TRAPS	Tumor necrosis factor receptor-associated periodic syndrome
WAS	Wiskott–Aldrich syndrome
WHIM	Warts hypogammaglobulinemia, infections, and myelokathexis

Introduction

Patients with primary immunodeficiency disease (PID) show susceptibility to infections due to congenital immune system defects. These patients are also associated with noninfectious complications including autoimmune diseases and malignant disorders. Recent studies have revealed the causes of many PIDs to be mutations in various genes encoding molecules involved in the host defense mechanisms [1]. In addition, various new PIDs including defects in innate immunity and autoinflammatory disorders were identified under the recent progress in immunology and molecular genetics [2]. PID classification has been revised according to the identification of new PIDs and on the basis of new findings in PID pathophysiology. For a more precise clinical analysis, data should be obtained in accordance with the latest PID classifications.

The first nationwide survey of patients with PID in Japan was conducted between 1974 and 1979, which included 497 registered cases [3]. By 2007, a total of 1,297 patients were cataloged by a small number of PID specialists into a registration system [4]. The approximate prevalence of PID patients in Japan in the first nationwide survey was 1.0 in 100,000 people, which was much lower than that in other countries [5–7]. This difference in PID prevalence between Japan and other countries suggested that some PID patients in Japan remained unregistered. To determine the prevalence and clinical characteristics of patients with PID in Japan on the basis of the recent international classification system for PID, we conducted a nationwide survey of PID for the first time in 30 years.

Methods

This study was performed according to the nationwide epidemiological survey manual of patients with intractable diseases (2nd edition 2006, Ministry of Health, Labour, and Welfare of Japan) as described previously [8]. PID classification was based on the International Union of Immunological Societies Primary Immunodeficiency Diseases Classification Committee in 2007 [2]. Patients with chronic benign neutropenia and syndrome of periodic fever, aphthous stomatitis, pharyngitis, and cervical adenitis were excluded because these were considered to be acquired diseases. The survey was conducted on PID patients who were alive on December 1, 2008 and those who were newly diagnosed and dead between December 1, 2007 and November 30, 2008 in Japan. Among the 2,291 pediatric departments and 8,026 internal medicine departments in Japan, hospitals participating in the survey were randomly selected after setting the selection ratio according to the number of beds (overall selection rate: 53.4% for pediatric departments, 20.8% for internal medicine departments; Table I). University hospitals and pediatric training hospitals, where many PID patients were considered to be treated, were stratified separately (Table I). Primary questionnaires regarding the number of patients and disease names based on PID classification were sent to the selected hospitals. Secondary questionnaires regarding age, gender, clinical manifestations, and complications of individual PID patients were sent to respondents who answered that they observed at least one PID patient with characteristics listed in the primary questionnaires.

Results

Questionnaires were distributed to 1,224 pediatric departments and 1,670 internal medicine departments of hospitals in Japan, and the response rate was 55.0% and 20.1%, respectively (Table I). A total of 1,240 patients (1,146 patients from pediatric departments and 94 patients from internal medicine departments) were registered (Table I). The estimated number of patients with PIDs in Japan was 2,900 (95% confidence interval: 2,300–3,500), and the prevalence was 2.3 per 100,000 inhabitants. We also determined the regional distribution on the basis of the patients' addresses. The estimated regional prevalence ranged from 1.7 to 4.0 per 100,000 inhabitants, and no significant differences were observed between different regions in Japan (Fig. 1). The most common form of PID was predominantly antibody deficiencies (40%), followed by congenital defects of phagocyte number, function, or both (19%) and other well-defined immunodeficiency syndromes (16%; Table II). Autoinflammatory disorders were observed in 108 cases (9%). The most common PID was Bruton's tyrosine kinase (BTK) deficiency (182 cases, 14.7%), followed by chronic granulomatous disease (CGD; 147 cases, 11.9%). However, common variable immunodeficiency disease (CVID) and selective IgA deficiency (SIgAD) were observed only in 136 (11.0%) and 49 cases (4.0%), respectively. Among patients registered from internal medicine departments, antibody deficiencies were the most common disorder (71%).

In the secondary survey, 923 cases were registered. The male-to-female ratio was 2.3:1 (n=914, unanswered: 9 cases) with a median age of 12.8 years (range: 0 to 75 years; n= 897, unanswered: 26 cases). The number of adolescent or

Table I Stratification and selection of hospitals and the survey results

	Stratification	Departments in Japan	Departments selected	Selection rate (%)	Return[a]	Response	Response rate (%)	PID Patient	Patients per department	Patients estimated
Pediatrics	University hospital	118	118	100	0	80	67.8	661	8.3	975
	Training hospital	402	402	100	4	242	60.8	376	1.6	618
	≥500 beds	92	92	100	5	48	55.2	24	0.5	44
	400–499 beds	118	118	100	3	63	54.8	42	0.7	77
	300–399 beds	287	230	80.1	4	122	54.0	31	0.3	72
	200–299 beds	289	116	40.1	4	53	47.3	6	0.1	32
	100–199 beds	486	98	20.2	0	44	44.9	4	0.1	44
	<99 beds	499	50	10.0	1	10	20.4	2	0.2	100
	Subtotal	2,291	1,224	53.4	21	662	55.0	1,146	1.7	1,961
Internal medicine	University hospital	156	156	100	1	47	30.3	37	0.8	122
	≥500 beds	374	374	100	1	86	23.1	35	0.4	152
	400–499 beds	328	263	80	1	54	20.6	6	0.1	36
	300–399 beds	692	278	40.2	6	49	18.0	10	0.2	140
	200–299 beds	1,008	202	20.0	0	36	17.8	2	0.1	56
	100–199 beds	2,460	246	10.0	1	36	14.7	1	0.0	68
	<99 beds	3,008	151	5.0	6	24	16.6	3	0.1	375
	Subtotal	8,026	1,670	20.8	16	332	20.1	94	0.3	950
Total		10,317	2,894	28.1	37	994	34.8	1,240		2,911

[a] Due to the closure of departments

adult cases (≥15 years) was 384 (42.8%; Fig. 2a). The male-to-female ratio of the younger generation (<15 years) was 2.7:1, while that of the older generation (≥15 years) was 2.0:1. Combined T and B cell immunodeficiencies (CIDs) were predominantly observed in the younger generation, while antibody deficiencies were more common with

Region	Reported PID Patients	Population ($x10^5$)	Estimated prevalence per 10^5 (95% C.I.)
Hokkaido	73	55.4	4.0 (0.2-7.8)
Tohoku	81	94.3	2.1 (0.9-3.3)
Kanto	387	419.8	1.8 (1.2-2.5)
Chubu	236	236.9	2.3 (1.4-3.1)
Kinki	158	208.4	2.0 (1.2-2.8)
Chugoku/Shikoku	105	116.1	1.7 (1.0-2.3)
Kyushu/Okinawa	200	146.0	2.5 (1.7-3.3)
Total	1240	1276.9	2.3 (1.8-2.7)

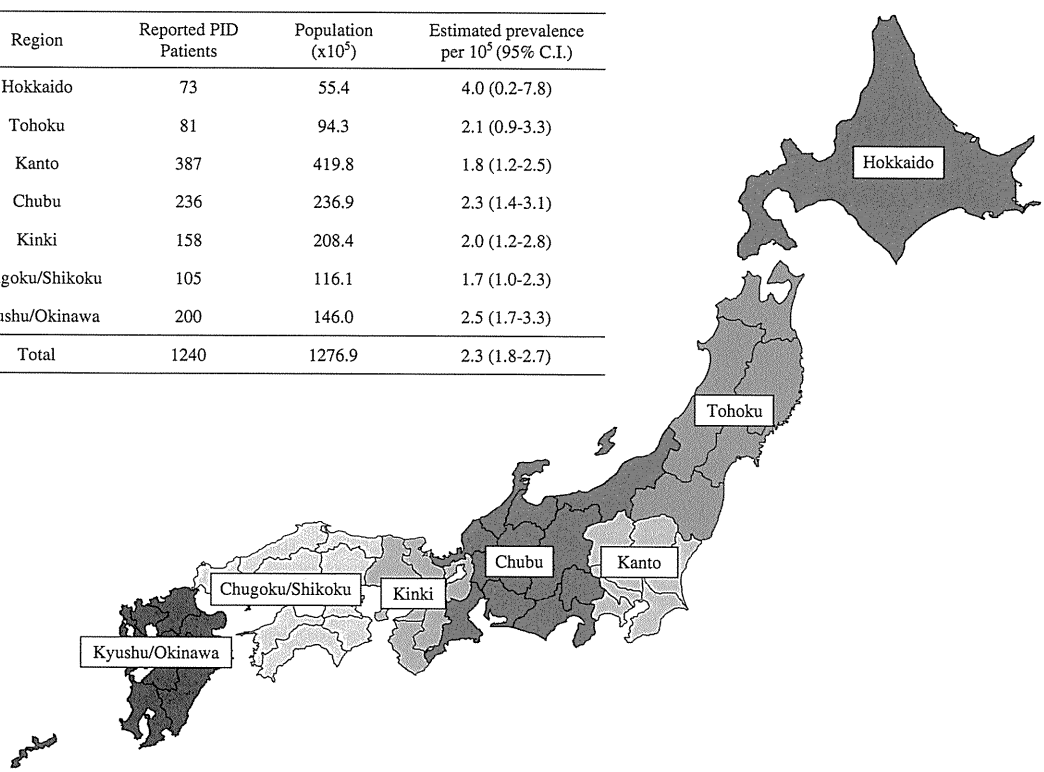

Fig. 1 Regional distribution of PID patients. *CI* Confidence interval

Table II Reported number of PID

Category	Total number	Pediatric department	Internal medicine department
I. Combined T and B cell immunodeficiencies	93 (7%)	93 (8%)	0 (0%)
γc deficiency	47	47	0
Adenosine deaminase deficiency	9	9	0
Omenn syndrome	4	4	0
Others	23	23	0
Untested or undetermined	10	10	0
II. Predominantly antibody deficiencies	501 (40%)	434 (38%)	67 (71%)
BTK deficiency	182	173	9
Common variable immunodeficiency disorders	136	107	29
Selective IgG subclass deficiency	66	58	8
Selective IgA deficiency	49	34	15
Hyper IgM syndrome	34	34	0
Transient hypogammaglobulinemia of infancy	7	7	0
Others	11	7	4
Untested or undetermined	16	14	2
III. Other well-defined immunodeficiency syndromes	194 (16%)	189 (17%)	5 (5%)
Wiskott–Aldrich syndrome	60	60	0
DNA repair defects (other than those in category I)	15	15	0
DiGeorge anomaly	38	38	0
Hyper-IgE syndrome	56	52	4
Chronic mucocutaneous candidiasis	17	16	1
Others	5	5	0
Untested or undetermined	3	3	0
IV. Diseases of immune dysregulation	49 (4%)	48 (4%)	1 (1%)
Chediak–Higashi syndrome	9	8	1
Familial hemophagocytic lymphohistiocytosis syndrome	5	5	0
X-linked lymphoproliferative syndrome	8	8	0
Autoimmune lymphoproliferative syndrome	8	8	0
APECED	4	4	0
IPEX syndrome	7	7	0
Others	2	2	0
Untested or undetermined	6	6	0
V. Congenital defects of phagocyte number, function, or both	230 (19%)	223 (19%)	7 (8%)
Severe congenital neutropenia	44	42	2
Cyclic neutropenia	19	17	2
Chronic granulomatous disease	147	144	3
Mendelian susceptibility to mycobacterial disease	5	5	0
Others	9	9	0
Untested or undetermined	6	6	0
VI. Defects in innate immunity	15 (1%)	15 (1%)	0
Anhidrotic ectodermal dysplasia with immunodeficiency	7	7	0
Interleukin-1 receptor-associated kinase 4 deficiency	2	2	0
Others	5	5	0
Untested or undetermined	1	1	0
VII. Autoinflammatory disorders	108 (9%)	101 (9%)	7 (8%)
Familial Mediterranean fever	44	40	4
TNF receptor-associated periodic syndrome	13	12	1
Hyper IgD syndrome	4	4	0
Cryopyrin-associated periodic syndrome	22	22	0

