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Analysis of mutations and recombination activity in RAG-deficient patients

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Abstract Mutations in the recombination activating genes (*RAG1* or *RAG2*) can lead to a variety of immunodeficiencies. Herein, we report 5 cases of RAG deficiency from 5 families: 3 of Omenn syndrome, 1 of severe combined immunodeficiency, and 1 of combined immunodeficiency with oligoclonal TCRγδ⁺ T cells, autoimmunity and cytomegalovirus infection. The genetic defects were heterogeneous and included 6 novel *RAG* mutations. All missense mutations except for Met443Ile in *RAG2* were located in active core regions of *RAG1* or *RAG2*. V(D)J recombination activity of each mutant was variable, ranging from half of the wild type activity to none, however, a significant decrease in average recombination activity was demonstrated in each patient. The reduced recombination activity of Met443Ile in *RAG2* may suggest a crucial role of the non-core region of RAG2 in V(D)J recombination. These findings suggest that functional evaluation together with molecular analysis contributes to our broader understanding of RAG deficiency.

1. Introduction

V(D)J recombination mediated by the recombination activating genes (RAG) 1 and RAG2 leads to the generation of diverse antigen receptors [1]. A complete lack of RAG activity causes severe combined immunodeficiency (SCID) with the

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absence of mature T and B cells, but the presence of natural killer (NK) cells (T^-B^- SCID) [2], whereas partial loss results in variant syndromes, such as Omenn syndrome (OS) [3] or combined immunodeficiency (CID) presenting with oligoclonal TCR$\gamma\delta^+$ T cells, autoimmunity and cytomegalovirus (CMV) infection (CID with $\gamma\delta$/CMV) [4,5]. OS is characterized by early-onset generalized erythroderma, lymphadenopathy, hepatosplenomegaly, protracted diarrhea, failure to thrive, eosinophilia, hypogammaglobulinemia, elevated serum IgE levels, the absence of B cells, and the presence of activated and oligoclonal T cells [6]. In contrast to T^-B^- SCID and OS, patients affected with CID with $\gamma\delta$/CMV exhibit autoimmune cytopenias, B cells, normal immunogulobulin levels, oligoclonal TCR$\gamma\delta^+$ T cells, and disseminated CMV infections [4,5]. Very recently, another distinct clinical syndrome caused by hypomorphic *RAG* mutations has been described. Schuetz et al. [7] reported 3 patients with late age of onset of illness characterized by hypogammaglobulinemia, diminished numbers of T and B cells, and the formation of granulomas in the skin, mucous membranes and internal organs. De Ravin et al. [8] described an adolescent patient presenting with destructive midline granulomatous disease who also exhibited autoimmunity, relatively normal numbers of T and B cells, and a diverse T-cell receptor (TCR) repertoire.

Herein, we report the identification of 8 *RAG* mutations including 6 novel mutations in a group of patients presenting with a variety of clinical phenotypes, and discuss the functional significance of these mutations by using the V(D)J recombination assay.

2. Materials and methods

2.1. Patients

We studied five patients with RAG deficiency from five families. Table 1 presents the immunological features of the patients. All patients except for patient 5 were born to non-consanguineous Japanese parents. The clinical and immunological data of patient 1 and patient 3 have been reported elsewhere [9]. Patient 2 was a 1-month-old boy who presented with generalized erythroderma, hepatosplenomegaly and *Pseudomonas aeruginosa* sepsis. Laboratory studies revealed hypereosinophilia, hypogammaglobulinemia, lack of B cells, and oligoclonal expansion of activated TCR$\alpha\beta^+$ T-cells. These findings were consistent with typical features of OS. Patient 4 was a 2-year-old girl who presented with prolonged diarrhea, bronchopneumonia, liver dysfunction and CMV infections. CMV was detected in her stool and sputum. Laboratory analysis revealed lymphopenia with normal immunoglobulin levels, an increased percentage of TCR$\gamma\delta^+$ T cells (61.7% of $CD3^+$), and multiple autoantibodies including anti-nuclear, anti-DNA, and antiparietal cell antibodies and Coombs test. In addition, IgG antibody against CMV was detected (20.7; normal, <2.0). Her elder sister suffered from autoimmune hemolytic anemia and immune mediated thrombocytopenia, and died of fatal interstitial pneumonia of adenovirus at age of 1 year. Patient 5 was the fourth child born to non-consanguineous parents of Indian origin. All of her 3 siblings were affected with immunodeficiency and died within the first year of life. Patient 5 showed lymphopenia, very low numbers of autologous T and B cells, preserved numbers of NK cells, and the presence of maternal $CD4^+$ T cell engraftment. At the age of 2 months, she remained asymptomatic except for oral thrush and microcephaly.

Table 1 Immunological features of the patients at diagnosis.

Patient	1[a]	2	3[a]	4	5
Diagnosis	OS	OS	Atypical OS	CID with $\gamma\delta$/CMV	Atypical SCID with MFT
Age at onset (month)	0	0	7	8	0
WBC	26,900	19,000	2800	3900	3280
Lymphocytes (/mm^3)	8339	5700	1300	546	459
$CD3^+$ (%)	84.8	41.3	20.0	53.9	7.8
$CD4^+$ (%)	56.7	16.6	17.3	9.9	7.4
$CD8^+$ (%)	27.0	37.8	1.3	35.4	0.1
$CD19^+$ or 20^+ (%)	0.0	0.2	0.1	11.6	0.1
IgG (mg/dl)	461	220	328	678	1475
IgA (mg/dl)	<4	<1	62	63	114
IgM (mg/dl)	<4	<2	31	65	147
IgE (IU/ml)	7	<2	16	NA	NA

OS, Omenn syndrome; CID, combined immunodeficiency; $\gamma\delta$, TCR$\gamma\delta^+$ T cells; CMV, cytomegalovirus; SCID, severe combined immunodeficiency; MFT, maternal T-cell engraftment; WBC, white blood cells; NA, not available.

[a] Data of patient 1 and patient 3 have been reported previously [9].

Approval for this study was obtained from the Human Research Committee of Kanazawa University Graduate School of Medical Science, and informed consent was provided according to the Declaration of Helsinki.

2.2. Mutation analysis of RAG1 and RAG2

DNA was extracted from blood samples using standard methods. The *RAG1* and *RAG2* genes were amplified in several segments from genomic DNA using specific primers, as previously described [10,11]. Sequencing was performed on purified polymerase chain reaction (PCR) products using the ABI Prism BigDye Terminator Cycle sequencing kit on an ABI 3100 automated sequencer (Applied Biosystems, Foster, CA).

2.3. V(D)J recombination assay

In vivo V(D)J recombination assay was performed by using the recombination substrate pJH200 as described previously with modifications [3,12]. The complete open reading frames of human *RAG1* and *RAG2*, and the active core regions of mouse *RAG1* (aa 330–1042) and *RAG2* (aa 1–388) were subcloned into the mammalian expression vector pEF-BOS [13]. PCR products carrying the patients' mutations were also subcloned into the vector. Cotransfections of full-length human *RAG1*, the mouse *RAG2* active core, and pJH200, or of full-length human *RAG2*, the mouse *RAG1* active core, and pJH200 into 293T cells were performed using 1 μg of each plasmid with Lipofectamine 2000 (Invitrogen, Carlsbad, CA).

Cells were harvested after 48-hours of culture, and the recombined products of signal joints were analyzed for recombination frequency by PCR using primers RA-CR2 and RA-14 [14]. After 30 cycles, the amplified products were visualized by ethidium bromide staining, and the intensity of each band was quantified using Image J software (NIH, Bethesda, MD).

2.4. Analysis of IgE production and somatic hypermutation (SHM) in variable regions of IgM

Peripheral blood mononuclear cells were isolated and incubated with 500 ng/ml of anti-CD40 (Diaclone, Besançon, France) and 100 U/ml of recombinant interleukin-4 (IL-4; R&D Systems, Minneapolis, MN) for 12 days. IgE production in culture supernatants was determined by enzyme-linked immunosorbent assay as previously described [15,16]. The frequency and characteristics of SHM in the V_H3-23 region of IgM were studied in purified $CD19^+$ $CD27^+$ B cells as previously described [15,16].

3. Results

3.1. RAG mutations

As shown in Table 2, we found 2 missense and 1 nonsense mutations in *RAG2* and 4 missense and 1 nonsense mutations in *RAG1*. Two distinct novel *RAG2* mutations, R73H and Q278X, were demonstrated in patient 1. Patient 2 was found to be homozygous for a novel M443I mutation in *RAG2*. Patient 3 was a compound heterozygote bearing R142X and R396H mutations in *RAG1*. The latter mutation has been repeatedly reported in OS patients [17]. Patient 4 was a compound heterozygote bearing R474C and L732P mutations in *RAG1*. These missense mutations are novel, although similar missense mutations, R474S, R474H and L732F, have been reported in patients with RAG deficiency [17–19]. Patient 5 carried a homozygotic novel E770K mutation in *RAG1*. All missense mutations but one (M443I in *RAG2*) were located in the active core regions of *RAG1* or *RAG2*, and all patients had at least one missense mutation. None of these mutations were found in 100 alleles of healthy controls.

Table 2 *RAG* mutations and recombination activity.

Patient	Gene	Nucleotide mutation	Effect	Relative recombination activity (%)[a]
1	RAG2	1419 G>A	R73H	59.3±4.7
		2033 C>T	Q278X	0.4±0.3
2	RAG2	2530 G>T[b]	M443I	8.7±1.2
3	RAG1	536 C>T	R142X	51.2±9.2
		1299 G>A	R396H	1.0±0.5
4	RAG1	1532 C>T	R474C	47.2±7.9
		2307 T>C	L732P	0.5±0.4
5	RAG1	2420 G>A[b]	E770K	15.6±9.1
Control	RAG2	wild type	–	100
	RAG1	wild type	–	100

[a] Data are expressed as the percentage of activity as compared with that of the wild type protein, and represent the mean±standard deviation of three independent experiments.
[b] Homozygous mutation.

