic; auto = autologous; Dx = diagnosis; SCT = stem cell transplantation.

The median age at SCT of 15 patients was 23 months (range, 13–178 months). At SCT, 14 patients had risk organ involvement. The affected organs before SCT were liver ($n=10$), hematopoietic system ($n=7$), and lung ($n=4$). Five of six patients in the low-risk group had risk organ involvement at SCT.

The disease status of six patients (patients 2–6 and patient 8) in the high-risk group was PD with risk organ involvement at SCT. The disease status of patient 2 was PD despite chemotherapy according to the JLSG-02 protocol, and liver dysfunction developed gradually. BM involvement was observed in patients 3–6, patient 8, patient 9, and patient 13 despite multi-drug chemotherapy. Patient 3, patient 6, and patient 8 suffered from serious infections before SCT. All six of these patients underwent SCT during 8–11 months after diagnosis. The disease status of the other three patients (patient 1, patient 7, and patient 9) was PR or NR with risk organ involvement at SCT. These three patients underwent SCT for 12–30 months after diagnosis.

Three of the six patients in the low-risk group (patient 10, patient 12 and patient 14) had suffered from recurrent active disease for 6, 13, and 7 years, respectively, despite chemotherapy consisting of vincristine, pirarubicin, prednisone, Ara-C, vinblastine, etoposide, and cyclophosphamide with or without cyclosporine. At recurrence, lung involvement and left phrenic nerve palsy were observed in patient 10, who required oxygen for 2 months. Patient 12 had recurrence of multiple bone lesions, including the ear, and needed a hearing aid. She relapsed twice with multiple

Table 2 Detailed characteristics of the 15 patients with refractory LCH who underwent SCT

Patient No.	*Sex*	*Onset*				*At SCT*					*Outcome*[b]
		Age	*Involved organ*	*Type of LCH*	*Initial response*	*Age*	*Involved organ*	*Disease status*	*Donor source*	*Conditioning regimen*[a]	
1	M	3 months	LIV, SPL, LU, skin, bone, LN	MS	PR	23 months	LIV, skin, bone, LN	PR	CB (sibling)	RIC (Flu, PAM)	Alive in CR (+73 months)
2	M	5 months	LU, skin	MS	PD	13 months	LIV	PD	PB (mother)	RIC (Flu, PAM)	Died (18 days)
3	F	5 months	LIV, SPL, LN	MS	NR	14 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, ATG)	Alive in CR (+92 months)
4 (1st)	F	8 months	BM, LIV, skin, bone, LN, thymus	MS	PD	17 months	BM, LIV	PD	PB (father)	RIC (Flu, CY, TBI)	
4 (2nd)						19 months	BM, LIV, LU	PD	PB (father)	RIC (TBI)	Relapse and died (271 days)
5	M	8 months	BM, LIV, SPL, LU, skin, bone	MS	PD	17 months	BM, LIV	PD	UCB	Myeloab (TBI, VP16, PAM)	Alive in CR (+107 months)
6	M	8 months	LIV, SPL, skin, middle ear, LN	MS	NR	16 months	BM	PD	UCB	RIC (Flu, PAM, TBI)	Alive in CR (+20 months)
7	F	9 months	BM, LIV, SPL, LU, skin, bone, thymus, pancreas	MS	PD	21 months	LIV, SPL, skin, bone, thymus	NR	UCB	Myeloab (TBI, CY)	Relapse and died (47 days)
8	F	15 months	LU, skin, bone	MS	PR	27 months	BM, LIV	PD	UCB	Myeloab (TBI, CY, VP16)	Died (188 days)
9	M	21 months	BM, pituitary, DI	MS	PR	51 months	BM, pituitary, DI, bone	NR	BM (sibling)	Myeloab (CY, VP16)	Alive in CR (+158 months)
10	M	28 days	Skin, LN, bone, mediastinal mass	MS	PD	83 months	LU	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+109 months)
11	F	6 months	Skin, intestine	MS	PD	16 months	LIV, LU	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+39 months)
12	F	13 months	LN, middle ear	MS	GR	178 months	Bone, pituitary, DI	PD	autoPB	Myeloab (VP16, TEPA, IFO)	Relapse and alive (+113 months)
13	M	4 months	Bone	SS-m	PR	35 months	BM, LIV	PD	UCB	RIC (Flu, PAM, ALG, TLI)	Alive in CR (+38 months)
14	M	28 months	Bone	SS-m	GR	122 months	LIV	PD	UCB	Myeloab (Flu, PAM, BU)	Alive in CR (+144 months)
15	M	9 months	Thymus	SS-s	PR	22 months	LU, skin, thymus, LN, gingiva	PR	UCB	Myeloab (TBI, CY, VP16)	Alive in CR (+110 months)

Abbreviations: ATG = antithymocyte globulin; auto = autologous; BM = bone marrow; CR = complete remission; CY = cyclophosphamide; DI = diabetes insipidus; F = female; LIV = liver; LN = lymph node; PAM = melphalan; LU = lung; M = male; MS = multisystem; myeloab = myeloablative; NR = non-response; PB = peripheral blood; PD = progressive disease; PR = partial response; RIC = reduced-intensity conditioning; SCT = stem cell transplantation; SPL = spleen; SS-m = single system multisite; SS-s = single system single site; TBI = total body irradiation; TLI = total lymphoid irradiation; UCB = unrelated cord blood; VP16 = etoposide.

Nine patients (patients 1–9) are classified in the low-risk group, and six (patients 10–15) are in the low-risk group.

[a]Dose of TBI/TLI was 10–12 Gy in the myeloablative regimen and 2 Gy in the reduced conditioning regimen.

[b]Values in parentheses indicate the duration from SCT to the final observation.

skull lesions and occurrence of DI, despite chemotherapy. Patient 14 developed systemic xanthogranuloma 3 years after diagnosis. He suffered from liver dysfunction, ascites, pleural effusion, fever, and pancytopenia before SCT. Among the remaining three patients, patient 13, who relapsed during maintenance therapy, became refractory to more intensive chemotherapy, and BM and CNS involvement occurred at SCT. Patient 11 suffered from diarrhea, bloody stool, and protein-losing gastroenteropathy at initial diagnosis. After 2 months, skin rash, hepatosplenomegaly, and disseminated intravascular coagulation were seen and she was diagnosed based on a rectal biopsy. She failed to achieve remission after JLSG Induction regimen A and B and received cisplatin according to the neuroblastoma regimen after disease activation. Patient 15 failed to achieve remission after the JLSG Induction regimen and received chemotherapy according to the non-Hodgkin lymphoma regimen. Patient 15, who had thymus involvement and respiratory dysfunction at diagnosis, obtained only a PR clinically and radiographically, and the skin, lung, gingiva, and palpebral conjunctiva became involved 6 months after diagnosis.

