Detection of T lymphocytes with a second-site mutation in skin lesions of atypical X-linked severe combined immunodeficiency mimicking Omenn syndrome

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Abstract

X-linked severe combined immunodeficiency (XSCID) is caused by mutations of the common gamma chain (γc) and usually characterized by the absence of T and natural killer (NK) cells. Here, we report an atypical case of XSCID presenting with autologous T and NK cells and Omenn syndrome-like manifestations. The patient carried a splice-site mutation (IVS1+5G>A) that caused most of the mRNA to be incorrectly spliced but produced normally spliced transcript in lesser amount, leading to residual γc expression and development of T and NK cells. The skin biopsy specimen showed massive infiltration of revertant T cells. Those T cells were found to have a second-site mutation and result in complete restoration of correct splicing. These findings suggest that the clinical spectrum of XSCID is quite broad and includes atypical cases mimicking Omenn syndrome, and highlight the importance of revertant mosaicism as a possible cause for variable phenotypic expression.
Introduction

Omenn syndrome (OS) is a peculiar immunodeficiency characterized by erythroderma, lymphadenopathy, hepatosplenomegaly, eosinophilia, hypogammaglobulinemia, elevated serum IgE, and activated/oligoclonal T cells.\textsuperscript{1,2} The genes responsible for OS include $RAG1$, $RAG2$, $Artemis$, $RMRP$, and $IL7RA$.\textsuperscript{3-8} Clinical manifestations resembling OS were demonstrated in cases of severe combined immunodeficiency (SCID) with maternal T-cell engraftment and of atypical DiGeorge syndrome.\textsuperscript{9,10} Additionally, we have recently reported an X-linked SCID (XSCID) patient with massive skin infiltration of natural killer (NK) cells, resulting in OS-like manifestations.\textsuperscript{11} Here, we describe another case of XSCID mimicking OS. The patient carried a splice site mutation that allowed development of peripheral T and NK cells, and showed revertant T-cell mosaicism caused by a second-site mutation predominately in the skin. Both of these findings may have contributed to his phenotypic variation of XSCID.
Study design

Patient

A 5-month-old Japanese boy from non-consanguineous parents was hospitalized because of failure to thrive, protracted diarrhea and hypoproteinemia. He developed generalized erythematous rash and alopecia at 1 month of age (Figure 1A-B), followed by persistent cough, fever, hepatosplenomegaly and lymphadenopathy. Laboratory studies revealed leukocytosis (60.0 x 10^9/L) with eosinophilia, liver dysfunction, and a low level of serum immunoglobulin G (IgG; 0.17 g/L). Serum IgE levels were significantly elevated at 1,300 kIU/L. Immunophenotypic analysis of lymphocytes showed an increased percentage of CD3+ T cells (93.1%), the majority expressing activation markers, CD45RO and HLA-DR. The ratio of CD4+ to CD8+ T cells was 1.1. The patient’s CD20+ B and CD56+ NK cells were detectable at 3.8% and 3.2%, restrictively. The level of soluble interleukin-2 receptor was markedly elevated at 10,895 kIU/L. He had a normal thymus shadow. A skin biopsy exhibited massive lymphocytic infiltration and mild spongiosis (Figure 1C-D). His maternal half-brother died of interstitial pneumonia at infancy.

Cellular and molecular studies

Cell isolation, fluorescence-activated cell sorting (FACS) analysis for the common gamma chain (γc) and T-cell receptor (TCR) repertoire, spectratyping, microsatellite analysis, and mutation analysis of γc were performed as described elsewhere.11-15 The purity of the sorted CD4+ T, CD8+ T, CD19+ B, and CD56+ NK cells
was 98.1\%, 99.3\%, 86.4\%, and 98.3\%, respectively. T-cell and B-cell lines from the patient were established by transformation with Herpes virus saimiri and Epstein-Barr virus, respectively. Approval for the 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.
Results and Discussion

