

EB ウイルス DNA PCR 陰性

NK 活性 18%

《生化学》

TP	6.1	g/dl
Alb	3.1	g/dl
AST	261	IU/l
ALT	137	IU/l
LDH	875	IU/l
T-bil	1.07	mg/dl
D-bil	0.36	mg/dl
BUN	6.3	mg/dl
Cre	0.5	mg/dl
Na	135	mEq/l
IgG	1360	mEq/l
IgA	202	mEq/l
IgM	167	mEq/l
Ferritin	10610	ng/ml
ハプトグロビン	<20	ng/ml

PT-INR 1.11

APTT 39.8 sec

FDP 4.7 μ g/ml

D-dimer 1.1 μ g/ml

《その他》

直接 Coombs (+)

間接 Coombs (+)

抗血 HLA 抗体 (+)

s-IL2R 3000 U/dl

B2-MG 3.5 mg/l

C3 92 mg/dl

C4 21 mg/dl

血清補体価 23.3 CH50/ml

抗核抗体、LE テスト、抗 DNA 抗体、

抗 SM 抗体、抗 SS-A 抗体 陰性

RF 26 U/ml、RA 1(+)

骨髓穿刺：NCC 10.7 万で血球貪食像を少数認めた。

<臨床経過>

ステロイド投与により、フェリチンは低下傾向となり、血小板低下は改善し、外来経過観察となったが、その後も発熱を契機に、血小板低下と貧血、心嚢液貯留を認めしたが、増悪と軽快を繰り返している。現在は溶血性貧血と血小板低下、心嚢液貯留を認めている。

(倫理面への配慮)

遺伝子解析にあたっては、三省合同のゲノム指針に則り、患者又は両親から同意を得、当センターの倫理委員会の承認を得て行っ

た。

C. 研究結果

- 遺伝子解析：*AIRE*、*CARD9*、*CLEC7A*、*IL2RA*、*FAS*、*NRAS*、*KRAS* 遺伝子の解析で変異は認められなかった。
- APECED で検出される *IL17A*、*IL-17F*、*IL-22*、*IL-23*、*IFN-alpha* に対する自己抗体は認めなかった。
- 慢性非悪性リンパ増殖症はなかった。
- TCR- α/β +CD4-CD8-T 細胞の増加はなく、*in vitro* での Fas を介したアポトーシスの欠如は認めなかった。

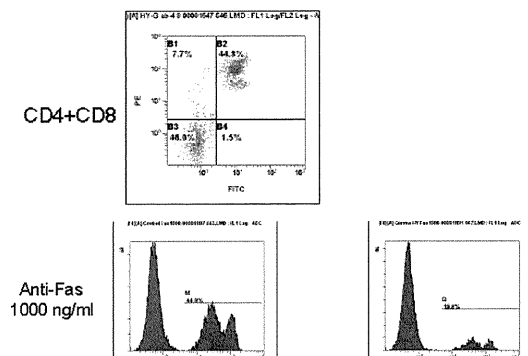
D. 考察

溶血性貧血、免疫性血小板減少症、心嚢液貯留、肝腫大、低身長、ばち指等がみられ、慢性皮膚粘膜カンジダ症の臨床診断であったが、種々の遺伝子検査の結果等からは ALPS の診断に一致しなかった。今後、経過観察を行いながら、FAS、RAS 以外の関連遺伝子を含む網羅的遺伝子解析を行う予定である。

E. 結論

皮膚粘膜カンジダ症候群の経過中に Evans 症候群と心嚢液貯留を認めた一例を経験し、種々の検査を行ったが ALPS ではなかった。今後網羅的遺伝子解析等の検討を行う予定である。

図. フローサイトメトリーによる検索



F. 健康危惧情報
特になし。

G. 研究発表

1. 論文発表

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- H. 知的所有権の取得状況
(予定を含む)
1. 特許取得
なし
 2. 実用新案登録

- なし
- 3. その他
- なし

IV. 研究成果に関する刊行の一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
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	management and outcome of the disease.				
Shiba N, Hasegawa D, Park MJ, Murata C, Matsubara A, Ogawa C, Manabe A, Arakawa H, Ogawa S, Hayashi Y.	CBL mutation in chronic myelomonocytic leukemia secondary to familial platelet disorder with propensity to develop acute myeloid leukemia.	Blood.	119 (11)	2612-4	2012 Mar. 15
Shiba N, Park MJ, Taki T, Takita J, Hiwatari M, Kanazawa T, Sotomatsu M, Ishii E, Arakawa H, Ogawa S, Hayashi Y.	CBL mutations in infant acute lymphoblastic leukaemia.	Br J Haematol.	156 (5)	672-4	2012 Mar.