Table II (continued)

Category	Total number	Pediatric department	Internal medicine department
Others	3	3	0
Untested or undetermined	22	20	2
VIII. Complement deficiencies	32 (3%)	29 (3%)	3 (3%)
IX. Undetermined	18 (1%)	14 (1%)	4 (4%)
Total	1,240	1,146	94

APECED Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy, *IPEX* immune dysregulation, polyendocrinopathy, enteropathy, X-linked

increasing age (Fig. 2b). The median age of CID, BTK deficiency, CVID, and CGD patients was 5.2, 12.8, 25.1, and 14.7 years, respectively.

It is well known that PID patients are susceptible to many pathogens and experience community-acquired or opportunistic infections. In this study, we focused on noninfectious complications of PID because they have been less well studied on a large scale and may provide important information for improving the quality of life of PID patients. Twenty-five PID patients developed malignant disorders (2.7%; Table III). Lymphoma, in particular, Epstein–Barr virus-related, and leukemia were dominant, while there were no patients with gastric carcinoma. CVID, Wiskott–Aldrich syndrome (WAS), and ataxia telangiectasia were more frequently associated with malignant diseases among PID patients. A case of Mendelian susceptibility

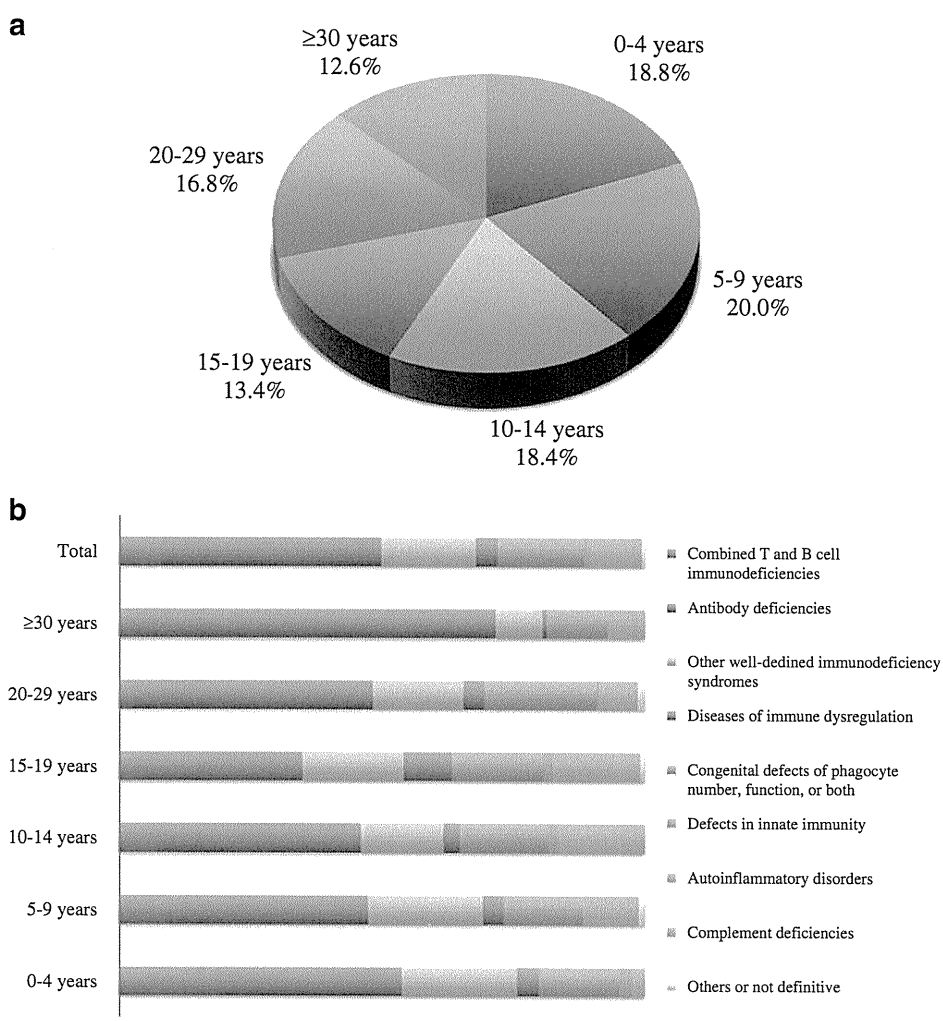

Fig. 2 **a** Age distribution of PID patients. **b** Distribution of PID in each age group

to mycobacterial disease with squamous cell carcinoma was also observed [9] (Table III).

Seventy-eight PID patients had immune-related (autoimmune) diseases (8.5%; Table IVa). Autoimmune lymphoproliferative syndrome, immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome, and nuclear factor kappa B essential modulator (NEMO) deficiency were associated with immune-related diseases at a very high incidence. In addition, immune-related diseases were relatively common in CGD and CVID patients (Table IVa). The most commonly observed immune-related disease was inflammatory bowel disease (33 cases), which was most frequently observed in CGD patients, followed by immune thrombocytopenic purpura (13 cases), autoimmune hemolytic anemia (8 cases), and systemic lupus erythematosus (SLE; 8 cases; Table IVa and b). Kawasaki disease occurred in WAS and CGD patients. In addition, this is the first report of Kawasaki disease in patients with complement deficiency (C9) and familial Mediterranean fever (FMF). A patient with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome and a patient with tumor necrosis factor receptor-associated periodic syndrome (TRAPS) were first reported as cases of type 1 diabetes mellitus and SLE, respectively [10, 11].

Discussion

We conducted a nationwide survey of PID for the first time in 30 years and report the prevalence of PID in Japan. We registered 1,240 PID patients and found that the estimated prevalence of PID (2.3/100,000) is higher than that previously reported (1.0/100,000) in Japan. Our results are equivalent to those reported in Singapore (2.7/100,000) and Taiwan (0.77–2.17/100,000) [12–14]. However, our values are lower than those reported in Middle Eastern countries such as Kuwait (11.98/100,000) or in European countries such as France (4.4/100,000) [5–7, 15]. The high rate of consanguinity may be a cause of the high prevalence rate of PID reported in Middle Eastern countries [6, 15]. There may has been sample selection bias in this study because some asymptomatic cases (SIgAD, etc.), clinically recovered cases (transient hypogammaglobulinemia of infancy, etc.), and cases in which patients were deceased were not registered. In addition, lack of recognition of PID in internal medicine departments, not just the low response rate, might also have influenced the estimated prevalence of PID as well as the age and disease distribution. The regional prevalence of PIDs in Japan was homogenous, unlike in other countries in which a higher prevalence was

Table III Malignancies in PID patients

Primary immunodeficiency	Total	*n*	Malignancy
I. Combined T and B cell immunodeficiencies	75	2	(2.7%)
Ommen syndrome	3	1	NHL (EBV+) 1[a]
Adenosine deaminase deficiency	4	1	Breast carcinoma 1
II. Predominantly antibody deficiencies	378	8	(2.1%)
Common variable immunodeficiency disorders	93	7	HL 2, ML 2, ALL 1, Basal cell carcinoma 1, Cervical carcinoma 1
Good syndrome	4	1	Double primary carcinoma of breast and colon 1
III. Other well-defined immunodeficiency syndromes	165	7	(4.2%)
Wiskott–Aldrich syndrome	57	5	NHL 3, NHL/HL 1, LPD (EBV-) 1
Ataxia telangiectasia	13	2	T-ALL 1, MDS 1
IV. Diseases of immune dysregulation	38	4	(10.5%)
X-linked lymphoproliferative syndrome	5	2	Burkitt lymphoma 2
Autoimmune lymphoproliferative syndrome	6	2	HL (EBV+) 1, Brain tumor 1
V. Congenital defects of phagocyte number, function, or both	153	4	(2.6%)
Severe congenital neutropenia	35	3	MDS 3 (including 2 cases with monosomy 7)
MSMD	7	1	Squamous cell carcinoma of finger 1
VI. Defects in innate immunity	12	0	(0%)
VII. Autoinflammatory disorders	74	0	(0%)
VIII. Complement deficiencies	23	0	(0%)
IX. Undetermined	5	0	(0%)
Total	923	25	(2.7%)

n Number of PID patients who had malignant disorders, *ALL* acute lymphoblastic leukemia, *EBV* Epstein-Barr virus, *HL* Hodgkin lymphoma, *LPD* lymphoproliferative disease, *MDS* myelodysplastic syndrome, *ML* malignant lymphoma, *MSMD* Mendelian susceptibility to mycobacterial disease, *NHL* non-Hodgkin lymphoma