Donor source and conditioning regimen

The donor source and conditioning regimen for SCT are also summarized in Table 2. A myeloablative conditioning regimen was used in 10 patients; total body irradiation was used in five, while the other five received a non-total body irradiation regimen. Five patients received a reduced-intensity conditioning regimen (RIC), which consisted of fludarabine, melphalan, or cyclophosphamide, and low-dose total body irradiation/total lymph node irradiation and/or antithymocyte globulin. Nine patients underwent unrelated cord blood transplantation. One patient received cord blood from an HLA-matched sibling. Overall, 10 of 15 patients received CBT with a median of 1.4×10^6/kg $CD34^+$ cells (range, 0.19–7.5×10^6/kg) or a median of 0.91×10^8/kg nucleated cells (range, 0.86–1.4×10^8/kg). Peripheral blood (PB) from haploidentical parental donors was used in two patients, and autologous PB was used in two patients, with a median of 10.9×10^6/kg $CD34^+$ cells (range, 7.5–13.0×10^6/kg). One patient received BM from an HLA 2 loci-mismatched sibling, with 3.0×10^8/kg nucleated cells. Prophylaxis for graft-versus-host disease and graft rejection consisted primarily of methotrexate and either cyclosporine or tacrolimus.

Clinical course of 15 patients after SCT

The clinical course of 15 patients after SCT is listed in Table 2. Engraftment with $>500/\mu l$ absolute neutrophil count was seen in all patients except for one who died of multi-organ failure on day 18. Regimen-related toxicity was seen in six of 15 patients; mucositis of grade 1 to grade 4 according to the common terminology criteria for adverse events[13] was the most common, and three patients had liver dysfunction of grade 2 or grade 3. One patient had thrombotic microangiopathy, which resolved without long-term sequelae. Four patients had various infections, such as sepsis, herpes simplex virus, and cytomegalovirus. Three patients who underwent unrelated cord blood transplantation had acute graft-versus-host disease (grades I, III, IV). One of them and another patient developed chronic graft-versus-host disease extensive type.

After SCT, two patients never entered remission and died on day 18 and day 188, respectively (patient 2 and patient 8). Patient 7 relapsed on day 20 and died on day 47 after SCT, due to sepsis, veno-occlusive disease, and gastrointestinal bleeding. Patient 4 underwent a second SCT on day 49 after the first SCT because of graft failure. She relapsed on day 194 and died on day 271 after the second SCT due to liver dysfunction, pancytopenia, and sepsis. Two patients (patient 10 and patient 12) relapsed at 8 months and 4 months after auto-PBSCT, respectively. Patient 10 had recurrence involving the cervical spine and received prednisone, vinblastine, and cyclosporine for 4 years. Patient 12 had recurrence of multiple bone lesions, including the femur, skull, and scapula in turn, and was treated with radiation therapy to control the bone lesions for 2 years. Patient 14 had macrophage activating syndrome after SCT, and TNF-α blocker and dexamethasone palmitate were administered for several months. Finally, 11 of 15 patients remain alive with no evidence of disease. The 10-year OS rate (median $\pm$ s.e.) in these patients was $73.3 \pm 11.4\%$ (Figure 1). The 10-year OS rate of nine patients who had risk organ involvement at diagnosis was $55.6 \pm 16.6\%$, whereas six patients who had no risk organ involvement at diagnosis have all survived with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$), because of the small number of patients.

Late toxicities associated with SCT included short stature with body height <-2.0 s.d. in five patients, whereas DI and hearing disturbance were seen in two patients each. One patient, who had a hip fracture and hearing disturbance, was evaluated as being intellectually retarded. Another patient, who suffered from a CNS lesion before SCT, had speech delay. Except for one infant who was too young to evaluate, the Karnofsky score[14] of the remaining eight survivors was 100%.

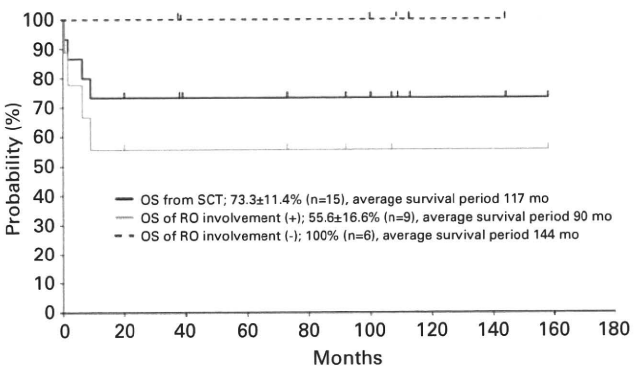

Figure 1 Overall survival (OS) of patients with LCH who underwent SCT. The 10-year OS rate of the 15 patients with refractory LCH who underwent SCT was $73.3 \pm 11.4\%$. The 10-year OS rate of the nine patients who had risk organ involvement at diagnosis was $55.6 \pm 16.6\%$, whereas all six patients who had no risk organ involvement at diagnosis remain alive with no evidence of disease. There was no significant difference in outcome between the two groups ($P = 0.07$). RO, risk organ.

Discussion

More than 40 patients with refractory LCH have been reported to have received SCT since 1987.[15] The OS of the 29 patients who underwent myeloablative SCT was 48% (14/29 patients), and the transplant-related mortality was 45%. However, in a recent study reported from Europe,[10] seven (78%) of nine patients survived with no signs of disease activity after RIC-SCT. The conditioning regimen consisted of fludarabine, melphalan, low-dose total lymph node irradiation (2 Gy), and either of antithymocyte globulin or MabCampath (anti-CD52 antibody). In Japan, four patients with LCH underwent SCT between 1994 and 1997, and two of them (50%) survived.[16] In this study, 11 of the 15 patients have survived with no evidence of disease; 8/10 (80%) with myeloablative conditioning and 3/5 (60%) with RIC-SCT, and the 10-year OS rate was superior (73.3 ± 11.4%). In particular, two patients with RIC-SCT had organ dysfunction before SCT, suggesting that a less toxic conditioning regimen is also effective for patients with organ dysfunction.

In the recent report of 22 patients who underwent SCT, the donor source was a sibling in 17 (77%) and a matched unrelated donor in five (23%).[11] The stem cell source was BM in 12 (55%), PB in six (27%), and CB in four (18%).[11] In this study, 10 of 15 patients received UCB, and eight of these have survived with no evidence of disease, including three with RIC-SCT. Therefore, our results support the use of UCB as an alternative donor source when neither a sibling donor nor a matched unrelated donor is available.