3.2. Recombination activity of RAG mutants

To elucidate the pathogenic significance of these novel mutations, we performed V(D)J recombination assay using the artificial extrachromosomal rearrangement substrate (Table 2). As expected, the recombined products were amplified from 293T cells transfected with both wild type RAG1 and RAG2, and no products were obtained from 293T cells transfected with either RAG1 or RAG2 (Fig. 1). Although the relative recombination activity of each mutant was variable, ranging from about half of the wild type activity to none, a significant decrease in average recombination activity was demonstrated in each patient (Fig. 1 and Table 2). The effects of the patients' missense mutations were also evaluated by the web-based analysis tools including Mutation@A Glance (http://rapid.rcai.riken.jp/mutation/) [20] and MutationTaster (http://www.mutationtaster.org/) [21]. Mutation@A Glance predicted all the mutation except for the E770K in *RAG1* to be deleterious on the basis of the SIFT program [22], whereas MutationTaster predicted all the missense mutations to be disease-causing.

3.3. B cell analysis of patient 4

The percentages of IgD^- $CD27^+$ and IgD^+ $CD27^+$ cells within $CD19^+$ B cells from patient 4 were found comparable to controls (Fig. 2A) [23]. After stimulation with anti-CD40 and IL-4, B cells from patient 4 produced levels of IgE equivalent to normal, indicating their capability of undergoing class

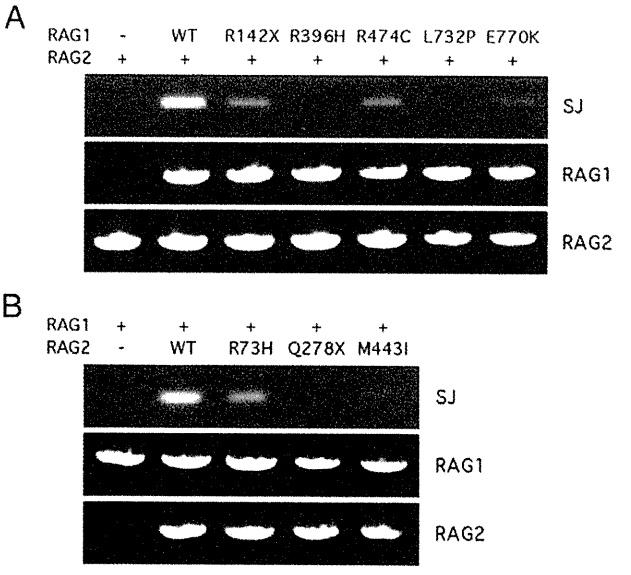
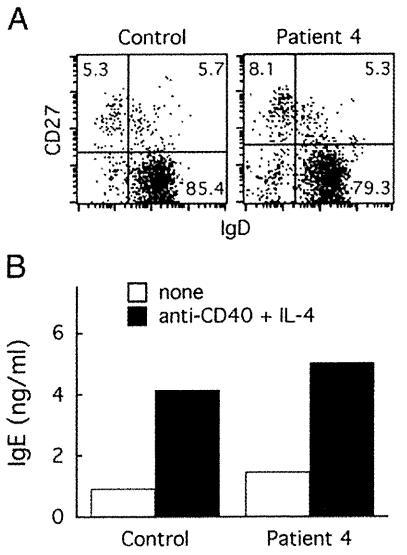


Figure 1 V(D)J recombination assay. V(D)J recombination activity was assessed by using the recombination substrate pJH200 in 293T cells that were cotransfected with mutant RAG1 and wild type RAG2 (A), or with wild type RAG1 and mutant RAG2 (B). Recombined products (signal joints, SJ) were analyzed by PCR (top). The presence of RAG1 and RAG2 was verified by vector specific PCR (middle and bottom).



C

%	Patient 4	Controls
SHM	4.8	2.6-6.3
GC target	56.0	62-66
GC transition	71.4	57-63
AT transition	50.0	33-67

To

From %	A	C	G	T	Total
A		5	13	10	28
C	2		6	11	19
G	29	6		2	37
T	2	9	5		16

Patient 4

From %	A	C	G	T	Total
A		6.2	12.9	4.1	23.2
C	2.6		6.0	11.4	20.0
G	26.1	13.2		4.3	43.6
T	4.8	5.5	3.0		13.2

Control

Figure 2 B cell analysis of patient 4. (A) B cell subpopulations. Peripheral bloods were stained with FITC-labeled anti-IgD, PE-labeled anti-CD27, and APC-labeled anti-CD19 monoclonal antibodies. The dot plot of immunofluorescence profiles of IgD and CD27 expression within $CD19^+$ B cells is shown. The number indicates the percentage of cells in each quadrant. (B) IgE production. After stimulation of peripheral blood mononuclear cells with anti-CD40 and IL-4 for 12 days, concentrations of IgE in the culture medium were quantified. (C) The frequency and pattern of somatic hypermutation in the V_H3-23 region of the IgM in memory B cells. RT-PCR products amplified from purified $CD19^+$ $CD27^+$ B cells by using V_H3-23 and $C\mu$ primers were subcloned and sequenced. Nucleotide changes were evaluated and shown as percentages.

switch recombination and IgE synthesis *in vitro* (Fig. 2B). In addition, the frequency and nucleotide substitution patterns of SHM were similar to those of healthy individuals (Fig. 2C).

4. Discussion

RAG deficiency has been considered to display a range of phenotype from classical T^-B^- SCID (complete RAG deficiency) to OS (partial RAG deficiency), depending on residual V(D)J recombination activity [24]. Atypical SCID/OS or leaky SCID may be also diagnosed in patients who show incomplete clinical and immunological characteristics and do not fulfill the criteria for SCID or OS [17]. However, it has recently been recognized that the clinical spectrum of RAG deficiency is much broader and includes CID with γδ/CMV [4,5], and CID with granulomatous inflammation [7], or destructive midline granulomatous disease [8]. In the present study, we studied 5 cases of RAG deficiency including 3 of OS, 1 of CID with γδ/CMV, and 1 of SCID with maternal T-cell engraftment, and identified 6 novel and 2 recurrent *RAG* mutations in these patients.

Hypomorphic RAG mutations leading to immunodeficiency have been shown to have up to 30% of wild type RAG activity by V(D)J recombination assay [7]. Although the R73H mutation in *RAG2* from patient 1, the R142X mutation in *RAG1* from patient 3, and the R474C mutation in *RAG1* from patient 4 exhibited around half of the wild type activity, all of these patients also had mutations with extremely low levels of recombination activity on the other allele, resulting in a substantial decrease in the average recombination activity due to a tetrameric complex formation of RAG1 and RAG2 during V(D)J recombination [1]. Similar results were obtained from an investigation of a RAG-deficient patient with destructive granulomatous disease who carried a W522C mutation with half of the recombination activity and a L541CfsX30 mutation with no recombination activity in *RAG1* [8]. It therefore seems reasonable that the clinical phenotype of partial RAG deficiency in patients 1, 3 and 4 is a consequence of these combinations of the mutations.

Biochemical studies have identified the core regions of RAG1 and RAG2 that are the minimal regions necessary for recombination of exogenous plasmid substrates *in vivo* and for DNA cleavage *in vitro* [1]. The M443I missense mutation demonstrated in patient 2 was located in the noncanonical plant homeodomain (PHD) of the non-core region of RAG2. Recent evidence indicates the importance of the non-core regions of RAG1 and RAG2 in V(D)J recombination and lymphocyte development [25]. The PHD of RAG2 has been shown to play crucial roles for chromatin and phosphoinositide binding, regulation of protein turnover, and cellular localization of RAG2 [26]. Additionally, the PHD of RAG2 is known to recognize histone H3 that has been trimethylated at the lysine at position 4 by interacting with 4 essential amino acids, Y415, M443, Y445, and W453 [27]. To date, 8 mutations of the non-core region in *RAG2* (W416L, K440N, W453R, A456T, C446W, N474S, C478Y, and H481P) have been reported in patients with T^-B^- SCID or OS [28]. A significant decrease in recombination activity of the M443I mutation from our patient further supports the important role of PHD of RAG2 in regulating V(D)J recombination.

Although the R142X nonsense mutation found in the N-terminal domain of RAG1 in patient 3 should have resulted in a complete loss of function, it remained partially functional for recombination unlike the Q278X mutation in RAG2 in our assay. On the other hand, the same R142X mutation has been described in a typical OS patient who also had a nonfunctional frameshift mutation in the core region of RAG1 on the other allele, thus suggesting that the residual V(D)J recombination activity exists

with the R142X mutation [29]. One explanation for these findings is alternative usage of methionine as a translation start site, which has been reported in OS patients with N-terminal RAG1 frameshift mutations [30,31]. A translation start prediction program NetStart 1.0 also indicated that methionines at codon 183 and 202, which were the first and second methionines found after the R142X mutations, could be alternative translation start sites with scores comparable to the conventional initiator codon 1 (http://www.cbs.dtu.dk/services/NetStart/) [32]. Therefore, it is possible that an N-terminal truncated and partially functional RAG1 protein generated by alternative usage of methionine led to the OS phenotype in our patient.