[a] The number of patients

Table IV Immune-related diseases in PID patients

(a) Immune-related diseases with each PID

Primary immunodeficiency	Total	*n*	Immune-related disease
I. Combined T and B cell immunodeficiencies	75	2	(2.6%)
MHC class II deficiency (suspected)	1	1	ITP with AIHA 1[a]
CD4 deficiency	1	1	Hashimoto disease 1
II. Predominantly antibody deficiencies	378	24	(6.3%)
Common variable immunodeficiency disorders	93	16	ITP 3, RA 2, AIHA 2, Hashimoto's disease 2, IBD 2, SLE 1, MG 1, ADEM 1, Autoimmune hepatitis 1, Uveitis 1
Hyper-IgM syndrome	32	3	JIA 1, SLE (complicated with C1q deficiency) 1, IBD 1
Selective IgA deficiency	28	3	SLE 1, SLE with Kikuchi disease 1, RA 1
IgG subclass deficiency	50	2	ITP with AIHA 1, ITP with MS 1
III. Other well-defined immunodeficiency syndromes	165	5	(3.0%)
Wiskott–Aldrich syndrome	57	3	AIHA 2, Kawasaki disease 1
DiGeorge syndrome	33	2	AIHA 1, ITP 1
IV. Diseases of immune dysregulation	38	10	(26.3%)
X-linked lymphoproliferative syndrome	5	1	IBD 1
Autoimmune lymphoproliferative syndrome	6	4	ITP 3, Graves' disease with IBD 1
APECED	5	1	T1DM with Hashimoto's disease and Vogt–Koyanagi–Harada disease 1
IPEX syndrome	6	4	T1DM 1, T1DM with ITP, AIN and IBD 1, Autoimmune enteritis 1, AIHA with Autoimmune enteritis and Hashimoto's disease 1
V. Congenital defects of phagocyte number, function, or both	153	25	(16.3%)
Chronic granulomatous disease	87	25	IBD 20, ITP 2, JIA 1, MCTD 1, Kawasaki disease 1
VI. Defects in innate immunity	12	5	(41.7%)
NEMO deficiency	7	4	IBD 3, IBD with JIA 1
WHIM syndrome	3	1	T1DM 1
VII. Autoinflammatory disorders	74	3	(4.0%)
Familial Mediterranean fever	36	2	SLE 1, Kawasaki disease 1
TNF receptor associated periodic syndrome	9	1	SLE 1
VIII. Complement deficiencies	23	3	(13.0%)
C4 deficiency	1	1	SLE with RA 1
C6 deficiency	1	1	IBD 1
C9 deficiency	11	1	Kawasaki disease 1
IX. Undetermined	5	1	(20%)
Nakajo syndrome	1	1	SLE 1
Total	923	78	(8.5 %)

(b) Immune-related manifestations associated with PID

Immune-related diseases	*n*
IBD (including autoimmune enteritis)	33
ITP	13
AIHA	8
SLE	8
RA/JIA	6
Hashimoto's disease/Graves' disease	5
Kawasaki disease	4
T1DM	4
Uveitis (including Vogt–Koyanagi–Harada disease)	2
ADEM/MS	2
Others	5

n Number of PID patients who had immune-related disorders, *ADEM* acute disseminated encephalomyelitis, *AIHA* autoimmune hemolytic anemia, *AIN* autoimmune neutropenia, *APECED* autoimmune polyendocrinopathy candidiasis ectodermal dystrophy, *IBD* inflammatory bowel disease, *IPEX* immunodysregulation, polyendocrinopathy, enteropathy X-linked, *ITP* immune thrombocytopenic purpura, *JIA* juvenile idiopathic arthritis, *MCTD* mixed connective tissue disease, *MG* myasthenia gravis, *MS* multiple sclerosis, *RA* rheumatoid arthritis, *SLE* systemic lupus erythematosus, *T1DM* type 1 diabetes mellitus, *WHIM* warts, hypogammaglobulinemia, infections, and myelokathexis

[a] The number of patients

observed in urban areas [5, 7, 16]. This may be because many PID patients were treated or followed by PID specialists distributed nationwide in Japan; this is assumed by the location of hospitals with which they were affiliated.

The distribution ratios of BTK deficiency (14.7%) and CGD (11.9%) in Japan were higher than those in a previous report from Europe (5.87% and 4.33%, respectively), while those of CIDs and other well-defined immunodeficiency syndromes were comparable [17]. The prevalence of BTK deficiency was previously reported to be 1/900,000–1,400,000 in a European cohort study [18]. In contrast, this value was estimated to be 1/300,000 in Japan in our study. BTK deficiency appears to be common in Japan, although this may be partially because more patients, including those showing atypical clinical manifestations, were diagnosed more accurately by the recently established genetic diagnostic network in Japan [19]. This is supported by the highest proportion of Japanese patients in the international mutation database for X-linked agammaglobulinemia (BTKbase) [20]. The reason for the low number of registered CGD patients in Europe in a recent report (1/620,000) [17] is unknown; the prevalence of CGD was 1 in 250,000 in a previous European survey [21], which was similar to our results (1 in 380,000 in this study and 1 in 280,000 in our previous study [22]). The percentage of BTK deficiency and CGD would be lower if more adult cases were registered because the prevalence of these disorders is low in adults. CVID was the most commonly reported PID (20.7%) in Europe, and the onset of symptoms was observed most commonly in the third decade of life in these patients [17, 23]. In this study, CVID constituted 11.0% (136 cases) of PID cases, and only 29 cases were reported from internal medicine departments (Table II). A lower number of registered CVID patients may have led to a lower number of reported patients with antibody deficiency and a lower prevalence of PID, although it is still possible that CVID is not as common in Japan as in European countries. There was no significant difference in the distribution rate of SIgAD between Japanese and Europeans, although SIgAD is rare in Japanese (1/18,500) compared with Caucasians (1/330–2,200) according to seroepidemiologic studies [24]. This may be because most SIgAD patients lack clinical manifestations. The distribution ratio of autoinflammatory disorders in Japan (9%) was much higher than that in Europe (1.02%) [17] (Table II). Considering the disease type of the autoinflammatory disorders was not specified in 22 cases (20%), it is possible that many other patients with autoinflammatory disorders remain undiagnosed in Japan as well as in other countries.

The percentage of men (69.7%) with PID is higher in Japan than in Europe (60.8%) or Kuwait (61.8%), but is equivalent to that in Taiwan (70.2%) [6, 13, 17]. The higher ratio of men, particularly in younger generation (<15 years), appears to be due to the larger number of X-linked PID patients (BTK deficiency, X-CGD, γc deficiency, etc.) in this study compared to that in Europe or Kuwait. Adolescents or adults (≥15 years) constituted 42.8% of the patients in this study, which is equivalent to the number in the European study (≥16 years: 46.6%), while those >16 years constituted only 10.9% in the previous survey [3, 17]. In this study, it was found that CVID and SIgAD are common in adults (Table II) and that antibody deficiencies are more common with increasing age (Fig. 2b). A reason for the increased number of adult PID patients may be long-term survival of PID patients due to improved treatments such as immunoglobulin replacement therapy. In addition, an increased likelihood of patients being diagnosed by internists as having late-onset PID, e.g., CVID and SIgAD, may have contributed to these values [17, 25, 26]. Therefore, it is important for internists to be well-informed regarding PID. In contrast, CIDs are fatal during infancy without hematopoietic stem cell transplantation or gene therapy. Because hematopoietic stem cell transplantation has been widely performed in Japan since the 1990s, surviving patients with CID are limited to the younger generation, similar to French patients (Fig. 2b) [5, 27, 28].

It has been reported that PID patients are at increased risk of developing malignant diseases, in particular, non-Hodgkin lymphoma, leukemia, and stomach cancer [29]. Although lymphoma and leukemia were relatively common, stomach cancer was not observed in our study. In the previous survey in Japan, eight of nine PID patients with malignant disorders (including one gastric cancer patient) died [3]. It is possible that some PID patients with malignant disorders were not registered because they were deceased. PID is also associated with immune-related diseases because of a defect in the mechanisms to control self-reactive B and T cells. The frequency of immune-related manifestations varied among individual PID patients, as reported previously [30, 31]. Four PID patients who had developed Kawasaki disease, one patient with WHIM syndrome and type 1 diabetes mellitus, and one patient with TRAPS and SLE in our study may provide new pathophysiological insights of these diseases and the association between PID and autoimmune diseases.

Conclusions

We report the prevalence and clinical characteristics of PIDs in Japan. Although the advances in diagnostic technologies and treatments have improved the prognoses of PID, many patients continue to experience severe complications such as malignancy and immune-related diseases as well as infections. To improve the quality of life of PID patients, it is necessary to pay attention to

complications and treat them appropriately. Web-based PID databases and consultation systems have been created in Japan (Primary Immunodeficiency Database in Japan [4] and Resource of Asian Primary Immunodeficiency Diseases in Asian countries [32]) to reveal precise information regarding PID and to promote cooperation between doctors and researchers [19].

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Conflict of Interest There is no actual or potential conflict of interest in relation to the study.