The appropriate timing of SCT for LCH is unclear. In this study, nine of 15 patients underwent SCT within 12 months after initial diagnosis, including seven with risk organ involvement at diagnosis. This group had a life-threatening clinical course, and they required prompt SCT as the only potentially curative treatment. On the other hand, three patients without risk organ involvement at initial diagnosis underwent SCT 7 years or later after diagnosis, resulting in no evidence of disease. A total of six patients in the low-risk group underwent SCT because of PD or disease refractory to conventional chemotherapy. Although it is not known which LCH patients really require SCT, our findings suggest that SCT should also be considered in patients in the low-risk group at diagnosis who develop active or PD even after long-term chemotherapy. A large-scale prospective study could provide useful information to select subsets of LCH that definitely need SCT.

It has recently been shown that various cytokines have an important role in the pathogenesis of LCH, suggesting that eradication of the pathologic cells associated with cytokine production could be effective for refractory LCH. In the previous report, two patients with refractory LCH who underwent RIC-SCT showed resolution of disease after SCT.[10] Kinugawa *et al.*[16] reported one patient who failed engraftment, followed by complete autologous recovery and resolution of disease activity.[16] In this study, one patient who underwent allo-BMT (patient 9) had a similar clinical course. Two patients who relapsed after auto-PBSCT showed resolution of disease following conventional chemotherapy (patients 10 and 12). Although the disease state of the two patients at 12 or 14 weeks after chemotherapy was GR, multiple recurrences had occurred, and their disease state at SCT was PD. These two patients were rescued by a myeloablative conditioning regimen with infusion of donor T lymphocytes, which prevented deterioration of the LCH. Steiner *et al.*[17] also reported one patient who achieved complete remission after RIC-SCT, despite post transplant mixed chimerism, in which only a T-cell subset proved to be of donor origin. He emphasized that a strong immunomodulating influence, mainly exerted by allogeneic T-cells, rather than eradication of the LCH cell clone, may be potentially curative in LCH. Correction of inappropriate immunological crosstalk by the replacement of allogeneic donor cells may be pivotal.

Bernard *et al.*[9] reported that 7 of 10 patients with refractory LCH had achieved sustained complete remission after treatment with 2-CdA and Ara-C. In this study, two patients (patients 6 and 8), who failed to respond to the combination of 2-CdA and Ara-C, underwent SCT, and one has been alive with no disease after RIC-SCT. Prior utilization of 2-CdA may help tailor the indications for SCT.

In conclusion, the improved outcomes of SCT for refractory LCH show that it is a promising new salvage approach. RIC-SCT is desirable for young children, especially with non-malignant disease. Further investigations are required to establish the SCT strategy for refractory LCH.

Conflict of interest

The authors declare no conflict of interest.

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Nationwide Survey of Bisphosphonate Therapy for Children With Reactivated Langerhans Cell Histiocytosis in Japan

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Background. Several studies have suggested that Langerhans cell histiocytosis (LCH) is responsive to treatment with bisphosphonates (BPs). However the efficacy and safety of BPs therapy for childhood LCH is unknown. ***Procedure.*** Data on children with LCH who had received BPs therapy were collected retrospectively from hospitals participating in the Japanese Pediatric Leukemia/Lymphoma Study Group. ***Results.*** Twenty-one children with histologically proven LCH were identified. Of these, the case histories of 16 children who had been treated with pamidronate (PAM) for disease reactivation were analyzed in detail. The median post-PAM therapy follow-up period was 2.8 years (range: 0.9–9.3 years). The median age at commencement of PAM therapy was 9.4 years (range: 2.3–15.0 years). All children had one or more bone lesions but none had risk organ (RO) involvement. In the majority of the children, six courses of PAM were administered at a dose of 1.0 mg/kg/course at 4-week intervals. In 12 of the 16 children, all active lesions including lesions of the skin (n = 3) and soft tissues (n = 3) resolved. Of these children, eight children had no active disease for a median of 3.3 years post-PAM therapy (range: 1.8–9.3 years). Progression-free survival (PFS) was 56.3 ± 12.4% at 3 years. PFS was significantly higher in children with a first reactivation compared with children experiencing a second or subsequent reactivation. ***Conclusions.*** PAM may be an effective treatment for reactivated LCH with bone lesions. A prospective trial of the efficacy of PAM in recurrent pediatric LCH is warranted. Pediatr Blood Cancer. 2011;56:110–115. © 2010 Wiley-Liss, Inc.

Key words: bisphosphonate; bone lesion; Langerhans cell histiocytosis; reactivation

INTRODUCTION

Langerhans cell histiocytosis (LCH) is a rare histiocytic disease that is characterized by uncontrolled clonal proliferation of CD1a-positive dendritic Langerhans cells (LCs). This occurs most commonly in bone tissue, but may also occur in the skin and in various other organs. Its clinical manifestation and course vary from the development of a solitary self-healing lesion to fatal multi-organ disease involving a risk organ (RO) such as the liver, spleen, lung, or hematopoietic system [1]. Although the survival rate for patients without RO involvement is close to 100% [1], recurrence is common and occurs most frequently in bone [2]. Reactivations can increase the risk for permanent consequences, such as orthopedic abnormalities, diabetes insipidus (DI), and neurological impairments [2,3]. The treatment of bone LCH involves curettage or biopsy for single bone lesions, and chemotherapy or indomethacin for multiple bone lesions or reactivated bone disease [4]. There is some evidence to suggest that prolonged low dose chemotherapy may reduce the likelihood of disease reactivation [4]. However, multiple reactivation occurs in some patients despite chemotherapy and prolonged chemotherapy with etoposide or antimetabolites may induce secondary hematological malignancies in patients with LCH [5,6].

Although the pathogenesis of LCH remains obscure, many types of immune cells other than LCs are present in LCH lesions, including lymphocytes, macrophages, eosinophils, and multi-nucleated giant cells (MGCs). The MGCs in bone, skin, and lymph node lesions express characteristic osteoclast markers such as tartrate-resistant acid phosphate, vitronectin receptor, cathepsin K, and matrix metalloproteinase-9 [7]. We previously reported that patients with LCH have high serum levels of the soluble receptor activator of NF-κB ligand (RANKL), a cytokine which induces the differentiation of pre-osteoclasts into osteoclasts and the activation of osteoclasts [8]. Since the osteoclast-like MGCs in LCH express

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various matrix-degrading enzymes involved in tissue destruction, the targeting of these cells in LCH lesions in bone and other tissues may represent a valid therapeutic approach [7].

Bisphosphonates (BPs) are pyrophosphate analogs that inhibit the recruitment of osteoclasts and reduce their activity and longevity. BPs are widely used in the treatment of a variety of bone diseases, including osteogenesis imperfecta (OI), osteoporosis, Paget's disease, and the osteolytic lesions of multiple myeloma and other malignancies [9,10]. The results of several studies have suggested that BPs may also be effective in LCH, although most of these studies have described single adult LCH cases [11–17]. To assess the efficacy and safety of BPs therapy in children with LCH, we conducted a retrospective nationwide survey in Japan.