V. 別刷

Brief report

Autoimmune lymphoproliferative syndrome–like disease with somatic *KRAS* mutation

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Autoimmune lymphoproliferative syndrome (ALPS) is classically defined as a disease with defective FAS-mediated apoptosis (type I-III). Germline *NRAS* mutation was recently identified in type IV ALPS. We report 2 cases with ALPS-like disease with somatic *KRAS* mutation. Both cases were characterized by prominent autoimmune cytopenia and lymphadenopathy/splenomegaly. These patients did not satisfy the diagnostic criteria for ALPS or juvenile myelomonocytic leukemia and are probably defined as a new disease entity of RAS-associated ALPS-like disease (RALD). (*Blood*. 2011;117(10):2887-2890)

Introduction

Autoimmune lymphoproliferative syndrome (ALPS) is a disease characterized by dysfunction of the FAS-mediated apoptotic pathway,^{1,2} currently categorized as: type Ia, germline *TNFRSF6/FAS* mutation; type Ib, germline *FAS ligand* mutation; type Is, somatic *TNFRSF6/FAS* mutation; and type II, germline *Caspase 10* mutation. Patients exhibit lymphadenopathy, hepatosplenomegaly, and autoimmune diseases, such as immune cytopenia and hyper- γ -globulinemia. An additional subclassification has been proposed that includes types III and IV, whereby type III has been defined as that with no known mutation but with a defect in FAS-mediated apoptosis and type IV as one showing germline *NRAS* mutation.³ Type IV is considered exceptional because the FAS-dependent apoptosis pathway is not involved in the pathogenesis, and this subclass is characterized by a resistance to interleukin-2 (IL-2) depletion-dependent apoptosis. Recent updated criteria and classification of ALPS suggested type IV ALPS as a RAS-associated leukoproliferative disease.⁴

Juvenile myelomonocytic leukemia (JMML) is a chronic leukemia in children. Patients show lymphadenopathy, hepatosplenomegaly, leukocytosis associated with monocytosis, anemia, thrombocytopenia, and occasional autoimmune phenotypes. Approximately 80% of patients with JMML have been shown to have a genetic abnormality in their leukemia cells, including mutations of *NF1*, *RAS* family,⁵ *CBL*, or *PTPN11*. The hallmarks of the laboratory findings of JMML include spontaneous colony formation in bone marrow (BM) or peripheral blood mononuclear cells (MNCs) and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) of CD34⁺ BM-MNCs.⁶

Germline RAS pathway mutations cause Costello (*HRAS*), Noonan (*PTPN11*, *KRAS*, and *SOS1*), and cardio-facio-cutaneous syndromes (*KRAS*, *BRAF*, *MEK1*, and *MEK2*). Patients with Costello and Noonan syndromes have an increased propensity to develop solid and hematopoietic tumors, respectively⁷; among these tumors, the incidence of JMML in patients with germline mutation of *NF1* or *PTPN11* is well known.

We present 2 cases with autoimmune cytopenia and remarkable lymphadenopathy and hepatosplenomegaly, both of which were identified as having a somatic *KRAS* G13D mutation without any clinical features of germline *RAS* mutation, such as cardio-facio-cutaneous or Noonan syndrome.

Methods

All studies were approved by the ethical board of Tokyo Medical and Dental University.

Case 1

A 9-month-old boy had enormous bilateral cervical lymphadenopathy and hepatosplenomegaly (supplemental Figure 1A-B, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Blood test revealed the presence of hemolytic anemia and autoimmune thrombocytopenia. Hyper- γ -globulinemia with various autoantibodies was also noted. ALPS and JMML were nominated as the diseases to be differentially diagnosed. Detailed clinical history and laboratory data are provided as Supplemental data. The patient did not satisfy the criteria for the diagnosis of ALPS or JMML as discussed in "Results and discussion."

