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A Case Report of Respiratory Syncytial Virus-Infected 8p Inverted Duplication Deletion Syndrome with Low Natural Killer Cell Activity

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The 8p inverted duplication deletion [inv dup del(8p)] is a complex structural rearrangement in chromosome 8. Patients with this chromosomal abnormality exhibit developmental delay, facial dysmorphism, central nervous abnormalities, hypotonia, orthopedic abnormalities, and congenital heart defects. However, cellular immune function in inv dup del(8p) syndrome has never been reported. We present the case of a 1-month-old boy with inv dup del(8p) syndrome who had severe respiratory syncytial (RS) virus bronchiolitis. Natural killer (NK) cells are recruited to airway epithelium in the early phase of RS viral infection. A cluster of defensin genes (*DEF*s), which are deleted in inv dup del(8p), are located in 8p23.1. Human defensins are involved in antiviral activity through the NK cell-mediated cytotoxic pathway and envelope disruption in the normal immune response. This patient showed lower NK cell activity and *α*-defensin level compared with healthy controls. These results suggest that decreased NK cell activity can result from *DEF* haploinsufficiency. In addition to a skeletal deformity with chromosomal abnormality, NK cell-mediated immune deficiency may account for the exacerbation of RS virus bronchiolitis.

Keywords: 8p inverted duplication deletion; cell-mediated immunity; defensin; natural killer cell; respiratory syncytial virus bronchiolitis
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Introduction

The 8p inverted duplication deletion [inv dup del(8p)] is a complex structural rearrangement in chromosome 8. The frequency of inv dup del(8p) syndrome is estimated to be one in 20,000-30,000 liveborn infants (Floridia et al. 1996). Patients with inv dup del(8p) syndrome exhibit developmental delay (100%), facial dysmorphism (97%), central nervous abnormalities (80%), hypotonia (66%), orthopedic abnormalities (58%), and congenital heart defects (26%) (Guo et al. 1995).

Immunodeficiency causes exacerbation of lower respiratory tract infection in patients with some chromosome abnormalities, including Down syndrome and DiGeorge syndrome (Hilton et al. 1999; Gennery et al. 2002). Respiratory syncytial (RS) virus bronchiolitis was reported to be exacerbated by mutation in several genes related to innate immunity (Janssen et al. 2007). Recurrent infection was reported to be a phenotype of 8p structural abnormalities in the DECIPHER database (https://decipher.sanger.ac.uk). *DEF* and *ERI1*, which encode defensins and exoribonuclease 1, respectively, are genes located near the 8p deletion breakpoint and are involved in cell-mediated immunity (Taudien et al. 2004; Logsdon et al. 2021). Natural killer (NK) cell activity may be affected by the disappearance of these genes. However, exacerbation of RS virus bronchiolitis and waning NK cell-mediated immunity in patients with inv dup del(8p) syndrome has not been reported. Here, we report the case of an infant with RS virus-infected inv dup del(8p) syndrome whose NK cell activity and defensin expression were attenuated.

Case Presentation

This patient was born at 37 weeks gestation by spontaneous vaginal delivery. His birth weight, length, and head circumference were 2,898 g, 48.7 cm, and 32.5 cm, respectively, which were appropriate for his gestational age. His symptoms included muscular hypotonia, poor suckling, and

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poor weight gain. Additionally, the patient had other health problems, including high arched palate, funnel chest, and right congenital inguinal hernia. Unilateral sensorineural hearing loss was also detected. Computed tomography of the brain showed hypoplasia of the corpus callosum. The presence of a congenital heart defect was not explored by ultrasonography.

G-banded chromosome analysis suggested the presence of inverted duplication of a short arm segment 8p11.2-8p23.1 and deletion of a region 8pter-8p23.1. Fluorescence *in situ* hybridization (FISH) analysis, undertaken using a chromosome 8 painting probe, confirmed fluorescent staining along the entire length of both normal and abnormal chromosome 8 (Fig. 1A). Using an 8pq subtelomeric probe, normal chromosome 8 showed signals at both the short and long arms, whereas abnormal chromosome 8 showed signals only at the long arm (Fig. 1B). This result suggested that the duplicated region was derived from chromosome 8, and the end of short arm was deleted. Thus, his karyotype was inv dup del(8)(qter→p23.1::p23.1→p11.2:) (Fig. 2).