The patient’s clinical findings were reminiscent of OS. However, he showed neither mutation in the RAG genes (data not shown) nor severely restricted TCR repertoire (Figure 1E-F). Microsatellite analysis ruled out maternal T-cell engraftment (Figure 1G). Because of possible X-linked inheritance in this family and our previous experience\textsuperscript{11}, we assessed \( \gamma c \) expression. FACS analysis clearly demonstrated defective \( \gamma c \) expression on his lymphocytes and lymphocyte subpopulations, leading to the diagnosis of XSCID (Figure 1H). The patient had a G to A substitution at the fifth nucleotide of intron 1 of the \( IL2RG \) (IVS1+5G>A) that was previously listed in the IL2RG database (http://research.nhgri.nih.gov/apps/scid/IL2RGbase.shtml), and that caused most of the mRNA to be incorrectly spliced but produced, in lesser amount, normally spliced wild-type transcript (Figure 2A-D). When we analyzed such sequences in subcloned RT-PCR products obtained from his peripheral blood mononuclear cells, the wild-type sequence was detected in 15\% of 20 clones. Accordingly, his lymphocytes showed the residual \( \gamma c \) protein that likely permitted T and NK cell development to occur. Similar results were obtained from the study in another case of atypical XSCID with poorly functioning peripheral T cells.\textsuperscript{16}

The presence of circulating T cells offered us a unique opportunity to establish T-cell lines from XSCID. Unexpectedly, FACS analysis showed a significant fraction of cells with normal \( \gamma c \) expression among his T-cell lines (Figure 2E). Therefore, sequence analysis was performed using the cloned T-cell lines. We found the IVS1+5G>A in DNA from the \( \gamma c \)-defective clone (#3-4), whereas DNA from the \( \gamma c \)-expressing clone (#3-1)
showed coexistence of the IVS1+5G>A and a second-site mutation (IVS1+29G>A) which was located at position +1 of the cryptic 5’ splice site (Figure 2F). Analysis of 100 normal chromosomes demonstrated the absence of the IVS1+5G>A and the IVS1+29G>A. The IVS1+29G>A was found to suppress the effect of the IVS1+5G>A and result in complete restoration of correct splicing that was consistent with the restored γc expression on the cell surface (Figure 2G). To determine whether the IVS1+29G>A was present in vivo, direct sequencing was performed with his various DNA samples. A peak of the IVS1+29G>A was only detectable in his skin specimen, but was absent among circulating CD4+ and CD8+ T and CD19+ B cells (Figure 2H). When we analyzed the sequence in subcloned PCR products obtained from the skin, the IVS1+29G>A was detected in 12.9% of 31 clones. RT-PCR and GeneScan analysis demonstrated that a peak of the normal-sized transcript but not the mutated one was evident in the skin. Because IL2RG mRNA is expressed only in hematopoietic cells, these findings suggest that the majority of skin-infiltrating lymphocytes carry the IVS1+29G>A, thus indicating revertant cells (Figure 2I).

Although revertant γc expression would confer selective advantage in vivo to T and NK cell progenitors, we could not detect the γc-expressing T cells in his circulation. This lack of in vivo selective advantage is in contrast to a previously described XSCID patient with revertant T-cell mosaicism in whom a reasonably diversified TCR repertoire was generated from a single T-cell progenitor, resulting in a mild clinical course. The reasons underlying these findings are presently unclear. It is possible that the substantial numbers of activated peripheral T cells with defective γc expression that were not seen in the previous case might have competed with the revertants, reduced the abilities of
selective advantage of $\gamma c$ expression, and provided insufficient time for the reversion to reach the detection threshold in our patient. On the other hand, selective proliferation of revertant CD8$^+$ T cells with TCR VB2 in the skin (Figure 2J-K) may be due to a clonal expansion in response to infections or autoantigens and be involved in pathogenesis of his skin lesions. However, his complicated condition including the presence of autologous $\gamma c$-defective T and NK cells in the peripheral blood and the expansion of revertant $\gamma c$-expressing T cells in the skin does not allow us to draw clear conclusion about which aspects are responsible for the development of OS phenotype. Since recent studies in OS patients and murine models have indicated that impaired immune tolerance and defective immune regulation play important roles in the pathophysiology of OS$^{2,20}$, the highly elevated numbers of activated, $\gamma c$-defective T cells with a moderate restricted TCR repertoire may have more contributed to the OS phenotype in our patient. If this is the case, it is possible that any SCID diseases having a profound but incomplete block in T-cell development could result in OS.