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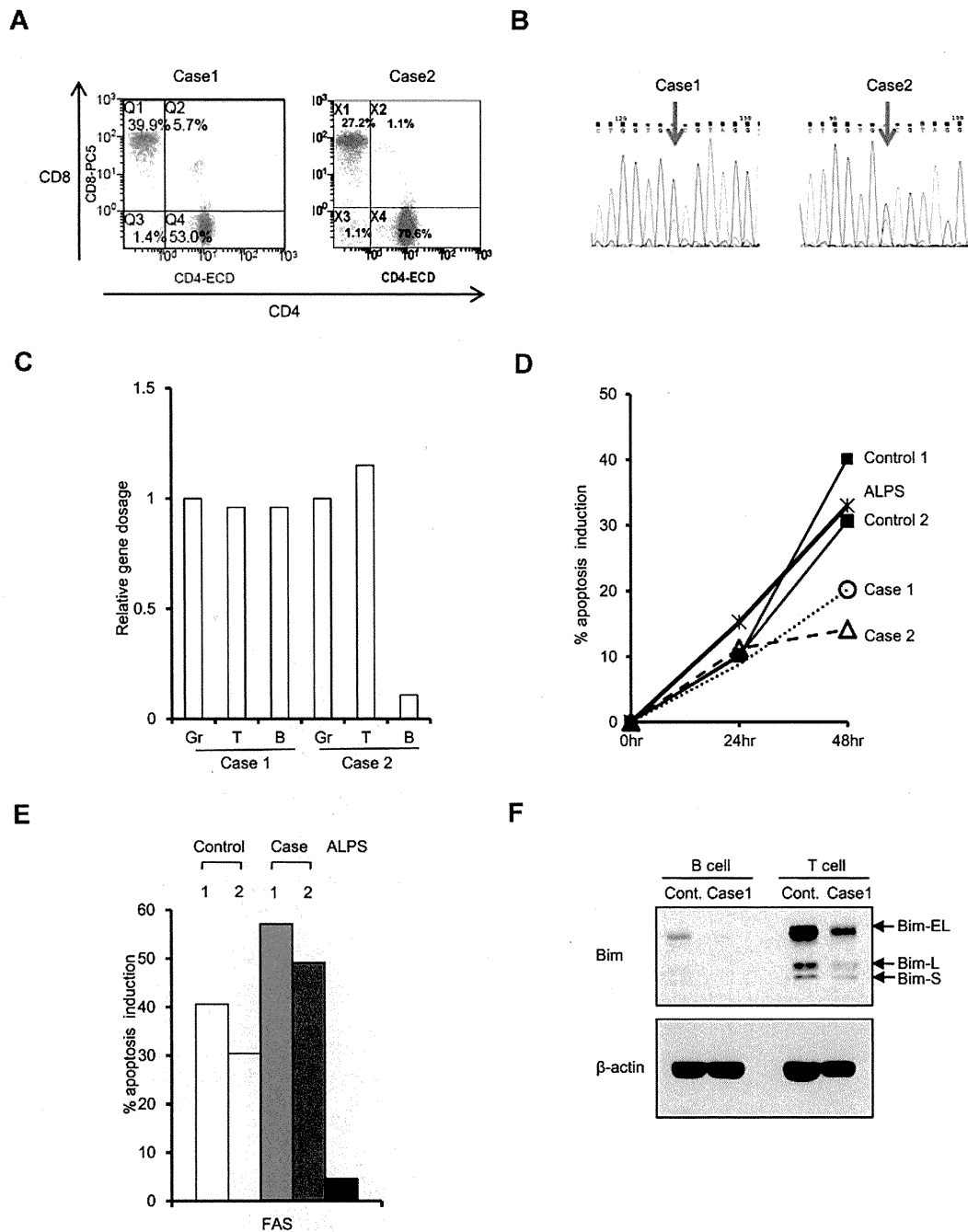


Figure 1. Molecular cell biologic assay of RALD. (A) Flow cytometric analysis of double-negative T cells. CD8 and CD4 double staining was performed in T-cell receptor- $\alpha\beta$ -expressing cells. (B) Electropherogram showing KRAS G13D mutation in BM-MNCs in case 1 (left panel) and case 2 (right panel). (C) Gene dosage of mutated allele in granulocytes (Gr), T cells (T), and B cells (B). Relative gene dosage was estimated by a mutant allele-specific polymerase chain reaction method in cases 1 and 2 using albumin gene as internal control. (D) Apoptosis assay using activated T cells. Apoptosis percentage was measured by flow cytometry with annexin V staining 24 and 48 hours after IL-2 depletion. (E) Apoptosis percentage was measured 24 hours after addition of anti-FAS CH11 antibody (final 100 ng/mL). (F) Western blotting analysis of Bim expression.