The clinical features of patient 4 were consistent with CID with γδ/CMV. Despite decreased recombination activity, patient 4 exhibited normal immunogulobulin levels and a normal percentage of peripheral B cells. These findings were in contrast to SCID and OS, but were in agreement with previously described cases of this disease [4,5]. Moreover, our B cell analysis of patient 4 revealed normal maturation, normal production of IgE after stimulation with anti-CD40 and interleukin-4, and normal somatic hypermutation in $CD27^+$ B cells. Taken together, our case provided additional data of the genetic and immunological features of this unique disease.

RAG mutations found in patients with typical T^-B^- SCID have been usually shown to abrogate recombination activity almost completely [2,33]. The residual V(D)J recombination activity resulting from the E770K mutation in RAG1 was associated with the SCID phenotype in patient 5. Despite trends towards more severe mutations, such as nonsense and frameshift mutations in SCID patients, missense mutations can lead to the SCID phenotype [33]. It is also known that the same mutations may cause different clinical phenotypes, presenting as either T^-B^- SCID or OS [18], and as either T^-B^- SCID or CID with γδ/CMV even within one family [34,35]. These findings suggest that that residual V(D)J recombination activity may not be solely responsible for the disease development. Further studies will be necessary to assess additional factors that influence the clinical phenotype of RAG deficiency.

In summary, our studies demonstrated the pathogenic significance of the 8 RAG mutations including 6 novel mutations from 5 patients with RAG deficiency. The characterization of the genetic defects and functional abnormalities in RAG-deficient patients will help define the role of RAG in V(D)J recombination and may lead to a better understanding of the variable phenotypic expression in RAG deficiency.

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References

[1] M. Gellert, V(D)J recombination: RAG proteins, repair factors, and regulation, Annu. Rev. Biochem. 71 (2002) 101–132.
[2] K. Schwarz, G.H. Gauss, L. Ludwig, U. Pannicke, Z. Li, D. Lindner, W. Friedrich, R.A. Seger, T.E. Hansen-Hagge, S. Desiderio, M.R. Lieber, C.R. Bartram, RAG mutations in human B cell-negative SCID, Science 274 (1996) 97–99.
[3] A. Villa, S. Santagata, F. Bozzi, S. Giliani, A. Frattini, L. Imberti, L.B. Gatta, H.D. Ochs, K. Schwarz, L.D. Notarangelo, P. Vezzoni, E. Spanopoulou, Partial V(D)J recombination activity leads to Omenn syndrome, Cell 93 (1998) 885–896.
[4] J.P. de Villartay, A. Lim, H. Al-Mousa, S. Dupont, J. Dechanet-Merville, E. Coumau-Gatbois, M.L. Gougeon, A. Lemainque, C. Eidenschenk, E. Jouanguy, L. Abel, J.L. Casanova, A. Fischer, F. Le Deist, A novel immunodeficiency associated with hypomorphic RAG1 mutations and CMV infection, J. Clin. Invest. 115 (2005) 3291–3299.
[5] S. Ehl, K. Schwarz, A. Enders, U. Duffner, U. Pannicke, J. Kuhr, F. Mascart, A. Schmitt-Graeff, C. Niemeyer, P. Fisch, A variant of SCID with specific immune responses and predominance of gamma delta T cells, J. Clin. Invest. 115 (2005) 3140–3148.
[6] A. Villa, L.D. Notarangelo, C.M. Roifman, Omenn syndrome: inflammation in leaky severe combined immunodeficiency, J. Allergy Clin. Immunol. 122 (2008) 1082–1086.
[7] C. Schuetz, K. Huck, S. Gudowius, M. Megahed, O. Feyen, B. Hubner, D.T. Schneider, B. Manfras, U. Pannicke, R. Willemze, R. Knuchel, U. Gobel, A. Schulz, A. Borkhardt, W. Friedrich, K. Schwarz, T. Niehues, An immunodeficiency disease with RAG mutations and granulomas, N. Engl. J. Med. 358 (2008) 2030–2038.
[8] S.S. De Ravin, E.W. Cowen, K.A. Zarember, N.L. Whiting-Theobald, D.B. Kuhns, N.G. Sandler, D.C. Douek, S. Pittaluga, P.L. Poliani, Y.N. Lee, L.D. Notarangelo, L. Wang, F.W. Alt, E. M. Kang, J.D. Milner, J.E. Niemela, M. Fontana-Penn, S.H. Sinal, and H.L. Malech, Hypomorphic Rag mutations can cause destructive midline granulomatous disease, Blood 116 (2010) 1263–1271.
[9] H. Okamoto, C. Arii, F. Shibata, T. Toma, T. Wada, M. Inoue, Y. Tone, Y. Kasahara, S. Koizumi, Y. Kamachi, Y. Ishida, J. Inagaki, M. Kato, T. Morio, A. Yachie, Clonotypic analysis of T cell reconstitution after haematopoietic stem cell transplantation (HSCT) in patients with severe combined immunodeficiency, Clin. Exp. Immunol. 148 (2007) 450–460.
[10] T. Wada, K. Takei, M. Kudo, S. Shimura, Y. Kasahara, S. Koizumi, K. Kawa-Ha, Y. Ishida, S. Imashuku, H. Seki, A. Yachie, Characterization of immune function and analysis of RAG gene mutations in Omenn syndrome and related disorders, Clin. Exp. Immunol. 119 (2000) 148–155.
[11] T. Wada, T. Toma, H. Okamoto, Y. Kasahara, S. Koizumi, K. Agematsu, H. Kimura, A. Shimada, Y. Hayashi, M. Kato, A. Yachie, Oligoclonal expansion of T lymphocytes with multiple second-site mutations leads to Omenn syndrome in a patient with RAG1-deficient severe combined immunodeficiency, Blood 106 (2005) 2099–2101.
[12] S. Kumaki, A. Villa, H. Asada, S. Kawai, Y. Ohashi, M. Takahashi, I. Hakozaki, E. Nitanai, M. Minegishi, S. Tsuchiya, Identification of anti-herpes simplex virus antibody-producing B cells in a patient with an atypical RAG1 immunodeficiency, Blood 98 (2001) 1464–1468.
[13] S. Mizushima, S. Nagata, pEF-BOS, a powerful mammalian expression vector, Nucleic Acids Res. 18 (1990) 5322.
[14] C.A. Roman, D. Baltimore, Genetic evidence that the RAG1 protein directly participates in V(D)J recombination through substrate recognition, Proc. Natl Acad. Sci. USA 93 (1996) 2333–2338.
[15] K. Imai, G. Slupphaug, W.I. Lee, P. Revy, S. Nonoyama, N. Catalan, L. Yel, M. Forveille, B. Kavli, H.E. Krokan, H.D. Ochs, A. Fischer, A. Durandy, Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination, Nat. Immunol. 4 (2003) 1023–1028.
[16] K. Imai, Y. Zhu, P. Revy, T. Morio, S. Mizutani, A. Fischer, S. Nonoyama, A. Durandy, Analysis of class switch recombination and somatic hypermutation in patients affected with autosomal

