Introduction

Omenn syndrome (OS) is an autosomal recessive primary immunodeficiency and caused by mutations of the recombination activating genes \( \text{RAG1} \) or \( \text{RAG2} \). \(^3\) OS mutations maintain a residual recombination activity that allows limited T-cell receptor (TCR) gene rearrangements in the thymus, whereas null mutations cause a complete block of T- and B-cell development and lead to severe combined immunodeficiency (SCID) with absence of mature T and B lymphocytes (T\(^-\)B\(^-\) SCID). \(^4\) However, the occurrence of the same mutations in patients with T\(^-\)B\(^-\) SCID and OS suggests that “leaky” mutations in \( \text{RAG} \) genes may not be solely responsible for the development of OS. \(^5\)

Somatic revertant mosaicism is a rare phenomenon that is increasingly being described in human genetic disorders. \(^6,7\) In all cases reported to date, revertant cells carried a single revertant sequence. \(^5,7\) It is also recognized that revertant mosaicism is an additional basis for milder phenotype in several primary immunodeficiencies such as adenosine deaminase deficiency, \(^8\) X-linked SCID, \(^9\) and Wiskott-Aldrich syndrome. \(^10\) Here we describe an unusual case of RAG1 deficiency presenting somatic T-cell mosaicism due to multiple second-site mutations and show that the patient’s revertant T-cell mosaicism might have contributed to the modification of his clinical features.

Study design

Patient

The patient was the second child born to consanguineous, healthy Japanese parents. He developed generalized exudative erythroderma at age 1 month, followed by failure to thrive and persistent cough. At age 2 months, the patient was hospitalized for upper respiratory infections and otitis media. Two weeks later, he suffered from sepsis due to \textit{Pseudomonas aeruginosa}. Laboratory evaluation at age 3 months showed moderate anemia, leukocytosis (104 × 10\(^9\)/L [104 000/\(\mu\)L]) with marked eosinophilia (21.8 × 10\(^9\)/L [21 800/\(\mu\)L]), and hypogammaglobulinemia (immunoglobulin G [IgG], 1.48 g/L [148 mg/dL]; IgA, less than 0.01 g/L [less than 1 mg/dL]; IgM 0.02 g/L [2 mg/dL]; and IgE less than 2 kIU/L). The level of soluble interleukin-2 receptor was markedly elevated at 19 400 kIU/L (normal, 220-530 kIU/L). Immunophenotypic analysis showed the absence of peripheral B cells and marked increase of both CD4\(^+\) and CD8\(^+\) T cells with activated/memory phenotypes. A skin biopsy revealed lymphocytic infiltration in the upper dermis with occasional eosinophils and destruction of epidermal-dermal junction. Based on these findings, a clinical diagnosis of OS was made.

Cell isolation, sequencing, and TCR\(\beta\) repertoire

CD4\(^+\) and CD8\(^+\) T cells were purified using magnetic beads as described. \(^11\) CD16\(^+\) natural killer (NK) cells and CD4\(^+\)TCR\(\beta\)B8\(^+\) and CD8\(^+\)TCR\(\beta\)B1\(^+\)
T cells were separated from peripheral blood mononuclear cells (PBMCs) by an EPICS Elite flow cytometer (Beckman Coulter Fullerton, CA). Approval was obtained from the human research committee of Kanazawa University Graduate School of Medical Science for these studies, and informed consent was provided according to the Declaration of Helsinki. Mutation analysis of RAG genes, fluorescence-activated cell sorter (FACS) analysis of TCRVβ repertoire, and complementarity-determining region 3 (CDR3) spectratyping were performed as described.12,13

Results and discussion

Inherited mutations in either the RAG1 or the RAG2 gene resulting in partial V(D)J recombination activity have been detected in most OS patients.14 We found that our patient is homozygous for a single base C deletion after nucleotide 2113 of the RAG1 gene (delC) in DNA from his granulocytes (Figure 1A). His parents were both heterozygous for this novel mutation. In contrast, DNA from the patient’s PBMCs showed coexistence of the delC and other unexpected sequences (Figure 1A). When we analyzed such sequences in subcloned polymerase chain reaction (PCR) products obtained from his T cells, 6 different second-site mutations (mut no. 1–mut no. 6) were detected in addition to the delC mutation (Figure 1B). All of them restored the RAG1 reading frame and resulted in missense mutations, which were located in the RAG2-interacting domain (Figure 1C). Sequencing analysis in the general population excluded the possibility that they could be functional polymorphisms. The possibility that his T cells were derived from the maternal T-cell engraftment was ruled out by fluorescence in situ hybridization analysis for the detection of the X/Y chromosome and by standard molecular study of HLA typing (data not shown). In addition, the second-site mutations were not detectable in the mother’s PBMCs. We therefore concluded that T cells carrying the second-site mutations originated from the patient’s own hematopoietic cells in vivo.

The incidence of revertant mosaicism is considered rare, and revertant cells have been shown to carry a single revertant sequence in reported cases.6,7 Our studies, however, provide evidence for the presence of multiple and different second-site mutations in a single

Figure 1. Characterization of RAG1 gene mutations and T-cell receptor (TCR) Vβ repertoire. (A) The RAG1 gene was amplified from DNA extracted from normal PBMCs, the patient’s granulocytes, and PBMCs, and the parents’ PBMCs. Direct sequencing was performed using an automated sequencer. A thin bar shows the position of the delC mutation. Pt indicates patient. (B) Sequence analysis of the same genomic region in subcloned PCR products obtained from the patient’s T cells. A thick bar highlights the delC sequence. (C) Expression profile of TCRVβ subfamilies. Peripheral blood samples were stained with monoclonal antibodies (mAbs) for individual TCRVβ expression within CD4+ or CD8+ T cells was analyzed by a flow cytometry. (E) CDR3 spectratyping. Each TCRVβ fragment was amplified from cDNA with one of the Vβ-specific primers. The size distribution of PCR products was determined by an automated sequencer and GeneScan software.
Table 1. Genotypic analysis of lymphocyte subsets

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<tr>
<th>Lymphocyte subsets</th>
<th>Second-site mutations</th>
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<tr>
<td></td>
<td>No.  del C</td>
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<tr>
<td>PBMCs</td>
<td>81</td>
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<tr>
<td>CD4+ T cells</td>
<td>16</td>
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<tr>
<td>CD8+ T cells</td>
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<td>CD16+ NK cells</td>
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<td>Monocytes</td>
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<td>Granulocytes</td>
<td>24</td>
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Sequence occurrence/total number of sequences.

Acknowledgments

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References

Oligoclonal expansion of T lymphocytes with multiple second-site mutations leads to Omenn syndrome in a patient with RAG1-deficient severe combined immunodeficiency

Taizo Wada, Tomoko Toma, Hiroyuki Okamoto, Yoshihito Kasahara, Shoichi Koizumi, Kazunaga Agematsu, Hirokazu Kimura, Akira Shimada, Yasuhide Hayashi, Masahiko Kato and Akihiro Yachie