References

1. Notarangelo LD. Primary immunodeficiencies. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S182–94.
2. Geha RS, Notarangelo LD, Casanova JL, Chapel H, Conley ME, Fischer A, et al. Primary immunodeficiency diseases: an update from the international union of immunological societies primary immunodeficiency diseases classification committee. J Allergy Clin Immunol. 2007;120(4):776–94.
3. Hayakawa H, Iwata T, Yata J, Kobayashi N. Primary immunodeficiency syndrome in Japan. I. overview of a nationwide survey on primary immunodeficiency syndrome. J Clin Immunol. 1981;1 (1):31–9.
4. Primary Immunodeficiency Database in Japan (PIDJ). http://pidj.rcai.riken.jp/ (in Japanese).
5. CEREDIH. The French PID study group. The French national registry of primary immunodeficiency diseases. Clin Immunol. 2010;135(2):264–72.
6. Al-Herz W. Primary immunodeficiency disorders in Kuwait: first report from Kuwait national primary immunodeficiency registry (2004–2006). J Clin Immunol. 2008;28(2):186–93.
7. Stray-Pedersen A, Abrahamsen TG, Froland SS. Primary immunodeficiency diseases in Norway. J Clin Immunol. 2000;20(6):477–85.
8. Nakamura Y, Matsumoto T, Tamakoshi A, Kawamura T, Seino Y, Kasuga M, et al. Prevalence of idiopathic hypoparathyroidism and pseudohypoparathyroidism in Japan. J Epidemiol. 2000;10(1):29–33.
9. Toyoda H, Ido M, Nakanishi K, Nakano T, Kamiya H, Matsumine A, et al. Multiple cutaneous squamous cell carcinomas in a patient with interferon gamma receptor 2 (IFN gamma R2) deficiency. J Med Genet. 2010;47(9):631–4.
10. Takaya J, Fujii Y, Higashino H, Taniuchi S, Nakamura M, Kaneko K. A case of WHIM syndrome associated with diabetes and hypothyroidism. Pediatr Diabetes. 2009;10(7):484–6.
11. Ida H, Kawasaki E, Miyashita T, Tanaka F, Kamachi M, Izumi Y, et al. A novel mutation (T61I) in the gene encoding tumour necrosis factor receptor superfamily 1A (TNFRSF1A) in a Japanese patient with tumour necrosis factor receptor-associated periodic syndrome (TRAPS) associated with systemic lupus erythematosus. Rheumatology (Oxford). 2004;43(10):1292–9.
12. Lim DL, Thong BY, Ho SY, Shek LP, Lou J, Leong KP, et al. Primary immunodeficiency diseases in Singapore—the last 11 years. Singapore Med J. 2003;44(11):579–86.
13. Lee WI, Kuo ML, Huang JL, Lin SJ, Wu CJ. Distribution and clinical aspects of primary immunodeficiencies in a Taiwan pediatric tertiary hospital during a 20-year period. J Clin Immunol. 2005;25(2):162–73.
14. Lee WI, Huang JL, Jaing TH, Shyur SD, Yang KD, Chien YH, et al. Distribution, clinical features and treatment in Taiwanese patients with symptomatic primary immunodeficiency diseases (PIDs) in a nationwide population-based study during 1985–2010. Immunobiology. 2011 Jun 21 [Epub ahead of print].
15. Shabestari MS, Maljaei SH, Baradaran R, Barzegar M, Hashemi F, Mesri A, et al. Distribution of primary immunodeficiency diseases in the Turk ethnic group, living in the northwestern Iran. J Clin Immunol. 2007;27(5):510–6.
16. Matamoros Flori N, Mila Llambi J, Espanol Boren T, Raga Borja S, Fontan Casariego G. Primary immunodeficiency syndrome in Spain: first report of the national registry in children and adults. J Clin Immunol. 1997;17(4):333–9.
17. Gathmann B, Grimbacher B, Beaute J, Dudoit Y, Mahlaoui N, Fischer A, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2006–2008. Clin Exp Immunol. 2009;157 Suppl 1:3–11.
18. Toth B, Volokha A, Mihas A, Pac M, Bernatowska E, Kondratenko I, et al. Genetic and demographic features of X-linked agammaglobulinemia in Eastern and Central Europe: a cohort study. Mol Immunol. 2009;46(10):2140–6.
19. Burrows PD, Fischer A. Building networks for immunodeficiency diseases and immunology training. Nat Immunol. 2008;9(9):1005–7.
20. Valiaho J, Smith CI, Vihinen M. BTKbase: the mutation database for X-linked agammaglobulinemia. Hum Mutat. 2006;27 (12):1209–17.
21. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PLoS One. 2009;4(4):e5234.
22. Hasui M. Chronic granulomatous disease in Japan: incidence and natural history. The study group of phagocyte disorders of Japan. Pediatr Int. 1999;41(5):589–93.
23. Chapel H, Lucas M, Lee M, Bjorkander J, Webster D, Grimbacher B, et al. Common variable immunodeficiency disorders: division into distinct clinical phenotypes. Blood. 2008;112(2):277–86.
24. Kanoh T, Mizumoto T, Yasuda N, Koya M, Ohno Y, Uchino H, et al. Selective IgA deficiency in Japanese blood donors: frequency and statistical analysis. Vox Sang. 1986;50(2):81–6.
25. Aghamohammadi A, Moin M, Farhoudi A, Rezaei N, Pourpak Z, Movahedi M, et al. Efficacy of intravenous immunoglobulin on the prevention of pneumonia in patients with agammaglobulinemia. FEMS Immunol Med Microbiol. 2004;40(2):113–8.
26. Quartier P, Debre M, De Blic J, de Sauverzac R, Sayegh N, Jabado N, et al. Early and prolonged intravenous immunoglobulin replacement therapy in childhood agammaglobulinemia: a retrospective survey of 31 patients. J Pediatr. 1999;134(5):589–96.
27. Sakata N, Kawa K, Kato K, Yabe H, Yabe M, Nagasawa M, et al. Unrelated donor marrow transplantation for congenital immunodeficiency and metabolic disease: an update of the experience of the Japan marrow donor program. Int J Hematol. 2004;80(2):174–82.
28. Morio T, Atsuta Y, Tomizawa D, Nagamura-Inoue T, Kato K, Ariga T, et al. Outcome of unrelated umbilical cord blood transplantation in 88 patients with primary immunodeficiency in Japan. Br J Haematol. 2011;154(3):363–72.
29. Vajdic CM, Mao L, van Leeuwen MT, Kirkpatrick P, Grulich AE, Riminton S. Are antibody deficiency disorders associated with a narrower range of cancers than other forms of immunodeficiency? Blood. 2010;116(8):1228–34.
30. Bussone G, Mouthon L. Autoimmune manifestations in primary immune deficiencies. Autoimmun Rev. 2009;8(4):332–6.
31. Arason GJ, Jorgensen GH, Ludviksson BR. Primary immunodeficiency and autoimmunity: lessons from human diseases. Scand J Immunol. 2010;71(5):317–28.
32. Resource of Asian primary immunodeficiency diseases (RAPID). http://rapid.rcai.riken.jp/RAPID/.

Clinical similarities and differences of patients with X-linked lymphoproliferative syndrome type 1 (XLP-1/SAP deficiency) versus type 2 (XLP-2/XIAP deficiency)

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X-linked lymphoproliferative syndromes (XLP) are primary immunodeficiencies characterized by a particular vulnerability toward Epstein-Barr virus infection, frequently resulting in hemophagocytic lymphohistiocytosis (HLH). XLP type 1 (XLP-1) is caused by mutations in the gene *SH2D1A* (also named *SAP*), whereas mutations in the gene *XIAP* underlie XLP type 2 (XLP-2). Here, a comparison of the clinical phenotypes associated with XLP-1 and XLP-2 was performed in cohorts of 33 and 30 patients, respectively. HLH (XLP-1, 55%; XLP-2, 76%) and hypogammaglobulinemia (XLP-1, 67%; XLP-2, 33%) occurred in both groups. Epstein-Barr virus infection in XLP-1 and XLP-2 was the common trigger of HLH (XLP-1, 92%; XLP-2, 83%). Survival rates and mean ages at the first HLH episode did not differ for both groups, but HLH was more severe with lethal outcome in XLP-1 (XLP-1, 61%; XLP-2, 23%). Although only XLP-1 patients developed lymphomas (30%), XLP-2 patients (17%) had chronic hemorrhagic colitis as documented by histopathology. Recurrent splenomegaly often associated with cytopenia and fever was preferentially observed in XLP-2 (XLP-1, 7%; XLP-2, 87%) and probably represents minimal forms of HLH as documented by histopathology. This first phenotypic comparison of XLP subtypes should help to improve the diagnosis and the care of patients with XLP conditions. (*Blood*. 2011;117(5):1522-1529)

Introduction

X-linked lymphoproliferative syndrome (XLP) is a rare immunodeficiency condition characterized by an extreme vulnerability to Epstein-Barr virus (EBV) infection, frequently resulting in hemophagocytic lymphohistiocytosis (HLH) or virus-associated hemophagocytic syndrome (VAHS).[1-3] HLH is caused by overwhelming T-cell and macrophage activation, leading to fever, splenomegaly, cytopenia, hypofibrinogenemia, or hypertriglyceridemia, hyperferritinemia, and hemophagocytosis.[4]

XLP belongs to the group of familial hemophagocytic lymphohistiocytosis (FHL) as originally proposed by Purtilo et al.[1] In the original description, the term "lymphoproliferative disease" in the Duncan kindred[1] was used for benign or malignant lymphoproliferation but also for the diffuse organ "infiltrates composed of lymphocytes, plasma cells, and histiocytes, some containing erythrocytes," describing histologic features of HLH. Thus, the term "X-linked lymphoproliferative disease or syndrome" used thereafter to name this condition refers not only to malignant lymphomas but also to HLH. Two genetic causes are responsible for XLP. XLP type 1 (XLP-1) is caused by hemizygous mutations in the gene *SH2D1A* encoding the signaling lymphocyte activation molecule (SLAM)–associated protein (SAP) (MIM no. 308240).[5,6] Hemizygous mutations in the gene encoding the X-linked inhibitor of

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apoptosis protein (XIAP; also termed *BIRC4*; MIM no. 300635) have been discovered in a cohort of patients with clinical XLP without any identified mutations in *SH2D1A* and normal SAP protein expression.[7] Thus, mutations in *XIAP* define the XLP type 2 (XLP-2). These findings were confirmed by the identification of additional patients with XIAP deficiency.[8,9] After EBV infection in most (but not all) cases, patients bearing mutations in *SH2D1A* (hereafter denoted SAP-deficient patients) may experience variable manifestations such as fulminant infectious mononucleosis corresponding pathophysiologically to HLH, malignant lymphoma, and hypogammaglobulinemia.[2,10,11] Less common findings are dysgammaglobulinemia, bone marrow hypoplasia, especially aplastic anemia, and lymphocytic vasculitis.[12,13] However, although HLH is almost always triggered by EBV, the other manifestations can be present even in SAP-deficient patients who have never encountered EBV.[2,3,10,11] The clinical features of the 12 patients with mutations in *XIAP* (hereafter denoted XIAP-deficient patients) initially described, slightly differed from the features described above. In some XIAP-deficient patients, splenomegaly was noticed as the first clinical symptom, and chronic colitis occurred during the disease course in 2 patients.[7]

The gene product affected in XLP-1 patients, SAP, is a small SH2-containing adaptor protein that is expressed in T, natural killer (NK), and invariant NKT (iNKT) cells.[5,14] SAP binds with high affinity and specificity to tyrosine-based motifs located in the cytoplasmic domains of the transmembrane receptors of the SLAM family. SAP couples SLAM family receptors to downstream signaling pathways and thereby enables SLAM receptors to mediate an array of activating or regulatory signals. In SAP-deficient humans and mice, multiple cellular defects have been documented, including altered $CD8^+$ T- and NK-cell cytotoxicity responses, $CD4^+$ T helper cell cytokine production and function, block of CD1d-restricted iNKT-cell development, defective antibody production associated with reduced numbers of switched memory B cells and defects in germinal center formation.[11,14] Studies of SAP-deficient humans and mice support the notion that the immune dysfunctions seen in SAP-deficiency are mostly caused by alterations in the signal transduction of SLAM family receptors.

The XLP-2 gene product, XIAP, belongs to the family of inhibitor of apoptosis proteins and is well known to be a potent physiologic inhibitor of caspases 3, 7, and 9.[15] XIAP is ubiquitously expressed.[7] In addition to its antiapoptotic role, XIAP is also involved in multiple signaling pathways, including copper metabolism, activation of the nuclear factor κB and the mitogen-activated protein kinases pathways and the transforming growth factor-β–receptor and bone morphogenetic protein–receptor signal transduction.[16] In XIAP-deficient patients, lymphocytes are characterized by an increased susceptibility to apoptosis in response to CD95 and tumor necrosis factor receptor–related apoptosis-inducing ligand receptor stimulation as well as enhanced activation-induced cell death.[7] XIAP-deficient patients also display low but detectable numbers of iNKT cells in blood although a recent study indicated that they can have normal numbers of iNKT cells.[9] NK cell–mediated cytotoxicity is apparently normal in XIAP-deficient patients.[7,9]

Our knowledge of the immune dysfunctions underlying the clinical manifestations in SAP-deficient patients has been largely improved in the past decade. However, this is not the case for XIAP-deficient patients. A better characterization of the clinical similarities and the differences between XLP-1 and XLP-2 could provide hints for a better understanding of the pathogenesis of these conditions and, furthermore, improve diagnostic and therapeutic procedures for these patients. Therefore, we performed a retrospective analysis of the clinical features observed in cohorts of 33 SAP- and 30 XIAP-deficient patients.