dominant hyper-IgM syndrome type 2, Clin. Immunol. 115 (2005) 277–285.
[17] A. Villa, C. Sobacchi, L.D. Notarangelo, F. Bozzi, M. Abinun, T.G. Abrahamsen, P.D. Arkwright, M. Baniyash, E.G. Brooks, M.E. Conley, P. Cortes, M. Duse, A. Fasth, A.M. Filipovich, A.J. Infante, A. Jones, E. Mazzolari, S.M. Muller, S. Pasic, G. Rechavi, M.G. Sacco, S. Santagata, M.L. Schroeder, R. Seger, D. Strina, A. Ugazio, J. Valiaho, M. Vihinen, L.B. Vogler, H. Ochs, P. Vezzoni, W. Friedrich, K. Schwarz, V(D)J recombination defects in lymphocytes due to RAG mutations: severe immunodeficiency with a spectrum of clinical presentations, Blood 97 (2001) 81–88.
[18] B. Corneo, D. Moshous, T. Gungor, N. Wulffraat, P. Philippet, F.L. Le Deist, A. Fischer, J.P. de Villartay, Identical mutations in RAG1 or RAG2 genes leading to defective V(D)J recombinase activity can cause either T–B-severe combined immune deficiency or Omenn syndrome, Blood 97 (2001) 2772–2776.
[19] C. Sobacchi, V. Marrella, F. Rucci, P. Vezzoni, A. Villa, RAG-dependent primary immunodeficiencies, Hum. Mutat. 27 (2006) 1174–1184.
[20] A. Hijikata, R. Raju, S. Keerthikumar, S. Ramabadran, L. Balakrishnan, S.K. Ramadoss, A. Pandey, S. Mohan, O. Ohara, Mutation@A Glance: an integrative web application for analysing mutations from human genetic diseases, DNA Res. 17 (2010) 197–208.
[21] J.M. Schwarz, C. Rodelsperger, M. Schuelke, D. Seelow, MutationTaster evaluates disease-causing potential of sequence alterations, Nat. Meth. 7 (2010) 575–576.
[22] P.C. Ng, S. Henikoff, SIFT: predicting amino acid changes that affect protein function, Nucleic Acids Res. 31 (2003) 3812–3814.
[23] K. Huck, O. Feyen, S. Ghosh, K. Beltz, S. Bellert, T. Niehues, Memory B-cells in healthy and antibody-deficient children, Clin. Immunol. 131 (2009) 50–59.
[24] T. Niehues, R. Perez-Becker, C. Schuetz, More than just SCID–the phenotypic range of combined immunodeficiencies associated with mutations in the recombinase activating genes (RAG) 1 and 2, Clin. Immunol. 135 (2010) 183–192.
[25] MatthewsA.G. , OettingerM.A. , RAG: a recombinase diversified, Nat. Immunol. 10 (2009) 817–821.
[26] J.M. Jones, C. Simkus, The roles of the RAG1 and RAG2 "non-core" regions in V(D)J recombination and lymphocyte development, Arch. Immunol. Ther. Exp. (Warsz) 57 (2009) 105–116.
[27] S. Ramon-Maiques, A.J. Kuo, D. Carney, A.G. Matthews, M.A. Oettinger, O. Gozani, W. Yang, The plant homeodomain finger of RAG2 recognizes histone H3 methylated at both lysine-4 and arginine-2, Proc. Natl Acad. Sci. USA 104 (2007) 18993–18998.
[28] C. Couedel, C. Roman, A. Jones, P. Vezzoni, A. Villa, P. Cortes, Analysis of mutations from SCID and Omenn syndrome patients reveals the central role of the Rag2 PHD domain in regulating V (D)J recombination, J. Clin. Invest. 120 (2010) 1337–1344.
[29] B. Cassani, P.L. Poliani, D. Moratto, C. Sobacchi, V. Marrella, L. Imperatori, D. Vairo, A. Plebani, S. Giliani, P. Vezzoni, F. Facchetti, F. Porta, L.D. Notarangelo, A. Villa, R. Badolato, Defect of regulatory T cells in patients with Omenn syndrome, J. Allergy Clin. Immunol. 125 (2010) 209–216.
[30] J.G. Noordzij, N.S. Verkaik, N.G. Hartwig, R. de Groot, D.C. van Gent, J.J. van Dongen, N-terminal truncated human RAG1 proteins can direct T-cell receptor but not immunoglobulin gene rearrangements, Blood 96 (2000) 203–209.
[31] S. Santagata, C.A. Gomez, C. Sobacchi, F. Bozzi, M. Abinun, S. Pasic, P. Cortes, P. Vezzoni, A. Villa, N-terminal RAG1 frameshift mutations in Omenn's syndrome: internal methionine usage leads to partial V(D)J recombination activity and reveals a fundamental role in vivo for the N-terminal domains, Proc. Natl Acad. Sci. USA 97 (2000) 14572–14577.
[32] A.G. Pedersen, H. Nielsen, Neural network prediction of translation initiation sites in eukaryotes: perspectives for EST and genome analysis, Proc. Int. Conf. Intell. Syst. Mol. Biol. 5 (1997) 226–233.
[33] J.G. Noordzij, S. de Bruin-Versteeg, N.S. Verkaik, J.M. Vossen, R. de Groot, E. Bernatowska, A.W. Langerak, D.C. van Gent, J.J. van Dongen, The immunophenotypic and immunogenotypic B-cell differentiation arrest in bone marrow of RAG-deficient SCID patients corresponds to residual recombination activities of mutated RAG proteins, Blood 100 (2002) 2145–2152.
[34] N.E. Karaca, G. Aksu, F. Genel, N. Gulez, S. Can, Y. Aydinok, S. Aksoylar, E. Karaca, I. Altuglu, N. Kutukculer, Diverse phenotypic and genotypic presentation of RAG1 mutations in two cases with SCID, Clin. Exp. Med. 9 (2009) 339–342.
[35] S. Pasic, S. Djuricic, G. Ristic, B. Slavkovic, Recombinase-activating gene 1 immunodeficiency: different immunological phenotypes in three siblings, Acta Paediatr. 98 (2009) 1062–1064.

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

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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 lymphoadenopathy/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, respectively[7]; 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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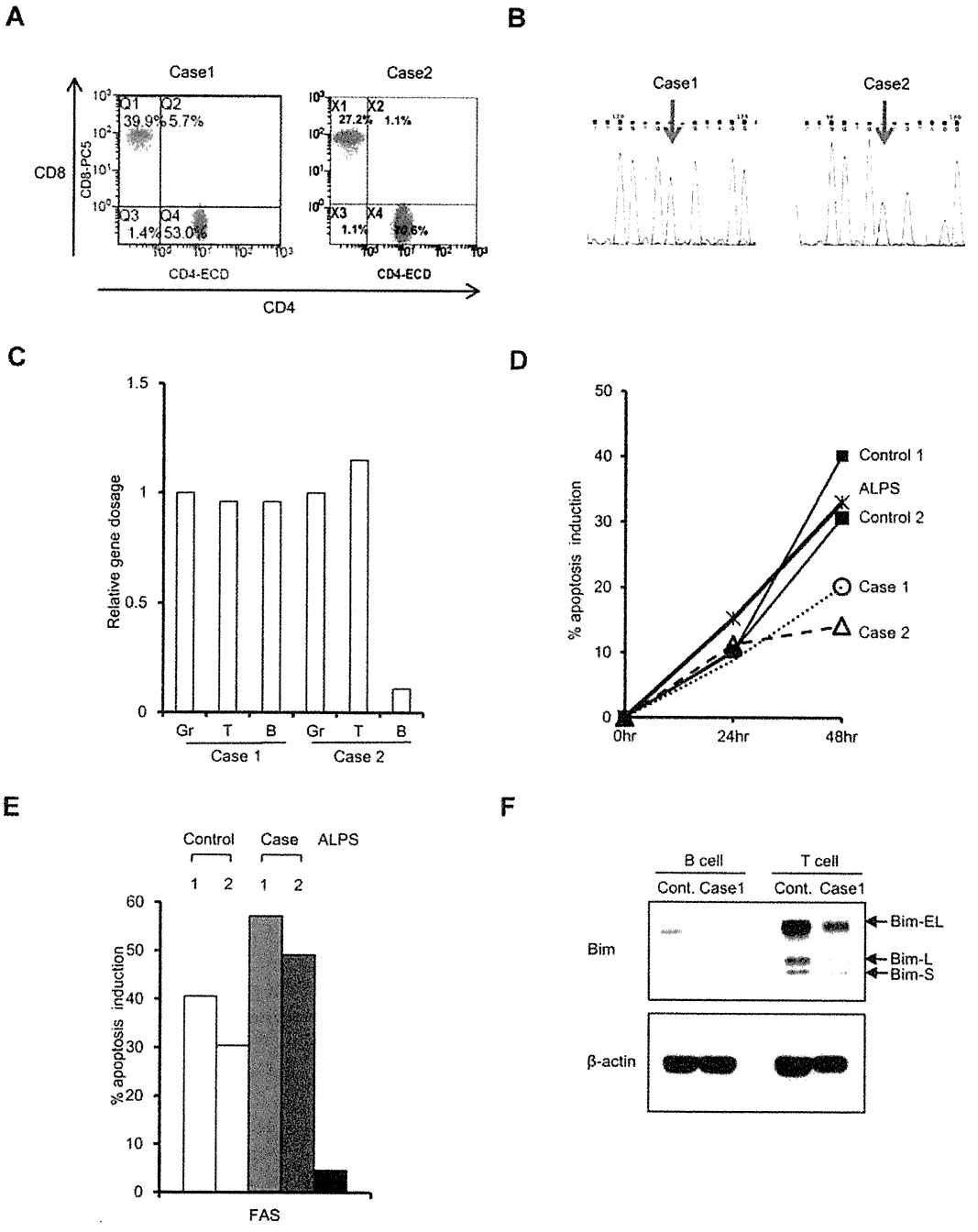

Figure 1. Molecular cell biologic assay of RALD. (A) Flow cytometric analysis of double-negative T cells. CD8 and CD4 double staining was performed in T-cell receptor-αβ-expressing cells. (B) Electropherogram showing KRAS G13D mutation in BM-MNCs in case 1 (left panel) and case 2 (right panel). (C) Gene 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-αβ cells (Figure 1A). JMML was also nominated as a disease to be differentiated because remarkable hepatosplenomegaly with thrombocythemia 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 Ia, 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 ALPS[2] 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 JMML[14] 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., S.K., Y.K., and A.T. supervised clinical and immunologic experiments or coordinated clinical information.