MATERIALS AND METHODS

Data Collection

The LCH committee of the Japanese Pediatric Leukemia/Lymphoma Study Group (JPLSG) sent out a questionnaire to all JPLSG-affiliated hospitals in the summer of 2008. This questionnaire enquired whether these hospitals had administered BPs therapy to any children with LCH (age younger than 18 years at the time of diagnosis). Replies were received from 157 of the 183 hospitals. Fourteen hospitals had administered BPs therapy to a total of 24 children with LCH. These hospitals were sent a second questionnaire requesting details of the following: (i) diagnostic procedure, (ii) age at diagnosis, (iii) sex, (iv) site(s) of the lesion(s), (v) treatment, (vi) complications, and (vii) outcome. Twelve hospitals responded to the second questionnaire and 21 children with histologically proven LCH who had been treated with various BP preparations were identified. Of these, the case histories of 16 children who had been treated with intravenous pamidronate (PAM) for disease reactivation were analyzed in detail.

Evaluation Criteria and Definitions

No active disease (NAD) was defined as the disappearance of all signs and symptoms of disease with the exception of DI, central nervous system degeneration (CNS-D), or residual radiological findings of bone lesions showing regression or stabilization. Partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter (LD) for all bone or mass lesions taking as reference the baseline sum LD evaluated by radiological findings or at least a 50% decrease in area of skin lesion without organ dysfunction or the occurrence of a new lesion. No response (NR) was defined as more than 70% residual in the sum of the LD for all bone or mass lesions evaluated by radiological findings or more than 50% residual in area of skin lesion with or without organ dysfunction. The evaluation of radiological findings was done by a radiologist at each institute. Progression-free survival (PFS) was defined as continuing NAD following the commencement of PAM therapy. Reactivation was defined as the reappearance of signs and/or symptoms of disease activity following a period of NAD. Adverse effects were assessed using the Common Terminology Criteria for Adverse Events (CTCAE) [18].

Statistical Analysis

Fisher's exact test was used to analyze factors with an influence on the attainment of continuous NAD post-PAM therapy. PFS was estimated using Kaplan–Meier analysis, and is expressed as rates ± standard error. The log-rank test was used to compare the factors affecting PFS. P values of less than 0.05 were considered statistically significant.

RESULTS

Of the 16 children with reactivated disease who had received PAM-therapy, 10 were males and 6 were females (Table I). The median age at disease onset was 3.1 years (range: 0.4–14.1 years). Ten children had single system disease (two of the skin and eight of bone). Six children had MS disease including bone lesion(s) (three without RO involvement and three with RO involvement of the hematopoietic system, spleen, and lung, respectively). All but one of the children had received initial systemic chemotherapy; for the majority of the children, treatment had been administered in accordance with the JLSG-96/02 protocol [19].

The median post-PAM therapy follow-up period was 2.8 years (range: 0.9–9.3 years). The median age at the commencement of PAM therapy was 9.4 years (range: 2.3–15.0 years) (Table I). Prior to the commencement of PAM therapy, 6 of the children had multiple disease reactivations, and 5 children had been receiving chemotherapy and 11 had completed chemotherapy. Two of the children (UPN 7141 and 7142) have been reported previously [14]. Prior to PAM therapy, these two children had received oral etidronate therapy for 15–18 months and had shown PR. The remaining children had received PAM therapy immediately following disease reactivation. At commencement of PAM therapy, ten children had only bone lesion(s), six of whom had soft tissue mass associated with the bone lesion, and five had bone pain. The remaining six had multisystem involvement including bone and skin lesions (n = 3), DI (n = 3), CNS-D (n = 2), and soft tissue (n = 1). None of the children had RO involvement. PAM was administered intravenously at a median dose of 1.0 mg/kg/course. Four children had received 1.0–1.25 mg/kg/day daily for 3 days per course (UPN 4123, 4122, 4121, and 7091). Twelve children had received six courses of PAM administered at 4-week intervals. Four children had received more than 10 courses of PAM administered at 4- to 8-week intervals (UPN 4123, 4122, 4121, and 5081); three of these children are still receiving this therapy at the time of writing. In addition to PAM, nine children received meloxicam (MC) daily at a dose of 0.2 mg/kg. Along with PAM therapy, three children received continuous cytoreductive agents (methotrexate, vinblastine, and 6-mercaptopurine) which had been prescribed prior to the disease reactivation that led to PAM therapy. Three children experienced mild adverse effects in response to PAM therapy including pyrexia, fatigue, gastrointestinal symptoms, and hypocalcemia, which were rated as grades 1–2 according to the CTCAE. One child (UPN 5081) with cranial bone lesions, an orbital soft tissue mass, DI, and CNS-D, developed blurred vision secondary to uvenitis after 11 courses of PAM therapy without an acute phase reaction. The child's vision improved following the discontinuation of PAM therapy and the administration of immunosuppressive therapy (dexamethasone and a calcineurin inhibitor). At cessation of therapy, 12 of the 16 children (75%) had attained NAD and radiological reossification and normalization, including 3 children with skin lesions and 3 patients who had

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TABLE I. Characteristics and Outcome of Children With Reactivated LCH Who Received Treatment With Pamidronate

UPN	Gender	At commencement of PAM therapy			Concomitant drugs	Adverse effects	Response to PAM therapy	Reactivation after NAD	Subsequent treatment	Survival post-PAM therapy
		Age	Status	Lesions						
7144	F	9y6m	1st Re*2	B, Sk	MC	None	NAD after 2 courses	1.4y in Sk	PSL	2.8y+, NAD
3533	F	7y7m	2nd Re*2	Bs with St, CNS-D	MTX*4	None	Bs: PR, St: NR	NE	MTX	0.9y+, NAD
7143	M	15y0m	1st Re	B	MC	None	NAD after 2 courses	None	None	7.3y +, NAD
7145	M	14y9m	1st Re	Bs	MC	None	NAD after 2 courses	None	None	3.8y+, NAD
4123	M	14y0m	1st Re	B*3	None	None	NAD after 6 courses	None	None	1.8y+, NAD
3091	F	13y7m	11th Re	B*3	MC	Fever, fatigue, hypo Ca	NAD after 2 courses	1.0y in B	ZOL	1.2y+, NAD
3532	F	11y6m	4th Re*2	B with St	VBL*4	Fever, vomiting, diarrhea	New B lesion at 0.2y, St: NAD	NE	AraC/VCR/PSL, MTX, VBL	1.0y+, NAD
4122	M	10y0m	5th Re*2	B*3	6MP*4	None	NAD after 4 courses	None	None	2.8y+, NAD
5081	M	10y0m	5th Re	Bs*3 with St, DI, CNS-D	MC	Uvenitis	Bs: NR, St: NR	NE	VBL/DEX/CSA, 2CdA	5.7y+, NAD
7161	M	4y8m	1st Re	B	MC	Hypo Ca	NAD after 2 course	None	None	2.6y+, NAD
3121	M	8y8m	3rd Re*2	Bs*3 with St, St, DI	MC	None	NAD after 5 courses	3.3y in St	PAM	5.2y+, NAD
7141*1	F	3y0m	1st Re	Bs, Sk, DI	None	None	NAD after 2 courses	None	None	9.3y+, NAD
7142*1	F	2y3m	1st Re	Bs, Sk	MC	None	NAD after 3 courses	None	None	6.7y+, NAD
4121	M	8y0m	1st Re	B with St	DEX	None	NAD after 4 courses	None	None	2.7y+, NAD
7181	M	2y10m	1st Re	Bs	MC, PSL	None	Bs: NR, new Sk lesion at 0.5y	NE	PAM, VCR/PSL	6.8y+, NAD
7091	M	2y10m	1st Re	Bs with St	None	None	NAD after 2 courses	1.0y in B	PSL	2.7y+, NAD