In summary, our studies indicate that the clinical spectrum of XSCID is broad and includes immunodeficient patients mimicking OS. In addition, the detection of the revertant T cells caused by the second-site suppressor mutation makes our case a second example of genetic reversion in XSCID and highlights the importance of somatic revertant mosaicism as a possible cause for atypical clinical presentations.
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Statement of authorship:

T.W., and A.Y. participated in designing and performing the research; T.T., Y.N., M.N., M.S., M.O., Y.K., and S.K. analyzed data; M.Y, M.I., and K.K. made clinical contribution and provided critical paper review; T.W. wrote the paper; and all authors checked the final version of the manuscript.

Conflict of Interest Disclosure: The authors declare no competing financial interests.
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Figure Legends

Figure 1. Skin lesions, skin biopsy specimen, T-cell receptor repertoire, microsatellite markers, and γc expression. (A) Alopecia. (B) Generalized scaling erythroderma. (C) Hematoxylin and eosin, original magnification x40 (D) Hematoxylin and eosin, original magnification x100. (E) Expression profile of TCR VB subfamilies. Peripheral blood samples were stained with monoclonal antibodies (mAbs) for individual TCR VB together with anti-CD4 and anti-CD8 mAbs. The percentage of each TCR VB expression within CD4+ or CD8+ T cells was analyzed by FACS. (F) CDR3 spectratyping. Each TCR VB fragment was amplified from cDNA with one of the VB-specific primers. The size distribution of PCR products was determined by an automated sequencer and GeneScan software. (G) Microsatellite analysis. Two different markers were amplified with FAM-labeled specific primers and subjected to GeneScan analysis. HO-1 indicates heme oxygenase-1. (H) Analysis of γc expression. Shown are the results of γc expression on lymphocytes and lymphocyte subsets. Solid peaks indicate control Ab; open peaks represent anti-CD132 mAb.

Figure 2. Characterization of inherited IL2RG gene mutation, second-site suppressor mutation, and skin-infiltrating lymphocytes. (A) The IL2RG gene was amplified from DNA extracted from normal, the patient’s, and the mother’s peripheral blood mononuclear cells (PBMCs). Direct sequence was performed using an automated sequencer. Bars show the locations of the mutation. (B) RT-PCR analysis for IL2RG mRNA using PBMCs obtained from normal control (lane 1) and the patient (lane 2).
Lane M contains a 100-bp molecular size marker. (C) Direct sequence analysis of IL2RG cDNA using PBMCs from normal control and the patient. (D) Schematic representation of effect of the inherited splice-site mutation. (E) Analysis of γc expression. Shown are the results of γc expression on B-cell and T-cell lines, and cloned T cells #3-4 and #3-1 established from the patient. (F) Direct sequence analysis of IL2RG gene using DNA obtained from the clone #3-1. A bar shows the locations of the inherited mutation and an arrow highlights the second-site mutation. (G) Direct sequence analysis of IL2RG cDNA from the clone #3-1. (H) Direct sequencing analysis of IL2RG gene using DNA obtained from the patient’s primary CD4+ and CD8+ T cells and CD19+ B cells, and his skin. (I) GeneScan analysis of IL2RG cDNA amplified from the clone #3-1, the primary lymphocytes including CD4+ and CD8+ T cells and CD19+ B cells, and the skin of the patient. A peak of the size of approximately 454 nucleotides represents wild-type mRNA and a second peak of approximately 482 nucleotides is generated by aberrant splicing. (J) FACS analysis of skin-infiltrating lymphocytes. The percentage of cells gated in each region is shown. (K) Immunohistochemical staining of the skin biopsy specimen. The samples were stained with anti-CD8 mAb.
Figure 1A-D
Figure 1E-H

E

Control | Patient
CD4+ T | CD4+ T
TCR VB | TCR VB
CD8+ T | CD8+ T

F

CD4+ T

CD4+ T

Fluorescence intensity

CD8+ T

CD8+ T

CDR3 size

G

Mother
Patient Granulocytes
Patient CD4+ T
Patient CD8+ T
Patient CD56+ NK
Patient Skin

D9S1198 | HO-1 promoter

H

Lymphocytes
CD4+ T
CD8+ T
CD19+ B
CD56+ NK

Control | Patient
Molecular size | Fluorescence intensity

Relative cell number
Figure 2
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