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References

1. Fisher GH, Rosenberg FJ, Straus SE, et al. Dominant interfering Fas gene mutations impair apoptosis in a human autoimmune lymphoproliferative syndrome. *Cell.* 1995;81(6):935-946.
2. Teachey DT, Seif AE, Grupp SA. Advances in the management and understanding of autoimmune lymphoproliferative syndrome (ALPS). *Br J Haematol.* 2010;148(2):205-216.
3. Oliveira JB, Bidere N, Niemela JE, et al. NRAS mutation causes a human autoimmune lymphoproliferative syndrome. *Proc Natl Acad Sci U S A.* 2007;104(21):8953-8958.
4. Oliveira J, Bleesing J, Dianzani U, et al. Revised diagnostic criteria and classification for the autoimmune lymphoproliferative syndrome (ALPS): report from the 2009 NIH International Workshop. *Blood.* 2010;116(14):e35-e40.
5. Miyauchi J, Asada M, Sasaki M, Tsunematsu Y, Kojima S, Mizutani S. Mutations of the N-ras gene in juvenile chronic myelogenous leukemia. *Blood.* 1994;83(8):2248-2254.
6. Emanuel PD. Juvenile myelomonocytic leukemia and chronic myelomonocytic leukemia. *Leukemia.* 2008;22(7):1335-1342.
7. Aoki Y, Niihori T, Narumi Y, Kure S, Matsubara Y. The RAS/MAPK syndromes: novel roles of the RAS pathway in human genetic disorders. *Hum Mutat.* 2008;29(8):992-1006.
8. Kitahara M, Koike K, Kurokawa Y, et al. Lupus nephritis in juvenile myelomonocytic leukemia. *Clin Nephrol.* 1999;51(5):314-318.
9. Oliver JW, Farnsworth B, Tonk VS. Juvenile myelomonocytic leukemia in a child with Crohn disease. *Cancer Genet Cytogenet.* 2006;167(1):70-73.
10. Saif MW, Hopkins JL, Gore SD. Autoimmune phenomena in patients with myelodysplastic syndromes and chronic myelomonocytic leukemia. *Leuk Lymphoma.* 2002;43(11):2083-2092.
11. Flotho C, Kratz CP, Bergstrasser E, et al. Genotype-phenotype correlation in cases of juvenile myelomonocytic leukemia with clonal RAS mutations. *Blood.* 2008;111(2):966-967.
12. Matsuda K, Shimada A, Yoshida N, et al. Spontaneous improvement of hematologic abnormalities in patients having juvenile myelomonocytic leukemia with specific RAS mutations. *Blood.* 2007; 109(12):5477-5480.
13. Flotho C, Valcamonica S, Mach-Pascual S, et al. RAS mutations and clonality analysis in children with juvenile myelomonocytic leukemia (JMML). *Leukemia.* 1999;13(1):32-37.
14. Matsuda K, Sakashita K, Taira C, et al. Quantitative assessment of PTPN11 or RAS mutations at the neonatal period and during the clinical course in patients with juvenile myelomonocytic leukaemia. *Br J Haematol.* 2010;148(4):593-599.
15. Imamura M, Imai C, Takachi T, Nemoto T, Tanaka A, Uchiyama M. Juvenile myelomonocytic leukemia with less aggressive clinical course and KRAS mutation. *Pediatr Blood Cancer.* 2008;51(4):569.
16. Zhang J, Wang J, Liu Y, et al. Oncogenic Kras-induced leukemogeneis: hematopoietic stem cells as the initial target and lineage-specific progenitors as the potential targets for final leukemic transformation. *Blood.* 2009;113(6):1304-1314.
17. Sabnis AJ, Cheung LS, Dail M, et al. Oncogenic Kras initiates leukemia in hematopoietic stem cells. *PLoS Biol.* 2009;7(3):0537-0548.
18. Teachey DT, Manno CS, Axsom KM, et al. Unmasking Evans syndrome: T-cell phenotype and apoptotic response reveal autoimmune lymphoproliferative syndrome (ALPS). *Blood.* 2005; 105(6):2443-2448.
19. Seif AE, Manno CS, Sheen C, Grupp SA, Teachey DT. Identifying autoimmune lymphoproliferative syndrome in children with Evans syndrome: a multi-institutional study. *Blood.* 2010; 115(11):2142-2145.

総　説

分類不能型免疫不全症 Update

森尾友宏

Common variable immunodeficiency: an update on etiology, pathophysiology, and classification

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summary

Common variable immunodeficiency is one of the most common primary immunodeficiency that is categorized into primary antibody deficiency. The responsible genes identified so far include *ICOS*, *TACI*, *CD19*, *CD20*, *CD21*, *CD81* and *BAFF-R*; and most of the CVID-causing genes are yet to be identified. *TACI* mutation is the most common one; however the direct contribution of TACI mutation to pathogenesis of CVID is not yet clear. One third to a half of the patients with CVID shows autoimmunity as well as malignancy in their course. It is of importance to develop diagnostic measure, to identify the disease causing genes, and to develop the optimal therapy.

Key words——Common variable immunodeficiency (CVID); classification; KRECs; TRECs; whole exome sequencing

抄　録

分類不能型免疫不全症は Common variable immunodeficiency (CVID) と呼ばれ，最も頻度の高い先天性免疫不全症，かつ最も頻度の高い抗体産生不全型免疫不全症である．今までに判明した責任遺伝子には *ICOS*, *TACI*, *CD19*, *CD20*, *CD21*, *CD81*, *BAFF-R* などがあるが，いずれもその頻度は低く，*TACI* ではその変異が直接的にかつ単一で病態に関わっているかどうか不明である．臨床症状としては感染症，自己免疫疾患や悪性腫瘍の合併などがあり，成人型の免疫不全症としてきわめて重要な位置を占める．特に単一遺伝子異常に基づく，易感染性，自己免疫疾患・悪性腫瘍の発症という点で，様々な疾患の免疫異常基盤探索においても重要な疾患である．鑑別診断に加え，病態に応じた分類，責任遺伝子の究明，至適治療法の開発が重要である．

はじめに

Common variable immunodeficiency (CVID) は従来分類不能型免疫不全症と訳されていたが，症例数が多く (common)，多彩な臨床症状をとる (variable)，分類不能な疾患であるために，暫定的につけられた名称がそのまま用いられている．抗体産生不全を主体とする疾患群であり，成人領域で判明する原発性免疫不全症としては数が最も多い．疫学的には 10,000 人から 100,000 人に 1 人とされているが，私たちが 2009 年に行った我が国における全国調査においても 200 名程度の患者が存在することが明らかになっている[1~6]．その数は欧米をはじめとして少しずつ増加しており，これも awareness campaign によるところが大きいと考えられている．ヨーロッパにおける J-Project，英国の "Is it PIS?" キャンペーン，ドイツの FIND ID などがそれにあたる．表 1 には免疫不全症の診断の遅れを防ぐために，Jeffery Modell Foundation が中心となって作成した PID の 10 awareness warning signs をあげた．成人領域においても，感染症の頻度が高い，感染症が重症化あるいは遷延化した，稀な感染症を起こした，などというエピソードをもつ患者ではまず一般的な血算と共に，IgG, A, M, E を測定することが重要であり，今後その数はますます増加する可能性がある．

東京医科歯科大学大学院発生発達病態学分野

I. CVID の定義

CVID は，欧州免疫不全症学会 (European Society for Immunodeficiencies : ESID) によれば，「2 歳

表1 Ten warning signs

1-1E) 10 Warning Signs of PID-General

1) Four or more new ear infections within 1 year.
2) Two or more serious sinus infections within 1 year.
3) Two or more months on antibiotics with little effect.
4) Two or more pneumonias within 1 year.
5) Failure of an infant to gain weight or grow normally.
6) Recurrent, deep skin or organ abscesses.
7) Persistent thrush in mouth or fungal infection on skin.
8) Need for intravenous antibiotics to clear infections.
9) Two or more deep-seated infections including septicemia.
10) A family history of PI.

1-1J) 原発性免疫不全症を疑う10の徴候（日本語版）

1) 乳児で呼吸器・消化管感染症を繰り返し，体重増加不良や発育不良が見られる．
2) 1年に2回以上肺炎にかかる．
3) 気管支拡張症を発症する．
4) 2回以上，髄膜炎，骨髄炎，蜂窩織炎，敗血症や，皮下膿瘍，臓器内膿瘍などの深部感染症にかかる．
5) 抗菌薬を服用しても2ヶ月以上感染症が治癒しない．
6) 重症副鼻腔炎を繰り返す．
7) 1年に4回以上，中耳炎にかかる．
8) 1歳以降に，持続性の鵞口瘡，皮膚真菌症，重度・広範な疣贅（いぼ）がみられる．
9) BCGによる重症副反応（骨髄炎など），単純ヘルペスウイルスによる脳炎，髄膜炎菌による髄膜炎，EBウイルスによる重症血球貪食症候群に罹患したことがある．
10) 家族が乳幼児期に感染症で死亡するなど，原発性免疫不全症候群を疑う家族歴がある．

1-2) 成人で免疫不全症を疑う6の徴候

The 6 ESID warning signs for ADULT primary immunodeficiency diseases

1. Four or more infections requiring antibiotics within one year (otitis, bronchitis, sinusitis, pneumonia)
2. Recurring infections or infection requiring prolonged antibiotic therapy
3. Two or more severe bacterial infections (osteomyelitis, meningitis, septicemia, cellulitis)
4. Two or more radiologically proven pneumonia within 3 years
5. Infection with unusual localization or unusual pathogen
6. PID in the family

表2 除外すべき疾患群（Diseases to be excluded : DE）

	責任遺伝子
1. XLA（X連鎖γグロブリン血症）：男性	*BTK*
その他のB細胞欠損症（IGHM, CD79A, CD70B, BKNK…）は稀	
2. XLP（X連鎖リンパ増殖症候群）：男性	*SAP*
3. X-HIGM（X連鎖高IgM症候群）：男性	*CD40L*
その他の高IgM症候群（*AID*, *UNG*）	
4. Good's syndrome（胸腺腫を伴う免疫不全症）	
5. 非典型的SCID（後期発症複合型免疫不全症）	*ADA*, *LIGIV*, *XLF1*…
$CD4<200/mm^3$ で疑う	
6. Bone-marrow failure（骨髄不全症候群：Fanconi貧血，先天性角化異常症など）	
7. Lymphoma/leukemia（白血病，リンパ腫）	
8. Protein loss via the kidney（腎疾患における蛋白漏出）	
9. Protein loss via the gastrointestinal tract（腸管疾患における蛋白漏出）screening with alpha-1-antitrypisin in stool	
10. 薬剤	

以上（多くは10歳代以降）で発症する低γグロブリン血症で，同種血球凝集素の欠損，あるいはワクチンへの低反応を示し，既知の免疫不全症ではない疾患」と定義されている．疾患概念は不明瞭かつ様々な疾患群を含んでいることは間違いない[1~6]．

現時点において基本的には除外診断となっているが，特徴的な疾患群が存在することもまた確かである．表2に除外すべき疾患とその責任遺伝子などの

情報につき記載した．特に注意すべき点は以下の通りである．

X連鎖無γグロブリン血症では*BTK*変異部位などによりB細胞の完全欠損や，無γグロブリン血症ではなく低γグロブリン血症となることがあり，男性ではまず否定が必要である．男性では同様に特にEBVへの脆弱性（血球貪食症候群など）を伴った場合には，*SH2D1A*あるいは*XIAP*を検討しておくべきである．また高IgM症候群の約2/3では実際にIgMが高値になっておらず，IgG, IgAは低値を示す．従って男性ではCD40Lを，また男女いずれの場合もAIDは検査しておいて良い．さらに，胸腺腫を伴う免疫不全症（Good症候群）もその数は比較的多く，年長での発症及びB細胞がほぼ欠損することが特徴であるが，年長者では胸腺腫を確認しておく必要がある[1~6]．最後に，原因不明（他の疾患の除外）という点では自己矛盾する内容であるが，CVIDの責任遺伝子として後述するものについても，いずれも稀な疾患であり，すぐに検査する必要はない．