Methods

Patients and diagnosis

We performed a retrospective analysis of the clinical and laboratory features of SAP- and XIAP-deficient patients in whom confirmative molecular diagnosis had been performed at the Necker Children's Hospital. Patient conditions were diagnosed as XLP-1 and XLP-2 on the basis of molecular results or on the basis of clinical features when disease had been molecularly proven in male relatives on the mother's side (supplemental Methods and Results, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Patients and families provided informed consent for genetic and immunologic studies in accordance to the 1975 Declaration of Helsinki, and the study was approved by the local ethics regulations (Necker-Enfants Malades Ethical Board Committee).

Protein expression

Expression of SAP and XIAP was analyzed by Western blotting or flow cytometry or both after intracellular staining in phytohemagglutinin-induced T-cell blasts or peripheral blood mononuclear cells or both as described.[7] The monoclonal antibody (mAb) anti-SAP was kindly provided by Dr A. Veillette, IRCM, Montréal. Intracellular SAP was stained by fluorescein isothiocyanate– or phycoerythrin-coupled anti-SAP mAb and XIAP detected with noncoupled anti-XIAP mouse mAb (clone 48; BD Biosciences) revealed with fluorescein isothiocyanate–coupled anti–mouse antibodies (Jackson ImmunoResearch Laboratories Inc) after cell permeabilization with Perm 2 (BD Biosciences).

Histology and immunohistochemistry

All diagnostic specimens were fixed in 10% buffered formalin and stained with hematoxylin and eosin, Giemsa, or trichrome dyes (for the liver). Immunohistochemistry was performed on fixed tissues with a peroxidase-based method (Dako). Antibodies used were raised against CD20, CD3, CD8, and latent membrane protein 1 (LMP-1) (Dako); CD25 (Novocastra); and T-cell intracellular antigen-1 (Immunotech). EVB-encoded RNA (EBER) was probed on some specimen with the use of in situ hybridization technique. Slides were observed using a Leica DM LB microscope with ×20, ×40, and ×100 objectives and a 10× eyepiece. Acquisition of images was with IM50 software (Leica Microsystems). All slides were analyzed by the same pathologist (D.C.), and an independent review was also performed (F.H.).

Clinical assessment

The patients' clinical events and laboratory features were assessed retrospectively by retrieval of data from medical records.

Statistical analysis

The statistical analyses were performed with Fisher exact tests or log-rank tests (for comparison of survival curves) with the use of the PRISM software (GraphPad Software Inc).

Results

XLP-1 was diagnosed in 33 patients from 19 families, and mutations of *SH2D1A* were found in 18 families, and XLP-2 was

Table 1. Characteristics of patients with mutations in *SH2D1A/SAP* (XLP-1)

Patient ID*	*SH2D1A/SAP* mutation	SAP protein	HLH (age in years at diagnosis)	EBV at first HLH	HLH relapses (age in years at relapse)	SM (age in years at diagnosis)	Hypo-γ (age in years at diagnosis)	Lymphoma (age in years at diagnosis)	Other manifestations (age in years at diagnosis)	Outcome (age in years)
S1.1	E67G		–	NA		–	–	13	–	Alive, well (19)
S1.2	E67G	–	3	+	+ (25)	–	+ (26)	34	–	Alive, under lymphoma treatment (34)
S1.3	E67G		15	?	–	–	–	7, 30	–	Alive, under lymphoma treatment (30)
S1.4	E67G		–	NA		–	+ (4)†	–	–	Alive, well, IVIG (10)
S2.1	I96X	–	4	?	–	?	?	–	–	Died (4, HLH)
S3.1	del. of exons 1-4		–	NA		–	+†	–	Chronic gastritis,	Alive, well, IVIG (20)
S3.2	del. of exons 1-4		–	NA		–	+†	–	IM (2), chronic gastritis	Alive, well, IVIG (20)
S4.1	R55X		–	NA		–	–	40	–	Alive, well (42)
S4.2	ND		6	+	–	–	–	–	–	Died (6, HLH)
S5.1	del. of exon 2		3.7	+	–	?	?	–	–	Died (3.7, HLH)
S5.2	ND		–	NA		–	?	5	–	Died (5, lymphoma)
S6.1	del. of exon 1		2.2	+	–	–	?	–	–	Died (2.2, HLH)
S7.1	R55X	–	2.5	+	–	–	?	–	Recurrent infections	HSCT (2.7), alive (11)
S8.1	X129RfsX141	–	2.4	+	+ (9)	–	+ (3)†	–	–	First HSCT (9); second HSCT (10); died (10.2)
S8.2	ND		2	+	–	–	?	–	–	Died (2, HLH)
S9.1	C42Y	+/–	–	NA		–	–	2	–	Alive (18)
S9.2	C42Y		–	NA		–	+ (1)†	–	–	Alive, well, IVIG (16)
S10.1	R55Q		14	?	–	?	?	–	–	Died (14, HLH)
S11.1	X129R fsX141	–	–	NA		–	+	–	–	Alive, well, N+T, IVIG (22)
S11.2	X129R fsX141		–	NA		?	?	–	Recurrent pneumonia	Alive, well (66)
S11.3	X129R fsX141		–	NA		–	+	–	–	Alive, well, IVIG (15)
S11.4	X129R fsX141		–	NA		–	+ (9)	7	–	Alive, well, IVIG (19)
S12.1	del. of exon 3	–	19	+	–	–	+ (10)†	11	T (22)	Alive, T, IVIG (23)
S12.2	del. of exon 3		19	?	–	–	+ (19)†	20	–	Died (21, lymphoma)
S13.1	N82FfsX103	ND	10§	–	+ (12, EBV+)	+ (9)‡	?	–	–	Died (12, HLH)
S14.1	del. of exons 1-4		3.5	+	–	–	–	–	HUS (3.5)	Died (3.6, HLH)
S15.1	A22P	–	–	NA		–	+ (13)†	–	–	Alive, well, IVIG (25)
S15.2	ND		3.6	?	–	–	?	–	–	Died (3.6, HLH)
S15.3	ND		–	NA		+ (45)‡	?	–	–	Died (69, myelodysplasia)
S16.1	del. of exons 2-4	–	3.1	+	–	–	?	–	–	Died (3.1, HLH)
S17.1	M1T	–	–	NA	–	–	+ (4)†	–	IM (2.4)	Alive, N+T, IVIG (20)
S18.1	No mutation	–	16§	?	–	–	+ (15)†	9	–	Died (17, HLH)
S19.1	del. of exons 1-4	–	3.3	+		–	–	–	Hypopigmented hair	HSCT (3.7), died (3.8)

SM indicates recurrent splenomegaly or hepatosplenomegaly; Hypo-γ, hypogammaglobulinemia; NA, not applicable; del., deletion; ?, unknown; IM, infectious mononucleosis; ND, not done; HSCT, hematopoietic stem cell transplantation; N, neutropenia; T, thrombocytopenia; and HUS, hemolytic uremic syndrome.

*Patient identification: S indicates SAP-deficiency, the first number corresponds to the family and the second to the individual patient.

†With recurrent respiratory infections; + indicates yes or positive; –, no or negative.

‡Recurrent splenomegaly or hepatosplenomegaly associated with intermittent fever, anemia, and cytopenia.

§Diagnosed as incomplete HLH.

diagnosed in 30 patients from 11 families (Tables 1 and 2). In one patient (PS18.1), no mutation in *SH2D1A* was found; however, no SAP protein expression was detected.[17] Six and 7 mutations in *SH2D1A* and *XIAP* were novel and not reported, respectively (supplemental Methods and Results).

Clinical manifestations included HLH, splenomegaly and incomplete forms of HLH, lymphoma, dysgammaglobulinemia, colitis, and rare clinical manifestations.

HLH

The mean age at first episode of HLH was 7.35 years (range, 2.0-19.0 years) in SAP-deficient and 6.5 years (range, 0.1-23.0 years) in XIAP-deficient patients ($P = .89$). The occurrence of HLH in SAP-deficient (18 of 33, 55%) and in XIAP-deficient (22 of 29, 76%, one unknown) patients did not differ significantly ($P = .112$) (Figure 1A; Table 3). XIAP-deficient patients with null mutations (families X1 to X7 and X11) more frequently developed HLH (19 of 20, 95%) compared with XIAP-deficient patients expressing non-null mutations (families X8, X9, and X10; 3 of 9, 33%; $^{**}P = .0011$; supplemental Figure 1A).

Overall, 11 of the 33 SAP-deficient patients (33%) and 5 of 30 the XIAP-deficient patients (17%) succumbed to HLH ($P = .1563$). Among patients with HLH, HLH-associated lethality was significantly higher in SAP-deficient patients (11 of 18, 61%) than in XIAP-deficient patients (5 of 22, 23%) ($^{*}P = .0230$). HLH

Table 2. Characteristics of patients with mutations in *XIAP* (XLP-2)

Patient ID*	*XIAP* mutation	XIAP protein	HLH (age in years at diagnosis)	EBV at first HLH	HLH relapses (age in years at relapse)	SM (age in years at diagnosis)	Hypo-γ (age in years at diagnosis)	Chronic colitis (age in years at diagnosis)	Other manifestations (age in years at diagnosis)	Outcome (age in years)
X1.1	E99KfsX129		5	+	?	?	–	–	?	Alive, well (8)
X1.2	E99KfsX129	–	5.3	+	?	+ (5)	–	–	?	Alive, well (11)
X1.3	E99KfsX129		2.5	+	?	+ (2.5)	+	–	?	Alive, well, IVIG (14)
X1.4	E99KfsX129	–	7.8	+	+	+ (6)	–	+ (4)	Cholangitis (23)	Alive, ileitis (23)
X1.5	E99KfsX129		3	+	+	+ (3)	–	–	–	Alive, well (30)
X1.6	E99KfsX129	–	0.8	– (HHV-6+/–)	+ (EBV+)	+ (1)‡	+(10)	–	–	HSCT (11), died (11)
X1.7	ND		1.5†	?	+	+ (1.5)‡	+(42)	+ (41)	Cholangitis (41)	Died (42, colitis)
X2.1	I397FfsX414	–	1.2	+	+	+ (1)‡	–	–	–	HSCT (1.6), died (d+13, HLH)
X3.1	E118X	–	23	+	+	+ (22)	+(22)	–	–	Alive, well, IVIG (39)
X3.2	ND		0.5	?	–	?	?		–	Died (0.5, HLH)
X3.3	ND		20	+	–	?	?		–	Died (20, HLH)
X3.4	E118X	–	–	NA	–	+ (7)	–	–	–	Alive, well, SM (10)
X4.1	del. of exon 2	–	20	+	+ (21,EBV+)	+ (1)‡	–	–	–	Alive, well (28)
X4.2	del. of exon 2	–	10	?	+ (11, EBV+)	+ (6)‡	–	–	–	Alive, well (15)
X5.1	D130GfsX140	–	2.5	+	+ (3.4-3.6)	+ (1)	–	–	–	HSCT (3.6), died (4)
X5.2	ND		0.1	?	–	?	?	–	–	Died (0.1, HLH)
X5.3	ND		3.5	+	–	?	?	–	–	Died (3.5, HLH)
X6.1	R238X	–	1.7	+	+	+‡	–	–	–	Alive, recurrent HLH (3)
X7.1	I397NfsX405	–	2.7	+	+ (3.2-3.5, EBV+)	+ (2.7)	–	–	–	Alive, recurrent HLH (3.5)
X8.1	E434AfsX457	+/–	15.5	+	–	–	–	–	–	Alive, well (16)
X9.1	G466X	+/–	8†	+	–	+ (8)‡	+ (8)§	–	–	Alive, well, SM (27)
X9.2	G466X	+/–	–	NA	–	+ (21)	+ (21)§	–	–	Alive, well, SM (30)
X9.3	G466X	+/–	–	NA	–	+ (4)‡	–	+ (12)	Recurrent infections	Alive, colitis (14)
X9.4	G466X	+/–	–	NA	–	+ (22)‡	+ (10)§	–	Chronic liver failure (22)	Died (29, liver failure), IVIG,
X9.5	G466X	+/–	–	NA	–	–	–	–	–	Alive, well (39)
X9.6	ND		–	NA	–	?	?	+	?	Died (27, colitis)
X9.7	ND		–	NA	–	+‡	?	–	Recurrent infections	Died (52, pneumonia)
X10.1	T470S	+	8†	– (HSV-1+)	–	–	+ (4)§	–	Cryptococcosis (4)	Alive, well, IVIG (8)
X11.1	R381X	–	0.9†	–	NA	+ (0.6)‡	–	–	–	HSCT (1.2), died (1.4)
X11.2	ND		?	?	?	?	?	+(4)	?	Died (4, colitis)