Case 2

A 5-month-old girl had a fever and massive hepatosplenomegaly (supplemental Figure 1D). She was initially diagnosed with Evans syndrome based on the presence of hemolytic anemia and autoimmune thrombocytopenia with hyper- γ -globulinemia and autoantibodies. Spontaneous colony formation assay and GM-CSF hypersensitivity of BM-MNCs showed positivity. Then, tentative diagnosis of JMML was given, even though she showed no massive monocytosis or increased fetal hemoglobin. Detailed clinical history and laboratory data are provided in supplemental data.

Detailed methods for experiments are described in supplemental data.

Results and discussion

Case 1 showed a high likelihood of being a case of ALPS according to the symptoms and clinical data presented (supplemental Table 1), except for number of double-negative T cells, which was only 1.4% of T-cell receptor- $\alpha\beta$ cells (Figure 1A). JMML was also nominated as a disease to be differentiated because remarkable hepatosplenomegaly with thrombocytopenia and moderate monocytosis was

noted. However, no hypersensitivity to GM-CSF as determined by colony formation assay for BM-MNCs (data not shown) or phosphor-STAT5 staining (data not shown) was observed. DNA sequence for JMML-associated genes, such as *NRAS*, *KRAS*, *HRAS*, *PTPN11*, and *CBL*, was determined, and *KRAS* G13D mutation was identified (Figure 1B). The mutation was seen exclusively in the hematopoietic cell lineage, and no mutation was seen in the oral mucosa or nail-derived DNA. Granulocytes, monocytes, T cells, and B cells were all positive for *KRAS* G13D mutation (data not shown). The proportion of mutated cells in each hematopoietic lineage was quantitated by mutation allele-specific quantitative polymerase chain reaction methods, which revealed that mutated allele was almost equally present in granulocytes, T cells, and B cells (Figure 1C). CD34⁺ hematopoietic stem cells (HSCs) were also positive for *KRAS* G13D mutation, and 60% of colony-forming units-granulocyte macrophage (CFU-GM) developed from isolated CD34 cells carried the *KRAS* G13D mutation (data not shown). These observations suggest that the mutation occurred at the HSCs level, and HSC consists of wild-type and mutant HSCs.

NRAS-mutated type IV ALPS was previously characterized by apoptosis resistance of T cells in IL-2 depletion.³ Then, activated T cells were subjected to an apoptosis assay by FAS stimulation or IL-2 depletion. Remarkable resistance to IL-2 depletion, but not to FAS-dependent apoptosis (Figure 1D-E), was seen. This was in contrast to T cells from FAS-mutated ALPS type 1a, which showed remarkable resistance to FAS-dependent apoptosis and normal apoptosis induction by IL-2 withdrawal (Figure 1D-E). Western blotting analysis of activated T cells or Epstein-Barr virus-transformed B cells showed reduced expression of Bim (Figure 1F).

In case 2, autoimmune phenotype and hepatosplenomegaly were remarkable, as shown in Supplemental data. The patient was initially diagnosed as Evans syndrome based on the presence of hemolytic anemia and autoimmune thrombocytopenia. Double-negative T cells were 1.1% of T-cell receptor- $\alpha\beta$ cells in the peripheral blood, which did not meet with the criteria of ALPS. Although spontaneous colony formation was shown in peripheral blood- and BM-MNCs, and GM-CSF hypersensitivity was demonstrated in BM-MNCs derived CD34⁺ cell (supplemental Table 2), she showed no massive monocytosis or increased fetal hemoglobin. Thus, the diagnosis was less likely to be ALPS or JMML. DNA sequencing of JMML-related genes, such as *NRAS*, *KRAS*, *HRAS*, *PTPN11*, and *CBL*, identified somatic, but not germline, *KRAS* G13D mutation (Figure 1B). *KRAS* G13D mutation was detected in granulocytes and T cells. Mutation was not identified in B cells by conventional DNA sequencing (data not shown). Mutant allele-specific quantitative polymerase chain reaction revealed that mutated allele was almost equally present in granulocytes and T cells, but barely in B cells (Figure 1C). Activated T cells showed resistance to IL-2 depletion but not to FAS-dependent apoptosis (Figure 1D-E).