II. CVIDの臨床症状

CVIDの症状は様々である．身体的特徴としては，肝脾腫を呈する症例が比較的多い程度であり，皮疹（アトピー様乾癬様，多型滲出性紅斑様など），神経症状，発達遅滞などの合併も認める．ただし発達遅滞を認めるCVIDが真のCVIDであるかは検証が必要である．感染症としては，いわゆるsino-pulmonary diseaseが多いが，Epstein Barrウイルス（EBV）感染症，サイトメガロウイルス（CMV）感染症，パピローマウイルス感染症などT細胞機能不全を疑わせる症例も散見される．全国調査においては，自己免疫疾患を合併するものは全体で19％，40歳以上で36％，悪性腫瘍の合併は全体で10％，40歳以上で19％であった．自己免疫疾患として最も多いのは，自己免疫性溶血性貧血や自己免疫性血小板減少症であるが，関節リウマチ，炎症性腸疾患，多発筋炎などさまざまな疾患を認める．悪性腫瘍ではリンパ系悪性腫瘍が多いが，甲状腺腫瘍，子宮頸癌，消化器系腫瘍も散見される．臨床症状には大きな差があり，全く無症状のままに他疾患のスクリーニングの中で発見されることもある．Bodo Grimbacherらのgroupは CVIDの重症度について表3に示すようなスコアリングシステムを提唱している[5]．

III. CVIDの病態

CVIDの病態は様々であり，あらゆる異常が報告されていると言っても良い．たとえば胸腺からのT細胞新生能の低下，T細胞増殖能の低下，T細胞シグナル伝達異常，サイトカイン産生異常，アポトーシスの異常や，TCR Vbeta repertoireの偏り，CD40L発現の低下等が報告されており，また樹状細胞の機能不全や，数の低下などを示す報告もある[7~9]．テロメア長の短縮を認める症例もある[10]．B細胞については，クラススイッチ記憶B細胞数

表3　分類不能型免疫不全症の重症度スコアリングシステム

Points	1	2	3	4
1. Chronic sinusitis	Absent	Present		
2. Past meningitis or encephalitis	Absent	One bout	Two bouts	>Two bouts
3. Past pneumonia	Absent	One bout	Two bouts	>Two bouts
4. Bronchiectasis	Absent	One bout	Two bouts	>Two bouts
5. Other parenchymal lung pathology such as fibrosis, LIP, BOOP, *etc.*	Absent	Suspected		Confirmed
6. Lung surgery (lobectomy or pneumonectomy)	Absent			Performed
7. Splenomegly	Absent	11–14.9 cm	15–20 cm	>20 cm
8. Splenectomy	Absent			Performed
9. Lymphadenopathy (largest node)	Absent	<2 cm	2–3 cm	>3 cm
10. CVID enteropathy	Absent	Intermittent	Chronic but mild	Severe
11. Autoimmune condition	Absent	Suspected		Confirmed
12. Other rheumatological complaints such as arthralgia	Absent	Suspected	Confirmed	
13. Granulomata	Absent	Skin only	Lung, liver or spleen	CNS (incl. eye)
14. Lymphoma	Absent			Present
15. Cancer (solid tumors)such as bowel, skin or stomach	Absent			Present

文献5)より改変

や形質芽球数の減少は明らかであるが，B 細胞数が1%未満というものもあり，B 細胞欠損症との異同も明確ではない．これらの混乱した情報は，CVID の一群を取り上げて，その欠陥を一般化しようとする研究から生じたものであり，CVID は heterogenous な疾患群から成り立っているというコンセンサスの元により本質的な病態解明が望まれる．

今井，野々山らはこれらの混乱を鑑みて，CVID を B 細胞欠損型，T 細胞欠損型，B/T 欠損型，B/T 正常の真の CVID に分類することを提唱している（表 4）．ここでは B 細胞新生能，B 細胞数の代替え指標として，sjKRECs (signal joint kappa-deleting recombination excision circles)，cjKRECs (coding joint KRECs) を[11]，T 細胞新生能の指標として，TRECs (T-cell receptor excision circles) を用いている[12,13]．この系は realtime PCR にて簡単に測定することができるが，B 細胞では CD19/CD20 陽性細胞数が cjKRECs の，また CD4＋CD45RA＋(＋CD31＋) 細胞が recent thymic emigrant cells として TRECs の，代替えとして用いることができる．一方，CD19/CD20 は sjKRECs を反映しない．

B 細胞数の減少あるいは，sjKRECs の減少を除

表 4　分類不能型免疫不全症の亜群(Subgroup of CVID: SC)

SC-A.	B 細胞新生能正常，naïve T 細胞数正常
SC-B.	B 細胞新生能減少，naïve T 細胞数正常
SC-C.	B 細胞新生能正常，naïve T 細胞数減少
SC-D.	B 細胞新生能減少，naïve T 細胞数減少

B 細胞数として CD19，CD20 を用いる以外に，cjKRECs (Kappa-deleting recombination excision circles) を B 細胞数の，sjKRECs を B 細胞新生能の指標として用いる提言がある（今井，野々山ら）．Naïve T 細胞（CD4＋CD45RA＋）の代替えとしては，T 細胞新生能として TRECs (T-cell receptor excision circles) を用いることがある．

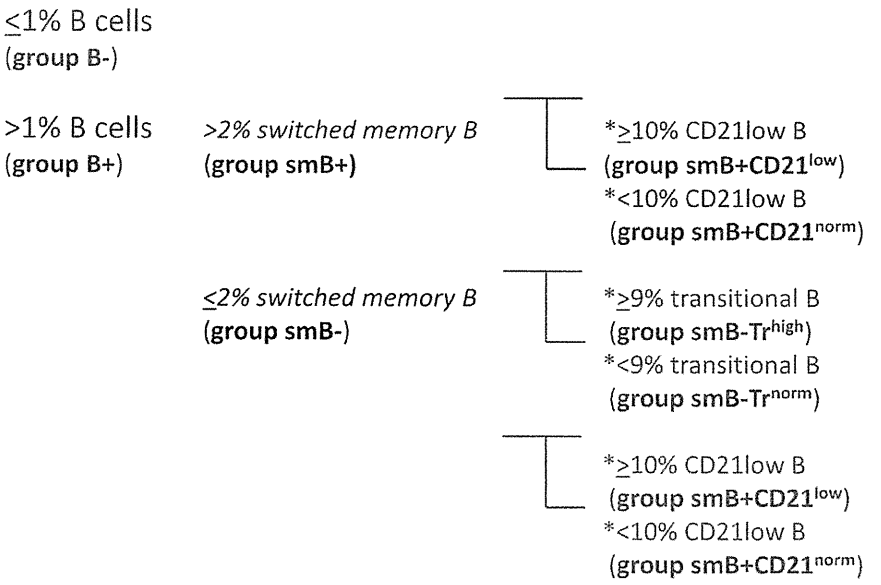

図 1　EURO Class of CVID（文献 14）より改変）

	B+	SmB+	SmB-	SmB+ 21low	SmB+ 21norm	SmB- Trhigh	SmB- Trnorm	SmB- 21low	SmB- 21norm
No of patients or %	303	42%	58%	33% of SmB+	67% of SmB+	19% of SmB-	81% of SmB-	49% of SmB-	51% of SmB-
Splenomegaly	41%	24%	52%	50%	14%	54%	51%	54%	51%
Lymphoadenopathy	26%	22%	24%	17%	20%	57%	22%	57%	22%
Granuloma	12%	4%	17%	14%	2%	24%	16%	24%	16%
Autoimmunity	20%	19%	21%	15%	10%	19%	26%	17%	26%

図 2　EURO Class of CVID と臨床症状（文献 14）より改変）

表5 いわゆる分類不能型免疫不全症に含まれる病態（Classification of CVID: CC）

1. 選択的 IgM 欠損症
2. 選択的 IgA 欠損症
3. IgG 欠損症及び IgG サブクラス異常症
4. 特異抗体欠損症
5. 高 IgM 症候群
6. IgG/IgA（IgM の程度は様々）：真の CVID
7. その他の低γグロブリン血症
それぞれに対し
Type (a) asymptomatic
Type (b) with recurrent infections
Type (c) without infections but with other associated pathology

文献 5）より改変

表6 分類不能型免疫不全症における特徴的表現型

1. 低身長，小頭症，発達遅滞	DE5, DE6 を示唆
2. 悪性腫瘍の合併	DE4, 5, 6, 7 の除外が必要
3. 自己免疫疾患が前面にでる疾患	
4. 特定の感染症への脆弱性	DE2 類似疾患など

DE は表2を参照

いた群においては，患者 B 細胞は CD20＋CD27＋IgM−IgD−の switched memory 群の減少を示す．時に抗体が関与すると思われる自己免疫疾患があるが，これらの症例における免疫グロブリンレパートア解析は今後の課題である．B 細胞が oligoclonal な場合も想定される．