SS, single system; MS, multi-system; Sk, skin; B, single bone; Bs, multiple bones; St, soft tissue; LN, lymph node; DI, diabetes insipidus; He, hematopoietic system; Sp, spleen; Thy, thymus; Lu, lung; PAM, pamidronate; Re, reactivation; CNS-D, central nerve system degeneration; MC, meloxicam; MTX, methotrexate; IVIG, intravenous immunoglobulin; DEX, dexamethasone; VBL, vinblastine; PSL, prednisolone; 6MP, 6-mercaptopurine; hypo Ca, hypocalcaemia; NAD, no active disease (apart from posterior pituitary lesion and CNS-D); PR, partial response; NR, no response; NE, non-evaluable; ZOL, zoledronate; AraC, cytarabine; VCR, vincristine; CSA, cyclosporine A; 2CdA, cladribine; *1 treated with etidronate for 15–18 months before receiving pamidronate (Ref. [14]); *2 reactivation on chemotherapy; *3 accompanied by bone pain; *4 administered continuously prior to the disease reactivation that led to PAM therapy.

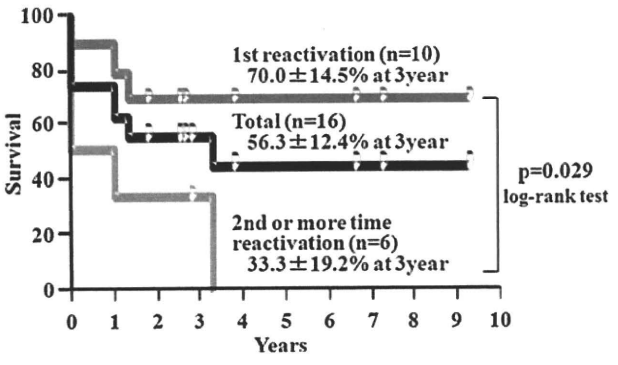

Fig. 1. Progression-free survival (PFS) post-PAM therapy. The overall PFS at 3 years was 56.3 ± 12.4%. PFS was significantly higher in children with a first reactivation compared with children experiencing a second or subsequent reactivation (70.0 ± 14.5% vs. 33.3 ± 19.2% at 3 years, $P = 0.029$).

had soft tissue masses at the commencement of PAM therapy. The median number of courses of PAM therapy in children attaining NAD was 2. Although the bone lesions of one patient showed a PR, the accompanying soft tissue masses showed NR. PAM therapy did not affect the bone lesions of three children; two of them developed a new bone lesion and a new skin lesion, respectively. Of the 12 children who had attained NAD, 8 have had NAD and complete resolution of radiographic findings in bone for a median of 3.3 years (range: 1.8–9.3 years) since the commencement of PAM therapy. The remaining four children experienced disease reactivation in bone (n = 2), skin (n = 1), and soft tissue (n = 1). These reactivated children were treated with prednisolone (PSL) (n = 2), zoledronate (ZOL) (n = 1), or PAM (n = 1), which again resulted in the complete disappearance of the lesions. The overall PFS at 3 years was 56.3 ± 12.4% (Fig. 1).

The ratio of maintaining NAD was significantly higher in children receiving PAM therapy at the first reactivation off chemotherapy compared to other patients (7/9 vs. 1/7, $P = 0.041$). PFS was significantly higher in children with a first reactivation than in children with a second or subsequent reactivation (70.0 ± 14.5% vs. 33.3 ± 19.2% at 3 years, $P = 0.029$) (Fig. 1). There was no significant difference in PFS between children who developed reactivation while off chemotherapy and those on chemotherapy (63.6 ± 14.5% vs. 40.0 ± 21.9% at 3 years, $P = 0.139$). Other factors, such as gender, age, type of disease, dose of PAM, number of PAM courses, and prescription of concomitant medication, also did not affect the PFS of children receiving PAM therapy.

DISCUSSION

Osteoclast-like MGCs in LCH lesions are a potential therapeutic target since they express the various matrix-degrading enzymes that mediate tissue destruction. In the present study, we demonstrated that intravenous PAM therapy appears to have considerable responses for 16 children with LCH. All 16 children had reactivated disease with bone lesion(s), and 6 had MS disease involving non-RO sites. In 12 of the 16 children, NAD after PAM therapy was observed for skin and soft tissue lesions as well as for bone lesions. Eight of the 16 children have had NAD for a median of 3.3 years since the cessation of PAM therapy.

Seven reports of BPs therapy for LCH have been published to date, and these studies have included a total of 14 patients, all of whom had bone lesion(s) [11–17]. Only three of these patients were children, and two of these were included in the present study. Eleven of the 14 patients had also presented with lesions in sites other than bone, including in the pituitary, skin, lung, and CNS. The preparation and dosage of the BPs administered to these 14 patients varied. In four patients, PAM was administered in 2–11 courses at a dose of 90–270 mg/course at 1–2 months intervals. With the exception of one case of renal failure, no serious adverse effects were reported. In most of the fourteen patients, BPs had been administered in order to relieve bone pain, and this was successful in all cases. Recalcification was also reported in some cases. However, these studies evaluated neither the response of LCH lesions in sites other than in bone, nor the long-term outcome of BP therapy.

The most widely used nitrogen-containing BP in children is PAM, and the most extensively investigated childhood disease for which PAM is prescribed is OI [20]. The most commonly used PAM protocol for OI is the administration of 1.0 mg/kg/day for 3 days every 4 months (i.e., an annual dose of 9 mg/kg) over a period of several years. In most of the LCH children in the present study, PAM was administered at a dose of around 1 mg/kg every month for 6 months. It may be possible to extend the duration of BPs therapy for LCH; in the present study, although PFS was not significantly higher in patients who received more courses of PAM because of the short period of follow-up and the small number of cases.