SM indicates recurrent splenomegaly or hepatosplenomegaly; Hypo-γ, hypogammaglobulinemia; ?, unknown; HSCT, hematopoietic stem cell transplantation; ND, not done; NA, not applicable; and del., deletion.

*Patient identification: X indicates XIAP deficiency, the first number corresponds to the family and the second to the individual patient; + indicates yes or positive; –, no or negative; and +/–, weakly positive.

†Diagnosed as incomplete HLH.

‡Recurrent splenomegaly or hepatosplenomegaly associated with intermittent fever, anemia, and cytopenia.

§With recurrent respiratory infections.

relapsed in 2 of 7 SAP-deficient HLH-survivors (29%), whereas 11 of 14 XIAP-deficient HLH-survivors (79%, 3 unknown) had ≥ 1 relapse of HLH ($P = .055$).

Six of the 18 SAP-deficient patients with HLH (33%) had proven neurologic involvement with mostly (5 of 6, 83%) lethal outcome, whereas 2 of 22 of XIAP-deficient patients with HLH (9%) had neurologic involvement with less mortality (1 of 2, 50%).

EBV infection was the most-frequent identified trigger of the first HLH episode in the SAP-deficient (11 of 12, 92%, 6 unknown) and XIAP-deficient (15 of 18, 83%, 4 unknown) patients ($P = .63$) (Table 3). Only PS13.1, PX1.6, PX10.1, and PX11.1 had a first HLH episode in the absence of a proven EBV-infection, whereas the EBV status of 6 SAP-deficient patients and 4 XIAP-deficient patients is not known. PX1.6 and PX4.2 subsequently experienced an HLH-relapse with positive EBV polymerase chain reaction. In 2 patients, herpes simplex virus type 1 (HSV-1) and human herpesvirus type 6 (HHV-6) were detected in the blood by polymerase chain reaction in the course of their first HLH episode. Of note, in several XIAP-deficient patients, other viruses than EBV were tested, including cytomegalovirus, parvovirus B19, HSV, HHV-6, HHV-8, HIV, human T-cell leukemia virus, adenovirus, and varicella-zoster virus. All were negative.

Splenomegaly and incomplete forms of HLH

Recurrent splenomegaly occurring in the absence of systemic HLH and often associated with fever and cytopenia (consisting of pancytopenia, bicytopenia, thrombocytopenia, and anemia) was frequently observed in XIAP-deficient patients (20 of 23, 87%, 7 unknown), whereas it was only found in 2 of 29 SAP-deficient patients (7%, 4 unknown; $***P < .0001$; Table 3). In 8 XIAP-deficient patients, episodes of splenomegaly occurred before they developed HLH and were the first clinical sign of the disease. Overall, although 3 patients with splenomegaly up to now did not

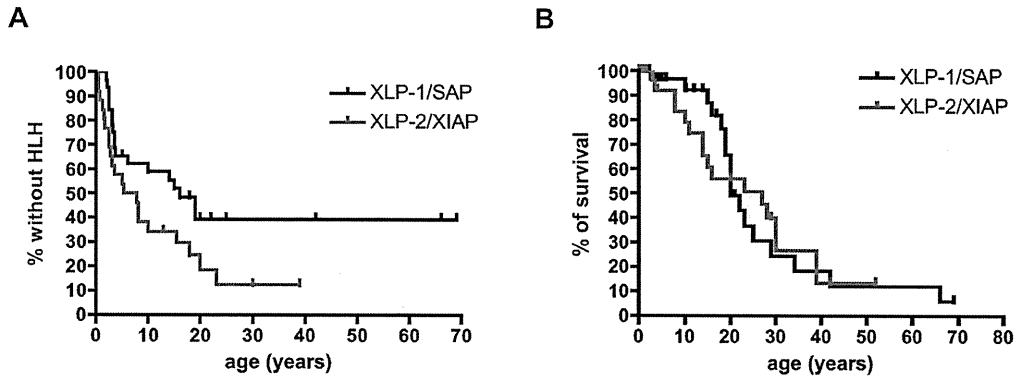

Figure 1. Comparison of HLH phenotypes and survival curves of SAP-deficient (XLP-1) and XIAP-deficient (XLP-2) patients. Kaplan-Meier survival curves were constructed on the basis of data presented in Table 1 and Table 2. Statistical analyses with log-rank tests. (A) Percentage of XLP-1/SAP and XLP-2/XIAP patients without HLH phenotype ($P = .099$). (B) Overall survival curves for XLP-1/SAP and XLP-2/XIAP patients ($P = .948$).

develop HLH, the others subsequently developed HLH within a period of time, varying from a few months to 19 years. In 2 XIAP-deficient patients, transient pancytopenia with splenomegaly was noticed after vaccinations against measles, mumps, and rubella or measles and rubella. Importantly, none of the XIAP-deficient patients developed B-cell lymphoproliferative disease.

PX4.1 underwent splenectomy at the age of 21 years, and histopathologic examination of the spleen showed reduced white pulp areas, and red pulp was extended with a mild fibrosis (supplemental Figure 2 top left). In the white pulp, most of the lymphocytes were $CD20^+$, whereas in the red pulp there was an accumulation of $CD3^+$ T cells that were mostly $CD8^+$ and cytotoxic (T-cell intracellular antigen-1^+; data not shown; supplemental Figure 2 bottom). Strikingly, features of hemophagocytosis were observed in the red pulp (supplemental Figure 2 upper right). Lymphocytes were negative for LMP-1 with very rare $EBER^+$ cells, suggesting that the infiltration was not related to EBV infection (data not shown). Altogether, these observations strongly suggest that these lymphoproliferative manifestations can be regarded as incomplete or attenuated forms of HLH.

In addition, 3 XIAP-deficient patients had liver disease (2 patients with cholangitis and 1 patient with chronic liver failure). In 2 of the patients, the cholangitis was associated with colitis, which are known to overlap.[18] For patient PX1.7, histopathologic examination of the liver showed granulomatous hepatitis in lobular areas with foci of macrophages around necrotic hepatocytes (supplemental Figure 3). Staining for LMP-1 was negative (data not shown). It is unclear whether these liver diseases should also be considered as an incomplete form of HLH.

Table 3. Comparison of XLP-1 and XLP-2 phenotypes

	SAP-/Y, n (%)	XIAP-/Y, n (%)	P*
HLH	18 of 33 (55)	22 of 29 (76)	NS
HLH relapses (/HLH-survivors)	2 of 7 (29)	11 of 14 (79)	NS
EBV at first HLH	11 of 12 (92)	15 of 18 (83)	NS
Fatal HLH	11 of 33 (33)	5 of 30 (17)	NS
Fatal HLH (/HLH patients)	11 of 18 (61)	5 of 22 (23)	.0230
Hypogammaglobulinemia	14 of 21 (67)	8 of 24 (33)	.0377
Lymphoma	10 of 33 (30)	0 of 30 (0)	.0010
Cytopenias (in the absence of full-blown HLH)	4 of 33 (12)	11 of 21 (52)	.0020
Splenomegaly (in the absence of full-blown HLH)	2 of 29 (7)	20 of 23 (87)	<.0001
Hemorrhagic colitis	0 of 33 (0)	5 of 30 (17)	.0203

*Calculated with Fisher exact tests.

Lymphoma

Ten of 33 SAP-deficient patients (30%) and none of the 30 XIAP-deficient patients developed lymphoma (Tables 1-3; supplemental Figure 1B; $***P = .001$). Mean age at diagnosis of lymphoma was 15 years (range, 2-40 years). Diagnoses were non-Hodgkin lymphoma (n = 9), including EBV-positive Burkitt lymphoma (n = 6) and EBV-negative (n = 3). Lymphomas were localized in the ileocecal (n = 5), cerebral[19] (n = 1), cervical (n = 2), and spinal (n = 2) regions, and for one the origin was not known. One patient (PS1.3) had a second lymphoma at the age of 30 years, 23 years after the first one, and one patient (PS15.3) had myelodysplasia.

Dysgammaglobulinemia

Hypogammaglobulinemia was documented in 14 SAP-deficient patients (14 of 21, 67%) and in 8 XIAP-deficient patients (8 of 24, 33%) ($*P = .0377$) (Tables 1-3). Thirty percent (10 of 33) of SAP-deficient patients and 13% (4 of 30) of XIAP-deficient patients received intravenous immunoglobulin (IVIG) substitution ($P = .1357$) (supplemental Figure 1C). Interestingly, hypogammaglobulinemia was transient in 2 of the 8 XIAP-deficient patients. PX3.1 was substituted with IVIG between the age of 23 and 35 years, currently, 4 years after stopping IVIG, immunoglobulin levels remain within the normal range, and the patient does not experience recurrent respiratory infections. Two XIAP-deficient patients developed hypergammaglobulinemia, with higher than normal IgA and IgM levels in PX9.3 and elevated IgG and IgM levels in PX11.1, respectively.

Severe infections were noted in several SAP- and XIAP-deficient patients with hypogammaglobulinemia before initiation of the IVIG substitution when treated. Ten of the 14 SAP-deficient and 4 of the 8 XIAP-deficient patients had recurrent respiratory tract infections. Rare severe infections caused by *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Cryptococcus neoformans* were also observed in SAP- and XIAP-deficient patients (supplemental Table 1).