Both of our cases were characterized by strong autoimmunity, immune cytopenia, and lymphadenopathy or hepatosplenomegaly with partial similarity with ALPS or JMML. However, they did not meet with the well-defined diagnostic criteria of ALPS² or JMML.⁶ It is interesting that case 2 presented GM-CSF hypersensitivity, which is one of the hallmarks of JMML. Given the strict clinical and laboratory criteria of JMML and ALPS, our 2 cases should be defined as a new disease entity, such as RAS-associated ALPS-like disease (RALD). Recently

defined *NRAS*-mutated ALPS type IV may also be included in a similar disease entity.

There are several cases of JMML reported simultaneously having clinical and laboratory findings compatible with autoimmune disease.^{8,9} Autoimmune syndromes are occasionally seen in patients with myelodysplastic syndromes, including chronic myelomonocytic leukemia.¹⁰ These previous findings may suggest a close relationship of autoimmune disease and JMML. Because *KRAS* G13D has been identified in JMML,¹¹⁻¹³ it is tempting to speculate that *KRAS* G13D mutation is involved in JMML as well as RALD. In JMML, erythroid cells reportedly carry mutant RAS, whereas B- and T-cell involvement was variable.¹³ In both of our cases, myeloid cells and T cells carried mutant RAS, whereas B cells were affected variably. These findings would support a hypothesis that the clinical and hematologic features are related to the differentiation stages of HSCs where RAS mutation is acquired. JMML-like myelomonocytic proliferation may predict an involvement of RAS mutation in myeloid stem/precursor cell level, whereas ALPS-like phenotype may predict that of stem/precursor cells of lymphoid lineage, especially of T cells. Under the light of subtle differences between the 2 cases presented, their hematologic and clinical features may reflect the characteristics of the stem cell level where *KRAS* mutation is acquired. Involvement of the precursors with higher propensity toward lymphoid lineage may lead to autoimmune phenotypes, whereas involvement of those with propensity toward the myeloid lineage may lead to GM-CSF hypersensitivity while still sharing some overlapping autoimmune characteristics.

One may argue from the other viewpoints with regard to the clinicopathologic features of these disorders. First, transformation in fetal HSCs might be obligatory for the development of JMML¹⁴ and, in HSCs later in life, may not have the same consequences. Second, certain *KRAS* mutations may be more potent than others. Codon 13 mutations are generally less deleterious biochemically than codon 12 substitutions, and patients with JMML with codon 13 mutations have been reported to show spontaneous hematologic improvement.^{12,15} Thus, further studies are needed to reveal in-depth clinicopathologic characteristics in this type of lympho-myeloproliferative disorder.

KRAS mutation may initiate the oncogenic pathway as one of the first genetic hits but is insufficient to cause frank malignancy by itself.^{16,17} Considering recent findings that additional mutations of the genes involved in DNA repair, cell cycle arrest, and apoptosis are required for full malignant transformation, one can argue that RALD patients will also develop malignancies during the course of the disease. Occasional association of myeloid blast crisis in JMML and that of lymphoid malignancies in ALPS will support this notion. Thus, the 2 patients are now being followed up carefully. It was recently revealed that half of the patients diagnosed with Evans syndrome, an autoimmune disease presenting with hemolytic anemia and thrombocytopenia, met the criteria for ALPS diagnosis.^{18,19} In this study, FAS-mediated apoptosis analysis was used for the screening. Considering the cases we presented, it will be intriguing to reevaluate Evans syndrome by IL-2 depletion-dependent apoptosis assay focusing on the overlapping autoimmunity with RALD.