Hypocalcemia and acute phase reaction are the most common adverse events following the intravenous administration of BPs, and both resolve with supportive care [9,10]. Of the 16 children in the present study, 2 had hypocalcemia and 1 had an acute phase reaction. Both effects subsided in response to the administration of appropriate medication. There have been rare reports of inflammatory ocular disease such as scleritis and uvenitis in adults secondary to BPs therapy, most of which were associated with an acute phase reaction, occurred within 6 hr to 2 days of treatment, and subsided after discontinuation of the BPs therapy [21]. In the present study, one child with an orbital LCH lesion developed blurred vision secondary to uvenitis after 11 courses of PAM therapy without an acute phase reaction. This presentation differs from those described in previous reports of BP-induced uvenitis. It is possible that an orbital inflammatory LCH lesion might affect the development of uvenitis. Another clinically significant adverse reaction to intravenous BPs is nephrotoxicity, which is dependent upon both the dose and the infusion time, and which can be avoided by dose reduction and a prolongation of infusion time to allow the monitoring of serum creatinine levels [22]. Although there has been one report of BPs induced renal failure in a patient with LCH [15], most cases of BPs-induced nephrotoxicity have been reported in patients with multiple myeloma receiving high dose PAM, and there have been no such reports in children [22]. Osteonecrosis of the jaw (ONJ) has been described as a serious complication of BPs therapy in adults with cancer [23], but not in children [24]. With respect to the long-term safety of BPs in children, a major concern is the suppression of longitudinal bone growth. This has been shown to be mildly suppressed by ZOL in growing rabbits [25], but intravenous PAM therapy does not appear to have a detrimental effect on the growth of children with OI [26]. Thus, while continued careful monitoring is required, particularly for the development of inflammatory ocular disease in children with an orbital LCH lesion, intravenous administration of

1 mg/kg PAM once every month for a total of 6 months for children with LCH may be a safe treatment.

In addition to having resolved the bone lesions, PAM therapy may have contributed to resolution of the skin and soft tissue lesion(s) of the children in the present study. No previous study has suggested that PAM is efficacious for LCH lesions in sites other than bone. PAM suppresses the activation and longevity of osteoclast-like MGCs, which are known to be present in LCH lesions of the skin and other sites as well as in LCH bone lesions [7]. However, it should be noted that none of the children in the present study had RO involvement at the commencement of PAM therapy. In addition, 2 children from our total cohort of 21 children with LCH who are not described in this report had RO involvement at the commencement of BPs therapy and experienced disease progression despite having received BPs and chemotherapy at disease onset. Children with a first reactivation had a statistically significantly better PFS, which may indicate that patients who experience more than one reactivation might have differing biological characteristics to those who experience no or only one reactivation. These findings suggest that BP therapy may be more effective for LCH children with a first reactivation involving a non-RO site.

In the present study, 9 of the 16 children received MC in addition to PAM. MC is a non-steroidal anti-inflammatory (NSAID) agent and a selective inhibitor of cyclooxygenase-2 (COX-2). NSAIDs block the arachidonic acid-prostaglandin pathway by inhibiting COX. It has been shown that COX and prostaglandins are over-expressed by LCH lesions and that prostaglandin E2 can induce bone resorption [27,28]. Several reports have described the effectiveness of NSAIDs in the treatment of LCH bone lesions [29–32]. The combined administration of MC and PAM did not affect PFS, however, and two children in the present study experienced disease reactivation shortly after the cessation of MC. MC might therefore have acted in an additive or synergistic manner with PAM in the children in the present study.

In conclusion, the intravenous administration of 1 mg/kg PAM once every month for 6 months may be an effective and safe treatment for children with LCH bone lesions. Although the long-term effects of BPs on LCH are unknown, it is possible that by preventing further disease reactivation in bone, PAM therapy may decrease or prevent late effects such as DI without the use of cytoreductive agents. However, a weakness of this study is its retrospective approach. Another weakness was that radiographs were not evaluated by independent radiologists but instead were evaluated by radiologists at each institution. A prospective study of children with reactivated LCH and no RO involvement is warranted to confirm these results, and this should be conducted with careful attention to the development of adverse effects.

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LCHの病態解明と治療の進歩

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1．はじめに

Langerhans cell histiocytosis（LCH）は、CD1a陽性で未熟樹状細胞の特性を示すランゲルハンス細胞が増殖する疾患で、主に乳幼児期に発症する。骨や皮膚、リンパ節、中枢神経などに浸潤し、その症状は多岐にわたる。自然治癒する例から、肝・脾・肺・造血器（リスク臓器）などに浸潤し致死的となる例まで、その予後もさまざまである。本疾患の成因・病態は依然として不明な点が多い。LCHの病態と治療の進歩につき最新の知見を紹介したい。

2．LCHの成因に関する知見

LCH細胞が単クローン性に増殖していることは1990年代前半に証明されたが、そのゲノム異常については長年不明のままであった[1]。2010年、既知の癌関連遺伝子の変異を網羅的に検索する手法によって、半数以上の症例でLCH細胞のBRAF遺伝子にV600E変異が認められたという驚くべき報告がなされた[2]。これはLCHが腫瘍性疾患であることを支持している。BRAFはRAS・ERK経路のシグナル伝達分子であり、BRAFはMEKをリン酸化することで下流にシグナル伝達し最終的にERKを活性化し細胞増殖を促進する[3]（図1）。