その他の CVID 分類の試みとして，どの免疫グロブリンサブクラスが主に障害を受けるのかと，臨床症状に着目するものがある（表5）．このような分類に至った1つの理由として，IgA 欠損症が CVID と相互移行することがあるという事実がある．実際に IV で記載する責任遺伝子の一部についてもその欠陥により IgA 欠損症あるいは CVID を呈している．その他 IgM が比較的高値で IgG, IgA が低値の症例は高 IgM 症候群 variant でクラススイッチ異常がベースにあり，IgM のみが低値という場合なども想定できる[5]．

ヨーロッパなどではさらに，B 細胞数，記憶 B 細胞の割合，transitional B 細胞の比率，CD21 の発現低下の有無，などにより CVID を分類する試みもある（図1及び図2）[14,15]．ここでは臨床症状との対比を行っているが，脾腫はクラススイッチ記憶 B 細胞が少ない群，比較的保たれているが CD21low 細胞が多い群により頻繁に認められる．リンパ節腫大は一方クラススイッチ記憶 B 細胞が少なく，CD21low 細胞が多い群あるいは transitional B 細胞が多い群に頻繁に認められる．

表6に，特徴的な臨床症状と除外診断の関連についてまとめた．

IV. 現在までに明らかになっている CVID の責任遺伝子

1. CD19/CD21/CD81 欠損症

CD19/CD21/CD81 は複合体を作っており，B 細胞への抗原刺激においては，抗原と C3d が会合し，B 細胞受容体と CD19/CD21/CD81 複合体の両者からのシグナルが伝達されることになる．いわゆる B 細胞における dual signaling model である．CD19 欠損においては，B 細胞数は正常で，クラススイッチした記憶 B 細胞数は減少している[16,17]．IgG は低値で，特異抗体産生能は低下しており，小児期から感染症を反復する．抗原受容体刺激による Ca 流入は低下している．CD21 欠損症においては，IgG, IgA は低値であるものの，抗原特異的抗体産生能は比較的保たれていた．比較的軽症な CVID 群である[15]．CD81 欠損症では IgG 低値，IgA やや低下，IgM 正常であり，血管性紫斑病が認められた．CD81 は CD19 の表出にも重要であり，欠損症では CD19 発現は低下し，CD19 欠損症と同様の B 細胞分化異常とシグナル異常が認められた[19]．

2. CD20 欠損症

CD20 は代表的な B 細胞抗原であり，骨髄の early pre-B から発現が認められる．ノックアウトマウスでは B 細胞数や抗体産生も正常であったが，Ca 流入に異常を認める．ヒトでは低 IgG（IgM, IgA 正常）の患者で CD20 異常が同定されている[20]．クラススイッチした記憶 B 細胞が減少し，抗原刺激による Ca 流入に欠陥があることから，CD19/CD21/CD81 欠損症と類似した状態ということができる．このように 1, 2 は Ca シグナルに異常のある群とも捕らえることができるが，そのシグナル系に関係する分子異常が CVID の新規責任遺伝子として同定される可能性がある．

3. ICOS 欠損症

ICOS は CD28 ファミリーに属する共刺激分子で，

CD28, CTLA-4, PD-1 などがその群に属する．活性化されたT細胞に発現し，T細胞分化，サイトカイン産生，T細胞依存性抗体産生などに重要とされている．ICOS 欠損症は分子異常が示された初めての CVID である．今までに同定された遺伝子変異は 2 種類だけであり，稀な CVID ということができる[21~24]．

患者では小児期から成人期に発症する様々な感染症を呈し，結節性リンパ様増生，間質性肺炎，自己免疫疾患（血球減少，炎症性腸疾患，関節リウマチ，乾癬など），パピローマウイルスによる子宮頸癌など，CVID が呈するほとんどの症状を示している．IgG は低下し，しかし IgM, IgA の低下の程度は様々である．IgM が比較的高値・IgA 低値のクラススイッチ異常パターンをとるケースも認められた．日本の症例の検討では Th1, Th2, Th17 サイトカインの産生低下，記憶 CD4T 細胞の減少，Treg サブセットの減少が認められ，さらに T-bet, GATA-3, MAF, RORC induction にも欠陥があることが示された．CTLA-4 の発現誘導にも問題がある．従って，ICOS 欠損症では，活性型及び抑制性両者の T 細胞の分化あるいは維持に問題があり，そのいずれが主に侵されるかによって，感染症が主体となるか，自己免疫疾患が主体となるかが決定される可能性がある．実際に ICOS 欠損マウスを用いた実験ではエフェクター T 細胞の機能低下が報告されているが，逆に autoimmunity への傾向を示唆した論文も少数認められる．CVID における易感染性と自己免疫疾患のバランスに関与する分子機構として興味深い．

4. TACI 異常症

TACI 異常症と IgA 欠損症，CVID との関連は 2005 年に示されたが，その因果関係は複雑である．ヘテロ異常で健常者と患者が混在する家系や，複合ヘテロ接合体異常，ホモ異常などが報告されているが，おそらく単独の因子ではなく，修飾因子が加わって発症するのではないかと考えられている[22~30]．TACI は transmembrane activator and calcium-modulating cyclophilin ligand interactor の略で，TNF 受容体スーパーファミリーに属し，BAFF（B cell activating factor), BCMA (B cell maturation antigen）や APRIL（a proliferation-inducing ligand）などをリガンドとしている．リガンドとの会合により，B 細胞ではクラススイッチが誘導される．多くの患者では TACI mutation があっても発現が認められることも解析を困難にしている．

患者ではしかし，CVID の大半の症状を呈しており，今までに 600 名近い患者が同定されている．B 細胞数は正常であるが，時に激減した患者も散見される．クラススイッチ記憶 B 細胞の減少は CVID 全般の傾向とかわらない．IgA, IgM は低値をとることが多いが，IgA が正常であったり，高 IgM となったりすることもある．両アリルでの変異では APRIL への会合が低下することが示されているが，最も頻度の高い変異は片アリルでの C104R, A181E であり，健常人でも同じ変異を有する集団が 2%程度存在する[27,31]．新たな変異では（あるいは既知のヘテロ変異でも）病的意義を検証することが難しい疾患群である．

5. BAFF-R 欠損症

BAFF-R 欠損症は姉弟例で報告されているのみである．BAFF-R は TNF 受容体スーパーファミリーに属し（TNFRSF13C），B 細胞に表出され，BAFF（B cell activation factor）によって刺激が入り，NFkB の誘導→抗アポトーシスに働き，B 細胞の生存に深く関与している．BAFF-R 欠損症では IgG, IgM の低下を認め，IgA は正常であった．しかし IgG の低下は一例ではごく軽度であった．姉弟での表現型の差異などから，BAFF-R 以外の因子が貢献するところが大きいと考えられている[32]．実際に，BAFF-R のリガンドは BAFF のみであるが，BAFF のリガンドとしては，先述の TACI, BCMA, APRIL などがある．

T 細胞依存性抗体産生は正常であるが，T 非依存的抗体産生に欠陥がある．また自己免疫やリンパ増殖などは認めていない．可溶性 BAFF-R が減少する CVID の一群があり，BAFF-R の調節領域異常があるのではと推測されている．

6. Msh5 異常症

Msh5 は DNA ミスマッチ修復や，減数分裂での相同組換えに関与するが，クラススイッチ組換えでの役割も明らかになっている．2 つの SNP の組み合わせ（L85F/P786S）が IgA 欠損症や CVID と関連していると報告されている[33]．

V. 病因へのアプローチ

CVID では家族例や遺伝歴のある症例が少なく

10%程度にすぎない．CVIDでは最低10以上の未知遺伝子が背景としてあるのではと推測されている．マウスモデルや分子機能から推測された共刺激分子については遺伝子変異が見つかっていない．具体的にはBAFF-RのリガンドであるBAFF, APRIL, BCMAは少なくともmajorなCVID遺伝子ではない[2,30,31,34,35]．重症複合型免疫不全症あるいは複合型免疫不全症の責任遺伝子である*ADA*, *RAG*, *LIG4*, *Artemis*などの遺伝子異常症の軽症型を見逃している可能性は十分にある．しかしおそらくは主たる責任遺伝子ではない．SC-AのKRECs, TRECs正常群においては，家族例の解析や体系的あるいは網羅的遺伝子解析に機能解析を加えて遺伝子探索が行われるべきである．

私たちは今までに3つのアプローチを用いて探索をおこなっている．1つは家系例からSNP arrayにおいてhomozygosity mappingを行い，その領域にmapされる遺伝子をFLX454にてlong read sequenceする方法である．実際の検討では候補遺伝子が4000前後程度残り，塩基配列決定においての省力化にはつながらなかった．2つめの方法は，免疫に関連した分子群を抽出して，濃縮チップを作成し，全エクソン領域を解析する方法である．私たちはRAPID（Resource of Asian Primary Immunodeficiency Diseases）: URL http://rapid.rcai.riken.jp/RAPIDをベースに，既知遺伝子，候補遺伝子を抽出し，さらにRAPIDでの候補遺伝子抽出にも用いられているMGI（Mouse genome informatics）URL: http://www.informatics.jax.org/, RefDIC（Reference database of immune cells）URL: http://refdic.rcai.riken.jp/welcome.cgi, NetPath URL: http://www.netpath.org/などと共同研究者が独自に収集したB細胞亜群に特徴的に発現する分子群の情報などから，約2,500遺伝子を抽出し，その全エクソン解析を行っている．さらに3番目の方法として，すでに定法となった全エクソン解析も開始した．いずれの場合にも，SNP databaseが重要であり，現時点ではdbSNP135を元に日本人SNP情報を収集しつつ，標的を絞っているところである．いずれにせよできるだけ均一な集団での解析が重要であり，かつ家族歴があるものが優先して解析されることにより，新たな責任遺伝子同定も遠くないものと予想している．