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Authorship

Contribution: Masatoshi Takagi and S.M. designed entire experiments and wrote the manuscript; K.S., N.M., and Mari Takagi treated patients and designed clinical laboratory test; J.P. performed experiments described in Figure 1B-F; K.M., H.M., and S.D. performed colony and mutational analysis; and M.N., T.M., K.K.,

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Nationwide Survey of Patients with Primary Immunodeficiency Diseases in Japan

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Abstract To determine the prevalence and clinical characteristics of patients with in Japan, we conducted a nationwide survey of primary immunodeficiency disease (PID) patients for the first time in 30 years. Questionnaires were sent to 1,224 pediatric departments and 1,670 internal medicine departments of Japanese hospitals. A total of 1,240 patients were registered. The estimated number of patients with PID was 2,900 with a prevalence of 2.3 per 100,000 people and homogenous regional distribution in Japan. The male-to-female ratio was 2.3:1 with a median age of 12.8 years. Adolescents or adults constituted 42.8% of the patients. A number of 25 (2.7%) and 78 (8.5%) patients developed malignant disorders and immune-related diseases, respectively, as complications of primary immunodeficiency disease. Close monitoring and appropriate management for these complications in addition to prevention of infectious diseases is important for improving the quality of life of PID patients.

Keywords Primary immunodeficiency disease · epidemiology · nationwide survey · Japan

Abbreviations

APECED	Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy
BTK	Bruton's tyrosine kinase
CGD	Chronic granulomatous disease
CID	Combined T and B cell immunodeficiency
CVID	Common variable immunodeficiency disease
FMF	Familial Mediterranean fever
IPEX	Immune dysregulation polyendocrinopathy enteropathy X-linked
NEMO	Nuclear factor kappa B essential modulator
PID	Primary immunodeficiency disease
SIgAD	Selective IgA deficiency
SLE	Systemic lupus erythematosus

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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

^aDue 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

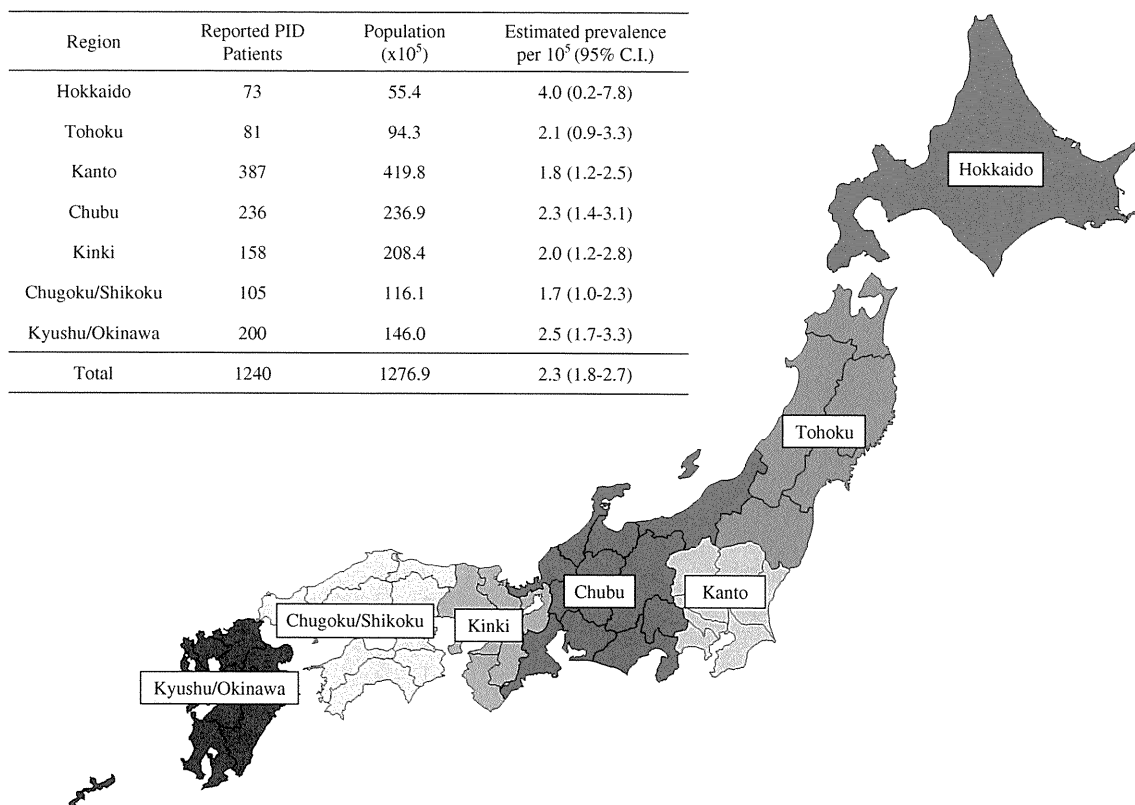


Fig. 1 Regional distribution of PID patients. *CI* Confidence interval

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%)
γ c 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