Colitis

Chronic colitis with hemorrhagic diarrheas or rectal bleeding or both evoking inflammatory bowel disease was observed in 5 of 30 XIAP-patients (17%) but in none of 33 SAP-deficient patients ($*P = .0203$; Tables 1-3). In PX1.4, colitis initially responded to immunosuppressive treatment with corticosteroids and cyclosporine A. However, corticosteroids could not be withdrawn, and the

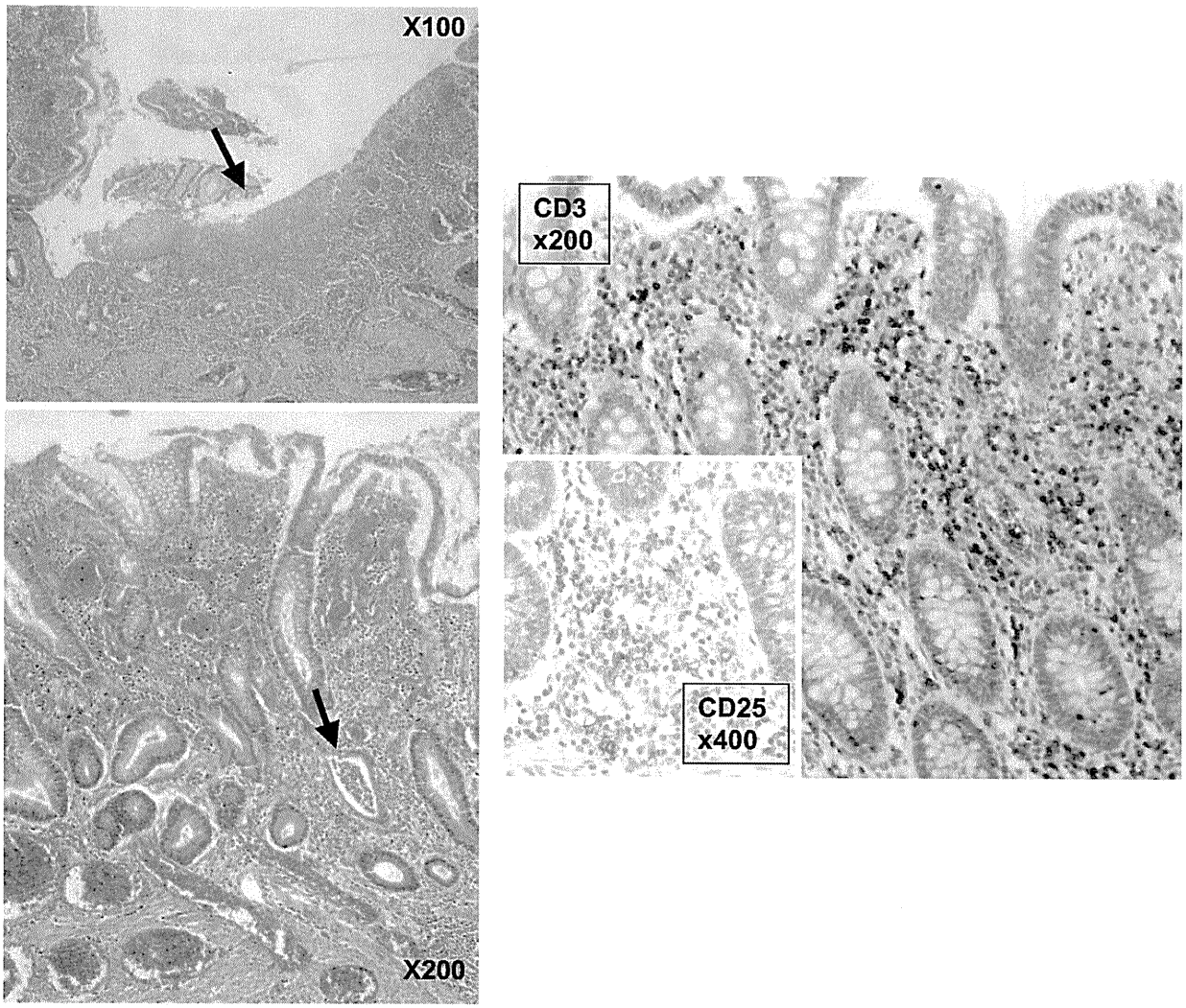

Figure 2. Histology of the large bowel of PX1.7 with XIAP deficiency. (Top left) On hematoxylin and eosin at low magnification (×100), a large ulceration is seen, indicated by an arrow. (Bottom left) Higher magnification (×200) shows a massive polymorphic inflammatory infiltrate associated with a crypt abscess (indicated by the arrow). (Central right) Immunostaining with anti-CD3 shows frequent lymphoid T cells (on the right, ×200), some of them express the activation marker CD25 (×400, inset).

addition of azathioprine could not prevent the recurrence of symptoms. Anti–tumor necrosis factor-α mAb treatment (infliximab) provided partial improvement. Recently, a colectomy was performed, but the patient now has terminal ileitis. In PX1.7, severe hemorrhagic colitis was associated with portal hypertension and massive gastroduodenal bleeding that lead to death of this patient. Patients PX9.6 and PX11.2 also suffered from chronic colitis and most probably died of intestinal hemorrhage.

Histopathologic examination of intestinal mucosa biopsy specimens was performed in 3 patients, PX1.4, PX1.7, and PX9.3. Representative images are shown in Figure 2. Hemorrhagic ulcerations of the colon associated with mononuclear infiltration consisting of lymphoid cells and plasma cells in the lamina propria were observed (Figure 2 left top). Crypt architecture was mostly preserved, except for rare crypt abscesses (Figure 2, left bottom), but frequent apoptotic crypt cells were seen (supplemental Figure 4). The lymphoid cells were mostly $CD3^+$ and $CD8^+$ with some lymphocytes expressing CD25 with numerous eosinophils (in PX1.4) (Figure 2; supplemental Figure 4). $CD20^+$ cells were rare, EBER staining was negative (not shown), and there was no granuloma formation. Microbiologic cultures were negative in all 3 cases.

Rare clinical manifestations

Rare clinical features (supplemental Table 1), each observed in 1 SAP-deficient patient, were hemolytic uremic syndrome associated with HLH, vasculitis, and arthritis. Clinical features, each observed in 1 XIAP-deficient patient, were Kawasaki syndrome and psoriasis. Additional infections in patients without hypogammaglobulinemia were caused by *Pseudomonas aeruginosa* (1 SAP-deficient patient), recurrent measles (1 XIAP-deficient patient), and HSV-(1 XIAP-deficient patient). Of note, 2 SAP-deficient patients (PS3.1 and PS3.2) had chronic gastritis.[20]

Survival and outcome

Sixteen of 33 SAP-deficient patients and 12 of 30 XIAP-deficient patients died at a mean age of 11 years (range, 2-69 years) and 16 years (range, 0.1-52 years), respectively. Survival rates did not differ between both patient groups ($P = .93$; Figure 1B), and the proportions of whom reached adulthood (age ≥ 16 years) were similar in both groups (17 of 33 SAP-deficient patients [52%] and

13 of 30 XIAP-deficient patients [43%]). Mortality was related to HLH (11 SAP- and 5 XIAP-deficient patients), lymphoma (2 SAP-deficient patients), myelodysplasia (1 SAP-deficient patient), colitis (3 XIAP-deficient patients), hepatitis (1 XIAP-deficient patient), complications of hematopoietic stem cell transplantation (2 SAP- and 4 XIAP-deficient patients), and pneumonia (1 XIAP-deficient patient). Mean age at last follow-up was 24.9 years (range, 10-66 years) for SAP-deficient patients and 17.5 years (range, 0.7- 39 years) for XIAP-deficient patients. Among the surviving 17 SAP-deficient patients, 4 are well without any treatment, 10 receive IVIG substitution, 2 are currently treated for a lymphoma, and 1 had successful hematopoietic stem cell transplantation. Among the surviving 17 XIAP-deficient patients, 10 are well without any treatment (among them 3 with splenomegaly), 2 received recently anti-CD20 antibody treatment because of EBV-related HLH, 2 are under IVIG substitution, 1 has terminal ileitis after colectomy, 1 has colitis treated with mesalazine and azathioprine, and 1 has recurrent HLH treated with cyclosporine A and dexamethasone. One XIAP-deficient and 2 SAP-deficient patients have never developed clinical signs and are considered to be asymptomatic.

Discussion

We report the first comparison of the clinical phenotypes of SAP- and XIAP-deficient patients. The present study was based on a retrospective analysis with data from medical records on 33 SAP- and 30 XIAP-deficient patients. The relatively small size of both cohorts obviously implies that data should be interpreted with caution.

The overall clinical phenotypes of the affected persons matched with the phenotypes previously reported.[2,7,9,21] In accordance to previous studies, we did not observe any genotype-phenotype correlation in the SAP-deficient patients. However, in our cohort of XIAP-deficient patients, we noticed that XIAP-deficient patients carrying non-null mutations had a tendency to be less prone to develop HLH by contrast to patients with null mutations. However, other genetic or environmental factors may contribute to the variety of phenotypes observed in XLP-1 and XLP-2.

HLH occurred both in SAP- and in XIAP-deficient patients but with more frequent neurologic involvement and fatal outcome in SAP-deficient patients than in XIAP-deficient patients. Splenomegaly often associated with cytopenia and fever was more frequent in XIAP-deficient patients than in SAP-deficient patients. Histologic analysis of one spleen showed accumulation of activated $CD8^+$ T cells and hemophagocytosis without EBV^+ cells. These symptoms probably represent incomplete forms of HLH. In addition, HLH relapses seemed to be more common in XIAP- than in SAP-deficient patients who survived HLH. Together, these findings suggest that HLH has a less severe disease course in XIAP- deficient patients than in SAP-deficient patients.

In most of the patients from both groups, the trigger of HLH was an EBV infection (> 80%); EBV may favor HLH by eliciting a potent CD8 T-cell response. It is also postulated that SAP and possibly XIAP are associated with activation pathways that are more important in triggering selective cytotoxicity toward B cells.[22-27] HLH in most hereditary conditions such as FHL, Griscelli syndrome type II, and Chediak-Higashi syndrome shares common pathophysiologic mechanisms, that is, global impaired cytotoxicity responses that lead to the inability of effector lymphocytes to kill infected cells and antigen-presenting cells.[28] In mice and humans, SAP-deficient $CD8^+$ T and NK cells exhibit defective cytotoxicity responses caused by abnormal functions of SLAM receptors.[29] This could explain the occurrence of HLH in SAP-deficient patients.[22-27] In contrast, NK-cell and T-cell cytotoxic responses appear to be preserved in XIAP-deficient patients[7,9] (C. Synaeve and S.L., unpublished data, 2009 and 2010). This might account for the lower severity of the HLH in the XIAP deficiency. Hence, the precise immune defects responsible for HLH in XIAP deficiency remain to be elucidated.