VI. 再び臨床症状[1~5,14]

1. 感染症

多くは細菌感染症であり上下気道炎が多い．特に呼吸器感染症による気管支拡張症は重要で30-50%程度の患者で認められると共に，生命予後に大きく関与する．慢性感染症よりも重症感染症がその成立に関与しているとされている．多く，一方ニューモシスチス肺炎やMAC感染症ではT細胞性免疫不全症を疑う．

2. 消化管症状

消化管症状を呈する症例は多く，全国調査でも約1/3で認められた．多くは下痢・消化管感染症であり，この場合キャンピロバクターなどに加えて，サルモネラなどの細胞内寄生菌，CMV腸炎なども報告されている．また結節性リンパ様増殖（nodular lymphoid hyperplasia : NLH），萎縮性胃炎，炎症性腸疾患も有名である．NLHは約8%の患者で認められる．

3. 自己免疫疾患

自己免疫性溶血性貧血（Autoimmune hemolytic anemia : AIHA）や特発性血小板減少性紫斑病（Idiopathic thrombocytopenic purpura : ITP）が最も多い．また乾癬，悪性貧血，関節リウマチ，SLE，シェーグレン症候群なども認められる．

4. 悪性腫瘍

胃がんの発症危険度は7-16倍，悪性リンパ腫の発症危険度は12-18倍とされている．悪性リンパ腫ではB細胞由来が多く，EBVは陰性が多いとされている．

VII. 治　　療

1) γグロブリン補充

IgGは（500-）700 mg/dL以上を目標に補充を行うが，個人により至適IgGレベルが異なることに注意が必要である．1つには抗体の質（特異抗体の有無や親和性）の問題があるからであろう．またIgGは最低レベルを（500-）700 mg/dLとし，かつ発見時のIgGレベル＋300-500 mg/dL程度にするべきとの意見もある．いずれにせよ1,000 mg/dL程度に保ってはじめて感染症の頻度が減少する症例も多く経験し，それ以上とせざるを得ない場合もあ

る．

2. 合併する自己免疫疾患

AIHA や ITP に対してはステロイドやγグロブリン大量療法が試みられる．リツキサンを用いた報告もある．炎症性腸疾患に対しては 5-Aminosalicylic acid やステロイドを用い，ステロイドや TNF アンタゴニストが用いられている．合併したリウマチ性疾患に対する治療も，CVID の背景に関わらず標準治療が行われる．

3. 根治療法

いわゆる表 4 の SC-C, SC-D では造血細胞移植を考慮される場合がある．SC-A に対する造血細胞移植はまだ本格的には行われていないが，たとえば自己免疫疾患の管理や血液リンパ系腫瘍の治療に難渋する症例では考慮しても良いと思われる．

VIII. おわりに

CVID の病像と研究の現況について記載した．真の CVID とは何かという問題が残されているが，基本的には CVID は抗体産生不全型免疫不全症であり，特異抗体産生は不良で，かついわゆる T 細胞免疫不全症に合併するような感染症は稀と考えるべきである．成人領域で診療の機会も多く，また除外する疾患も多いため，もし診断に苦慮する場合も多い．筆者まで遠慮なくご相談いただければと思っている．

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文　献

1) Bonilla, F. A., Geha, R. S. : Common variable immunodeficiency. *Pediatric Research* **65** : 13R-19R, 2009.
2) Takahashi, N., Morio, T. : Common variable immunodeficiency. *Nihon Rinsho Meneki Gakkai Kaishi* **31** : 9-16, 2009.
3) Park, M. A., Li, J. T., Hagan, J. B., Maddox, D. E., Abraham, R. S. : Common variable immunodeficiency : a new look at an old disease. *Lancet* **372** : 489-502, 2008.
4) Yong, P. F. K., Tarzi, M., Chua, I., Grimbacher, B., Chee, R. : Common variable immunodeficiency : an update on etiology and management. *Immunology & Allergy Clinics of North America* **28** : 367-386, ix-x, 2008.
5) Yong, P. F. K., Thaventhiran, J.E.D., Grimbacher, B : "A rose is a rose is a rose," but CVID is Not CVID common variable immune deficiency (CVID), what do we know in 2011? *Advances in Immunology* **111** : 47-107, 2011.
6) 森尾友宏：分類不能型免疫不全症．炎症と免疫 **19** : 17-22, 2011.
7) Bayry, J. et al. : Common variable immunodeficiency is associated with defective functions of dendritic cells. *Blood* **104** : 2441-2443, 2004.
8) Borte, S. et al. : Interleukin-21 restores immunoglobulin production ex vivo in patients with common variable immunodeficiency and selective IgA deficiency. *Blood* **114** : 4089-4098, 2009.
9) Rigaud, S. et al. : Human X-linked variable immunodeficiency caused by a hypomorphic mutation in XIAP in association with a rare polymorphism in CD40LG. *Blood* **118** : 252-261, 2011.
10) Visentini, M. *et al.* : Telomere-dependent replicative senescence of B and T cells from patients with type 1a common variable immunodeficiency. *European Journal of Immunology* **41** : 854-862, 2011.
11) van Zelm, M.C., Szczepanski, T., van der Burg, M., van Dongen, J. J. M. : Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *Journal of Experimental Medicine* **204** : 645-655, 2007.
12) Morinishi, Y. et al. : Identification of severe combined immunodeficiency by T-cell receptor

excision circles quantification using neonatal guthrie cards. *Journal of Pediatrics* **155** : 829–833, 2009.

13) Sottini, A. et al. : Simultaneous quantification of recent thymic T-cell and bone marrow B-cell emigrants in patients with primary immunodeficiency undergone to stem cell transplantation. *Clinical Immunology* **136** : 217–227, 2010.

14) Chapel, H. et al. : Common variable immunodeficiency disorders : division into distinct clinical phenotypes. *Blood* **112** : 277–286, 2008.

15) Wehr, C. et al. : The EUROclass trial : defining subgroups in common variable immunodeficiency. *Blood* **111** : 77–85, 2007.

16) Kanegane, H. et al. : Novel mutations in a Japanese patient with CD19 deficiency. *Genes & Immunity* **8** : 663–670, 2007.

17) van Zelm, M. C. et al. : An antibody-deficiency syndrome due to mutations in the CD19 gene. *New England Journal of Medicine* **354** : 1901–1912, 2006.

18) Isnardi, I. et al. : Complement receptor 2/CD21– human naive B cells contain mostly autoreactive unresponsive clones. *Blood* **115** : 5026–5036, 2010.

19) van Zelm, M. C. et al. : CD81 gene defect in humans disrupts CD19 complex formation and leads to antibody deficiency. *Journal of Clinical Investigation* **120** : 1265–1274, 2010.

20) Kuijpers, T. W. et al. : CD20 deficiency in humans results in impaired T cell-independent antibody responses. *Journal of Clinical Investigation* **120** : 214–222, 2010.

21) Grimbacher, B. et al. : Homozygous loss of ICOS is associated with adult-onset common variable immunodeficiency. *Nature Immunology* **4** : 261–268, 2003.

22) Takahashi, N. et al. : Impaired CD4 and CD8 effector function and decreased memory T cell populations in ICOS-deficient patients. *Journal of Immunology* **182** : 5515–5527, 2009.

23) Warnatz, K. et al. : Human ICOS deficiency abrogates the germinal center reaction and provides a monogenic model for common variable immunodeficiency. *Blood* **107** : 3045–3052, 2006.

24) Yong, P. F. K., Salzer, U., Grimbacher, B. : The role of costimulation in antibody deficiencies : ICOS and common variable immunodeficiency. *Immunological Reviews* **229** : 101–113, 2009.

25) Castigli, E., Geha, R. S. : TACI, isotype switching, CVID and IgAD. *Immunologic Research* **38** : 102–111, 2007.

26) Castigli, E. et al. : TACI is mutant in common variable immunodeficiency and IgA deficiency. *Nature Genetics* **37** : 829–834, 2005.

27) Lee, J. J. et al. : The C104R mutant impairs the function of transmembrane activator and calcium modulator and cyclophilin ligand interactor (TACI) through haploinsufficiency. *Journal of Allergy & Clinical Immunology* **126** : 1234–1241, 2010.

28) Pan-Hammarstrom, Q. et al. : Reexamining the role of TACI coding variants in common variable immunodeficiency and selective IgA deficiency. *Nature Genetics* **39** : 429–430, 2007.

29) Salzer, U. et al. Mutations in TNFRSF13B encoding TACI are associated with common variable immunodeficiency in humans. *Nature Genetics* **37** : 820–828, 2005.

30) Zhang, L. et al. : Transmembrane activator and calcium-modulating cyclophilin ligand interactor mutations in common variable immunodeficiency: clinical and immunologic outcomes in heterozygotes. *Journal of Allergy & Clinical Immunology* **120** : 1178–1185, 2007.

31) Garibyan, L. et al. : Dominant-negative effect of the heterozygous C104R TACI mutation in common variable immunodeficiency (CVID). *Journal of Clinical Investigation* **117** : 1550–1557, 2007.

32) Warnatz, K. et al. : B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome in humans. *Proceedings of the National Academy of Sciences of the United States of America* **106** : 13945–13950, 2009.

33) Sekine, H. et al. : Role for Msh5 in the regulation of Ig class switch recombination. *Proceedings of the National Academy of Sciences of the United States of America* **104** : 7193–7198, 2007.

34) Salzer, U. et al. : Screening of functional and positional candidate genes in families with common variable immunodeficiency. *BMC Immunology* **9** : 3, 2008.

35) Tampella, G. et al. : Evaluation of CARMA1/CARD11 and Bobl as candidate genes in common variable immunodeficiency. *Journal of Investigational Allergology & Clinical Immunology* **21** : 348–353, 2011.