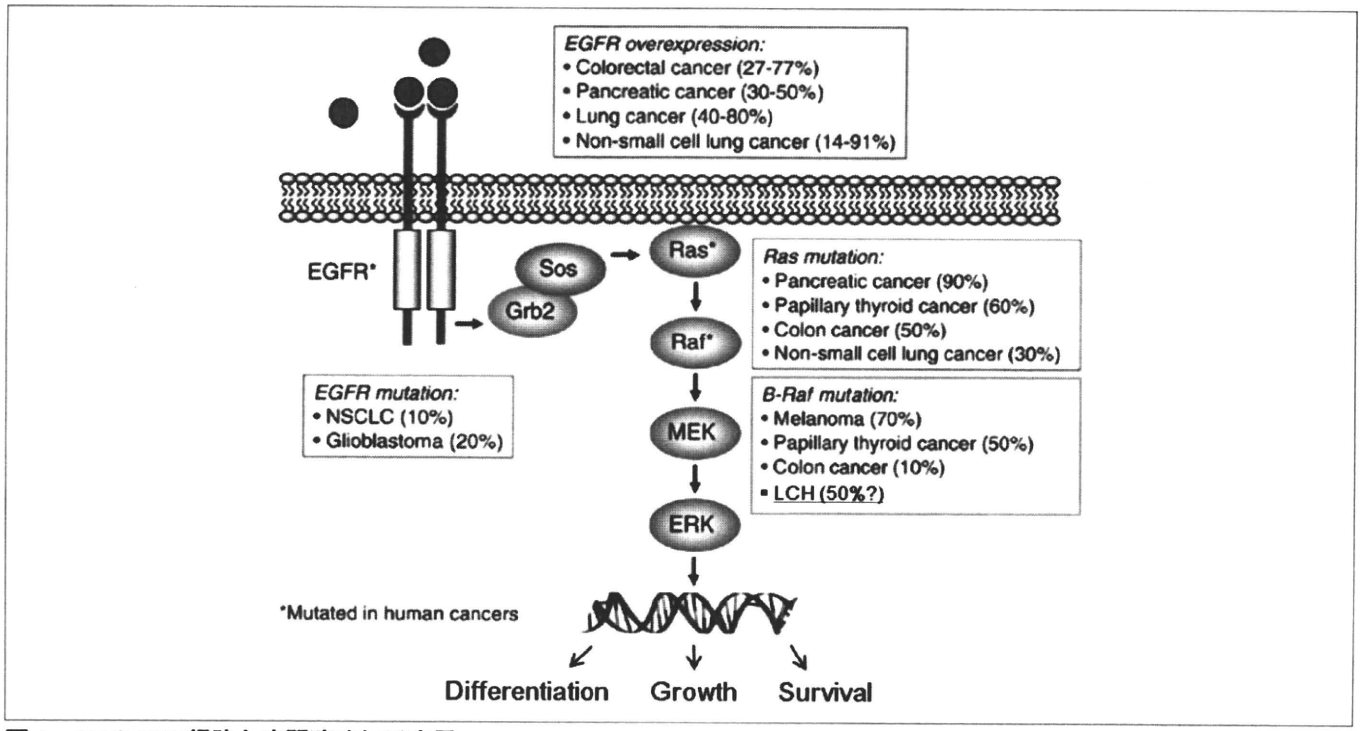

図1　RAS-ERK経路と癌関連遺伝子変異
文献3より改変引用

V600Eを代表とする癌関連BRAF変異には強いERK活性化作用がある。BRAF遺伝子の活性化変異は、メラノーマや大腸がん、甲状腺癌においてもみられ予後不良と関連する[3]。LCHとBRAF変異についての報告はこの1篇だけであり、病型や予後との関連については不明であるが、今後、RAF阻害剤のLCH治療への導入の可能性を含め、研究の進展が期待される。

3．LCHの病態に関する知見(図2)

LCHの病変部位には、LCH細胞のほか、T細胞・マクロファージ・好酸球・破骨細胞様多核巨細胞(MNGC)など多彩な細胞浸潤がある。これらの細胞は互いに刺激し合い活性化しサイトカインストームが形成される[4]。多臓器型LCH、特にリスク臓器浸潤陽性例では、血清中の炎症性ケモカインが高値であり[5]、これらの細胞遊走因子がLCH病変の全身への進展に関わっている。MNGCは、IL-17Aなどの働きによりLCH細胞から分化し[6]、骨のみならず皮膚やリンパ節病変部位にも存在し[7]、組織破壊に重要な役割をしている。また、LCH細胞は、炎症や骨代謝、癌転移などに関わる多機能分子オステオポンチンを高発現しており[8]、オステオポンチンをラットの肺に強制発現させると肺LCHの組織像が再現される[9]。今後、サイトカインやケモカイン、オステオポンチンが、LCHの治療ターゲットとなる可能性がある。

4．LCHの治療に関する知見(表1)

1)First line therapy

治療法は病変の浸潤部位と進展によって選択される[10~12]。単一骨型では、無治療経過観察または掻爬

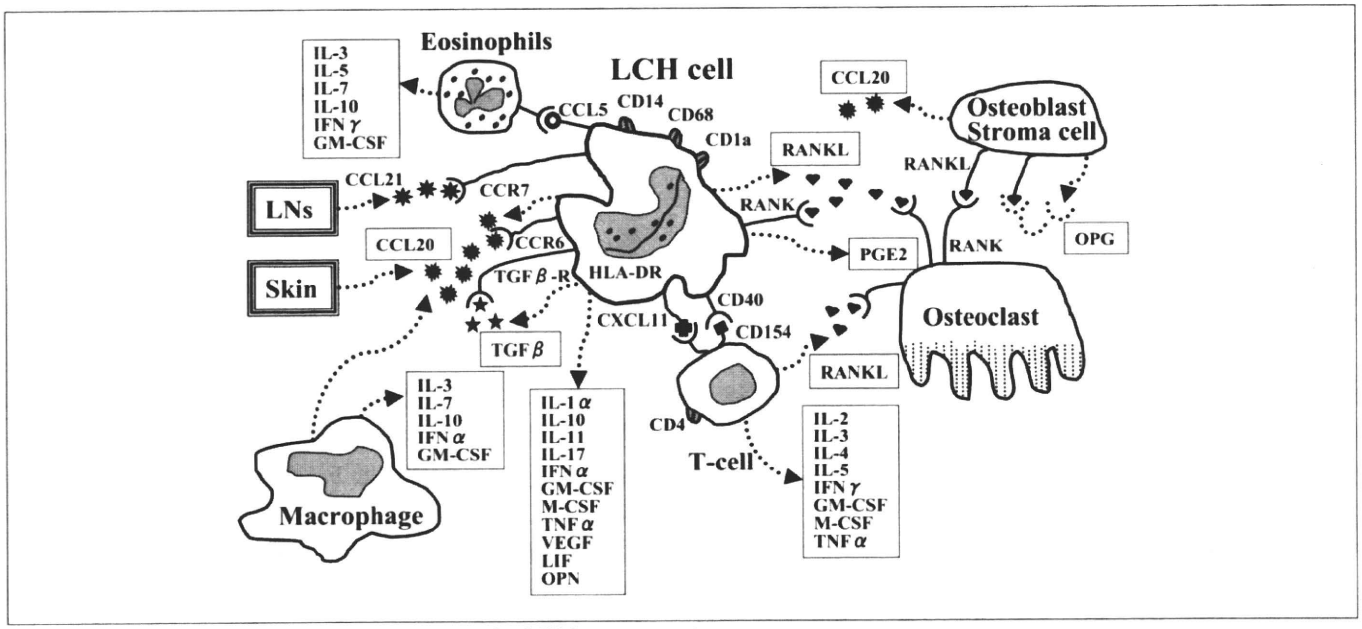

図2　LCH病変部位におけるサイトカイン/細胞間ネットワーク
森本哲. 小児科臨床. 2005；58：1807-1819. より改変引用

表1　LCHの各病型の治療ガイドライン

病型		推奨治療
単一病変型	骨型	無治療経過観察、掻爬術またはステロイド局所療法。 ただし、CNSリスク型*、または、圧迫症状や著しい疼痛がある例、病変の拡大がある例には、ビンクアルカロイドとステロイド剤を中心とする化学療法。
	皮膚型	ステロイド外用剤で経過観察。 ただし、皮疹が拡大する場合、ビンクアルカロイドとステロイド剤を中心とする化学療法。 多臓器型に移行する可能性があり注意深い経過観察を要する。
多発骨型		VCR/AraC/PSLまたはVBL/PSLによる導入療法を用いた化学療法を6-12か月。
多臓器型		VCR/AraC/PSLによる導入療法を用いた化学療法を12か月。