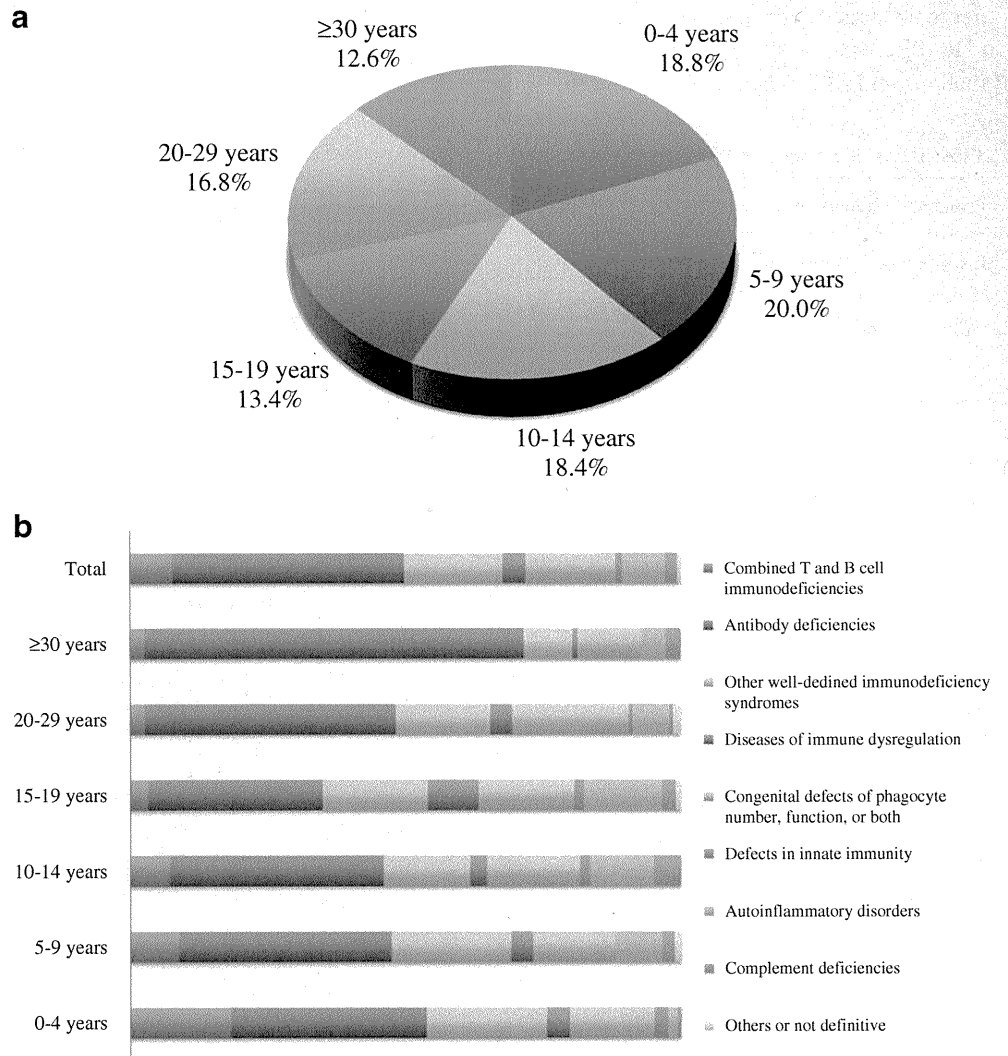
APECED Autoimmune polyendocrinopathy with candidiasis and ectodermal dystrophy, *IPEX* immune dysregulation, polyendocrinopathy, enteropathy, X-linked

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 have 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	<i>n</i>	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

^aThe number of patients

Table IV Immune-related diseases in PID patients*(a) Immune-related diseases with each PID*

Primary immunodeficiency	Total	<i>n</i>	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	<i>n</i>
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