Only XIAP-deficient patients were at risk for chronic colitis with often a lethal outcome. This phenotype seems that is may be even worse than HLH, because the mortality in the group of patients with colitis (3 of 5) has a tendency to be higher than in the group with HLH (5 of 22). Histopathologic analysis of intestinal mucosal biopsy specimens showed an inflammatory process with an accumulation of activated T cells (and eosinophils in one patient) that could evoke inflammatory bowel disease. Interestingly, a recent report indicates that XIAP is involved in nucleotide-binding oligomerization domain containing 2 (NOD2) activation which is an intracellular pattern recognition receptor of the NOD-like receptor family.[30] Importantly, *NOD2* is a key susceptibility gene for Crohn disease.[31] Thus, defects in XIAP might lead to defective NOD2 responses as an additive risk factor for colitis in some of these patients. Of note, however, *NOD2* was sequenced in 2 XIAP-deficient patients with colitis, and none had the genotype shown to be a risk factor for Crohn disease (J.P. Hugot and S.L., unpublished data, June 2006).

One striking difference between XLP-1 and XLP-2 was that only SAP-deficient patients developed lymphoma, although it could not be formally excluded that XIAP-patients might develop lymphomas in the future. In SAP-deficiency, the occurrence of lymphomas may be explained by defective immunosurveillance of hematopoietic cells, resulting from alterations in SLAM receptor-mediated NK- and T-cell cytotoxicity responses,[22-24,26] but also by the proapoptotic functions that have been assigned to SAP.[32,33]

Another common finding shared by XLP-1 and XLP-2 is the hypogammaglobulinemia. Interestingly, 2 XLP-2 patients recovered from hypogammaglobulinemia, which so far seems not to be the case for XLP-1 patients. Numerous studies in mice and humans have documented that impaired antibody production found in XLP-1 resulted from a block in germinal center formation, leading to defects in the differentiation of Ig-isotype–switched memory B cells.[34-36] In most of the XIAP-deficient patients, Ig-isotype–switched memory B cells are not found to be decreased[7] (S. Siberil and S.L., unpublished data, 2008 and 2009). In XIAP deficiency, hypogammaglobunemia could be the consequence of increased activation-induced cell death of B cells, a hypothesis that needs to be tested.

In conclusion, the present comparison of the clinical features of SAP- and XIAP-deficient patients shows that SAP deficiency and XIAP deficiency share a main phenotype, that is, EBV-induced HLH. This similarity raises the possibility of a functional/molecular link between SAP and XIAP proteins. Alternatively, impairment of 2 independent pathways, both important in EBV immunity, could lead to a shared phenotype. Nevertheless, we also demonstrate that XLP-1 and XLP-2 can be distinguished on several clinical aspects, which could be helpful for diagnosis and therapeutic decisions.

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Authorship

Contribution: J.P.S. collected and analyzed the data and participated in study design, writing of the report, and patients' care; D.C. performed the immunohistochemistry experiments and analyzed histopathologic findings; F.H. participated in histopathologic analysis and writing of the report; C.L., N.L., and S.R. realized gene sequencing and protein expression tests; G.S.B. participated in data analysis; A.F. contributed to study design, data analysis, writing of the report, and patients' care; S.L. coordinated the study collected the data and contributed to sequencing, expression tests, data analysis, and wrote the report. The other authors provided and collected the clinical data on patients' status and contributed to the data analysis and patients' care.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References

1. Purtilo DT, Cassel C, Yang JP. Letter: fatal infectious mononucleosis in familial lymphohistiocytosis. *N Engl J Med.* 1974;291(14):736.
2. Purtilo DT, Cassel CK, Yang JP, Harper R. X-linked recessive progressive combined variable immunodeficiency (Duncan's disease). *Lancet.* 1975;1(7913):935-940.
3. Seemayer TA, Gross TG, Egeler RM, et al. X-linked lymphoproliferative disease: twenty-five years after the discovery. *Pediatr Res.* 1995;38(4):471-478.
4. Henter JI, Horne A, Arico M, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer.* 2007;48(2):124-131.
5. Sayos J, Wu C, Morra M, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature.* 1998;395(6701):462-469.
6. Coffey AJ, Brooksbank RA, Brandau O, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet.* 1998; 20(2):129-135.
7. Rigaud S, Fondaneche MC, Lambert N, et al. XIAP deficiency in humans causes an X-linked lymphoproliferative syndrome. *Nature.* 2006;444 (7115):110-114.
8. Zhao M, Kanegane H, Ouchi K, Imamura T, Latour S, Miyawaki T. A novel XIAP mutation in a Japanese boy with recurrent pancytopenia and splenomegaly. *Haematologica.* 2009;95(4):688-689.
9. Marsh RA, Madden L, Kitchen BJ, et al. XIAP deficiency: a unique primary immunodeficiency best classified as X-linked familial hemophagocytic lymphohistiocytosis and not as X-linked lymphoproliferative disease. *Blood.* 2010;7(116):1079-1082.
10. Gaspar HB, Sharifi R, Gilmour KC, Thrasher AJ. X-linked lymphoproliferative disease: clinical, diagnostic and molecular perspective. *Br J Haematol.* 2002;119(3):585-595.
11. Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol Rev.* 2005;203:180-199.
12. Grierson HL, Skare J, Hawk J, Pauza M, Purtilo DT. Immunoglobulin class and subclass deficiencies prior to Epstein-Barr virus infection in males with X-linked lymphoproliferative disease. *Am J Med Genet.* 1991;40(3):294-297.
13. Dutz JP, Benoit L, Wang X, et al. Lymphocytic vasculitis in X-linked lymphoproliferative disease. *Blood.* 2001;97(1):95-100.
14. Ma CS, Nichols KE, Tangye SG. Regulation of cellular and humoral immune responses by the SLAM and SAP families of molecules. *Annu Rev Immunol.* 2007;25:337-379.
15. Eckelman BP, Salvesen GS, Scott FL. Human inhibitor of apoptosis proteins: why XIAP is the black sheep of the family. *EMBO Rep.* 2006;7(10): 988-994.
16. Galban S, Duckett CS. XIAP as a ubiquitin ligase in cellular signaling. *Cell Death Differ.* 2009;17(1): 54-60.
17. Verhelst H, Van Coster R, Bockaert N, et al. Limbic encephalitis as presentation of a SAP deficiency. *Neurology.* 2007;69(2):218-219.
18. Knight C, Murray KF. Hepatobiliary associations with inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol.* 2009;3(6):681-691.
19. Hervier B, Latour S, Loussouarn D, et al. An atypical case of X-linked lymphoproliferative disease revealed as a late cerebral lymphoma. *J Neuroimmunol.* 2010;218(1-2):125-128.
20. Rougemont AL, Fournet JC, Martin SR, et al. Chronic active gastritis in X-linked lymphoproliferative disease. *Am J Surg Pathol.* 2008;32(2): 323-328.
21. Sumegi J, Huang D, Lanyi A, et al. Correlation of mutations of the SH2D1A gene and Epstein-Barr virus infection with clinical phenotype and outcome in X-linked lymphoproliferative disease. *Blood.* 2000;96(9):3118-3125.
22. Dong Z, Cruz-Munoz ME, Zhong MC, Chen R, Latour S, Veillette A. Essential function for SAP family adaptors in the surveillance of hematopoietic cells by natural killer cells. *Nat Immunol.* 2009;10(9):973-980.
23. Bloch-Queyrat C, Fondaneche MC, Chen R, et al. Regulation of natural cytotoxicity by the adaptor SAP and the Src-related kinase Fyn. *J Exp Med.* 2005;202(1):181-192.
24. Parolini S, Bottino C, Falco M, et al. X-linked lymphoproliferative disease. 2B4 molecules displaying inhibitory rather than activating function are responsible for the inability of natural killer cells to kill Epstein-Barr virus-infected cells. *J Exp Med.* 2000;192(3):337-346.
25. Dupre L, Andolfi G, Tangye SG, et al. SAP controls the cytolytic activity of CD8+ T cells against EBV-infected cells. *Blood.* 2005;105(11):4383-4389.
26. Sharifi R, Sinclair JC, Gilmour KC, et al. SAP mediates specific cytotoxic T-cell functions in X-linked lymphoproliferative disease. *Blood.* 2004;103(10):3821-3827.
27. Hislop AD, Palendira U, Leese AM, et al. Impaired Epstein-Barr virus-specific CD8+ T cell function in X-linked lymphoproliferative disease is restricted to SLAM family positive B cell targets. *Blood. Blood.* 2010;116(17):3249-3257.
28. Fischer A, Latour S, de Saint Basile G. Genetic defects affecting lymphocyte cytotoxicity. *Curr Opin Immunol.* 2007;19(3):348-353.
29. Veillette A, Dong Z, Perez-Quintero LA, Zhong MC, Cruz-Munoz ME. Importance and mechanism of 'switch' function of SAP family adapters. *Immunol Rev.* 2009;232(1):229-239.
30. Krieg A, Correa RG, Garrison JB, et al. XIAP mediates NOD signaling via interaction with RIP2. *Proc Natl Acad Sci U S A.* 2009;106(34):14524-14529.
31. Hugot JP, Chamaillard M, Zouali H, et al. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature.* 2001; 411(6837):599-603.
32. Snow AL, Marsh RA, Krummey SM, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest.* 2009;119(10):2976-2989.
33. Chen G, Tai AK, Lin M, Chang F, Terhorst C, Huber BT. Signaling lymphocyte activation molecule-associated protein is a negative regulator of the CD8 T cell response in mice. *J Immunol.* 2005;175(4):2212-2218.
34. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R. SAP is required for generating long-term humoral immunity. *Nature.* 2003;421(6920):282-287.
35. Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature.* 2008; 455(7214):764-769.
36. Ma CS, Hare NJ, Nichols KE, et al. Impaired humoral immunity in X-linked lymphoproliferative disease is associated with defective IL-10 production by CD4+ T cells. *J Clin Invest.* 2005; 115(4):1049-1059.

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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

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.

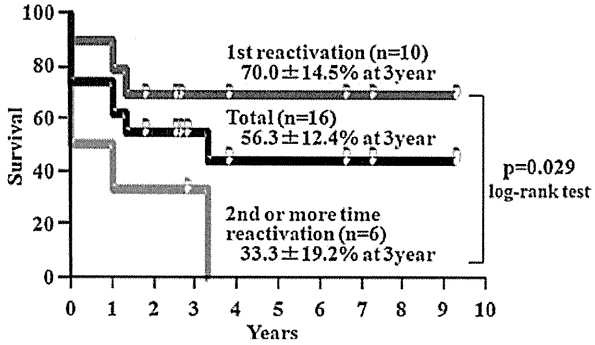

Fig. 1. Progression-free survival (PFS) post-PAM therapy. The overall PFS at 3 years was 56.3 ± 12.4%. PFS was significantly higher in children with a first reactivation compared with children experiencing a second or subsequent reactivation (70.0 ± 14.5% vs. 33.3 ± 19.2% at 3 years, $P = 0.029$).

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