＊CNSリスク型：眼窩、側頭骨、乳突洞、蝶形骨、頬骨、篩骨、上顎骨、副鼻腔、前頭蓋窩、中頭蓋窩に病変があり軟部組織腫瘤を伴う例。
(厚生労働省「乳児ランゲルハンス細胞組織球症の病態解明と診療研究」班、乳児LCH治療ガイドライン(2010)より抜粋)

術、ステロイド局所療法が行われる。骨病変の完全除去を目的とした外科的処置は骨欠損を生じるため推奨されない。CNSリスク病変と呼ばれる側頭骨や眼窩、副鼻腔、顔面骨の病変や、圧迫症状や著しい疼痛がある場合には、単一病変であっても、ビンクアルカロイド(VA)とステロイド剤を中心とした数か月の化学療法が推奨される。皮膚単独型では、ステロイド外用剤で経過観察する。皮疹が拡大する場合、VAとステロイド剤を中心とした数か月の化学療法が推奨される。多臓器型に移行する可能性があり注意深い観察を要する。多発骨型では、整形学的障害や尿崩症、難聴などの不可逆的病変が発現する可能性があり、VAを中心とした数か月から1年間の全身化学療法が行われる。多臓器型では、全身化学療法が必須である。シタラビンとビンクリスチン、プレドニゾロンに、メソトレキセート、6-MP、ビンブラスチンを組み合わせた1年間の多剤併用化学療法(JLSG-02プロトコール)が、現在最も有効と考えられる治療法である。

2)Salvage therapy

①2-Chlorodeoxyadenosine(2-CdA：クラドリビン)

2-CdAは免疫抑制作用と殺細胞効果をもつプリン誘導体である。単球に対する殺細胞効果が高く、中枢神経系への移行が良好とされ、リスク臓器浸潤を伴わない再発例や中枢神経浸潤例に対し単剤で効果がある[13,14]。また、2-CdAは腫瘍細胞内のara-CTP(Ara-Cの活性化代謝産物)の濃度を高めるためAra-Cとの相乗効果があり、リスク臓器浸潤を伴う不応例に対して試みられている[15]。

②同種造血幹細胞移植(SCT)

リスク臓器浸潤を伴う急速進行例や再発例は致死率が極めて高く、SCTが最も有望な治療法である[16]。RIST[17]やUCBT[18]の成功例の報告も散見される。

③抗破骨細胞療法

ビスフォスホネートは、破骨細胞による骨吸収を抑制し破骨細胞をアポトーシスに導く。LCHの骨病変に対しても効果が期待できる[19]。

5．LCHによる神経変性病変に関する知見

LCHの長期経過の中で約半数の例に尿崩症や難聴、整形外科的異常、神経学的異常、成長障害などの不可逆的障害が生じる[20]。その中で、診断後数年以上経過しLCHが治癒したと思われる頃に現れる中枢神経変性病変が最も重大である。

中枢神経変性LCHの特徴は次のようである[21]。①下垂体浸潤は危険因子である。②頭蓋顔面部位の病変、下垂体ホルモン分泌不全、神経症状のあるLCH症例の半数以上に、LCHの診断後3年時点で、脳MRIで異常が見つかる。③左右対称性の小脳虫部や基底核部の病変が特徴的(図3)で、進行性で改善することはない。④脳MRI異常の見つかったLCH症例の1/4は、LCH診断から数年後に、企図振戦、小脳失調、運動協調障害、集中力低下、知能低下などの精神神経症状が出現し進行する。⑤神経変性部位にはLCH細胞の浸潤は見られず、CD8陽性T細胞の浸潤による、神経・軸索の破壊、二次的な脱髄が特徴で、悪性腫瘍に伴う脳炎やRasmussen脳炎の病理像に類似する。

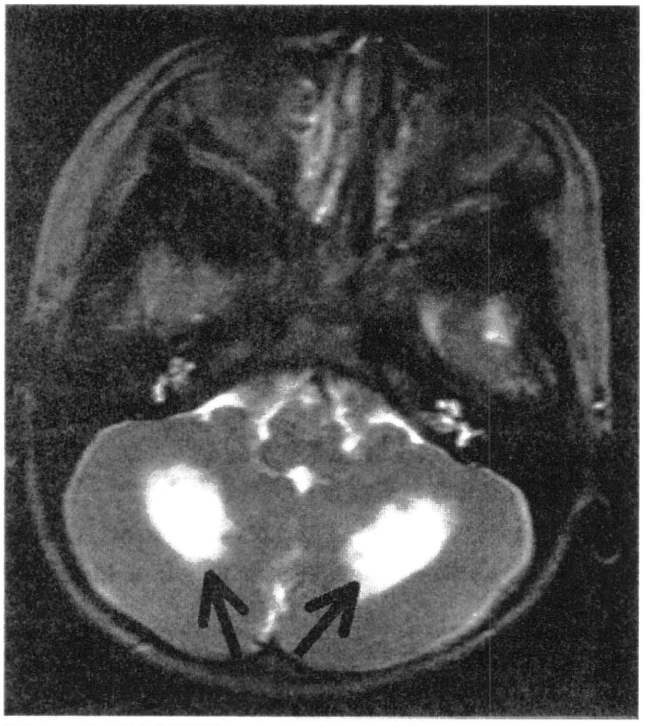

図3　中枢神経変性LCHの脳MRI所見
小脳歯状核に左右対称性にFLAIR画像で高信号域を認める。
文献22より引用

中枢神経変性LCHに対する有効な治療法はなく、いかに発症を減らすかが重要な課題である。γグロブリン大量療法によって進行を阻止する試みがなされている[22]。

6．おわりに

LCHの病態解明、最適な治療法の開発にはまだ時間を要するが、少しずつ前進してきている。LCHに関する情報は、日本LCH研究会のホームページ(http://www.jlsg.jp)により得られる。

文献

1) da Costa CE et al. Genes Chromosomes Cancer. 2009；48：239-249.
2) Badalian-Very G et al. Blood. 2010 Jun 2. [Epub