He was admitted to hospital at 1 month of age with a diagnosis of RS virus bronchiolitis. RS viral infection was diagnosed using the Quick Navi^{TM}-RSV2 (Otsuka Pharmaceutical Co., Ltd, Tokyo, Japan). Although tachypnea with a respiratory rate of 50/min and retractive breathing were observed, percutaneous oxygen saturation was maintained within the range of 95%-99%. The peripheral blood cell examination showed white blood cell counts of 7,900/μL (4,471/μL neutrophils). C-reactive protein was negative. Venous pH was 7.386, partial pressure of carbon dioxide (pCO_2) was 50 mmHg, and bicarbonate ions were 29.9 mmol/L, indicating compensatory respiratory acidosis. Chest X-ray showed focal air trapped in the lower left lobe. High-flow nasal cannula therapy with a FiO_2 of 0.3 and 8 L/min was performed and supported by biphasic cuirass ventilation starting on day 2 after admission because of the worsened respiratory conditions. His respiratory condition was the worst on day 5 after admission. Expiratory wheezes were clearly observed in the bilateral chest, and venous blood gas analysis showed decompensated respiratory acidosis (pH 7.21; pCO_2 85.2 mmHg). He was immediately treated with transvenous hydrocortisone (5 mg/kg) followed by prednisolone sodium succinate (0.5 mg/kg three times a day for 2 days). He recovered from respiratory failure in 1 week. Intubation and artificial ventilation were avoided.

NK cell activity and absolute counts were examined at 5 months of age. His NK cell activity was measured 9.9% using a ^{51}Cr release assay at an Effector/Target ratio of 20:1 (Table 1). His NK cell absolute count, which was defined using flow cytometry as CD16 and CD56-positive cells, was 564 cells/μL. A reference value for NK cell activity was previously reported as 36.1% ± 12.3% (mean ± SD) in 1-5-month-old infants (Yabuhara et al. 1990), and that of NK cell absolute counts was 420 cells/μL (median) in 5-9-month-old infants (Shearer et al. 2003). Thus, NK cell activity was lower in this patient compared with age-matched healthy controls. Additional immunological investigations were performed at 11 months of age. All other laboratory data were in the normal range (Table 1). The patient was vaccinated in accordance with the recommended schedule, and there were no complications following live vaccines, including rotavirus and BCG vaccines.

Plasma α-defensin level

Defensins, which are encoded by defensin genes (*DEF*s), are divided into three subfamilies (defensin α, β, and θ). Among six isoforms of the α-defensin family, human neutrophil peptide (HNP)1, 2, and 3 isoforms exist

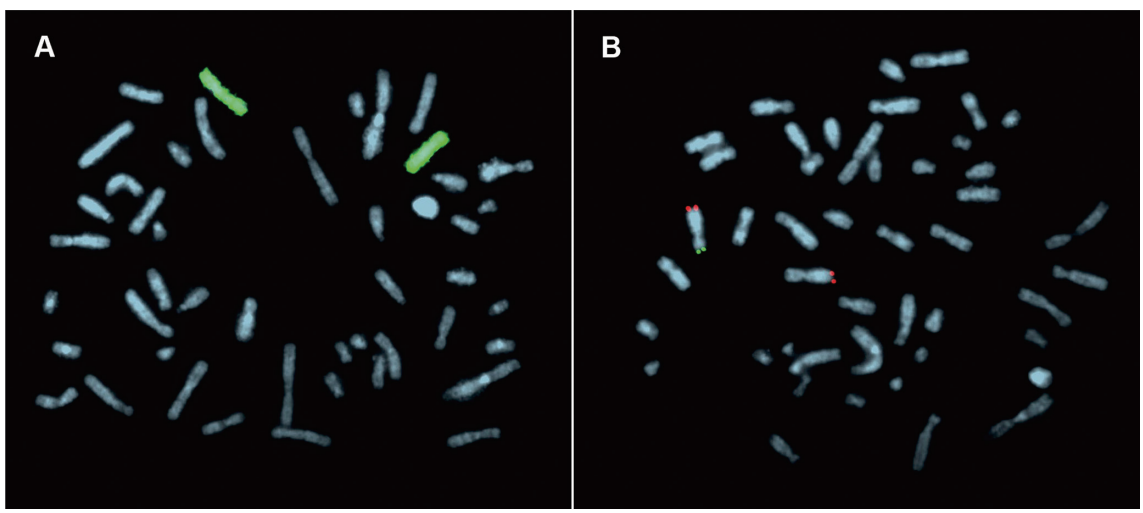

Fig. 1. Fluorescence in situ hybridization (FISH) analysis.
(A) Chromosome 8 painting probe indicated the entire length of both normal and aberrant chromosome 8 (green). (B) Chromosome 8pq subtelomeric probe indicated subtelomeres of short arms (green) and long arms (red). The absence of green signals on the aberrant chromosome 8 shows a deletion of the 8p terminal region.

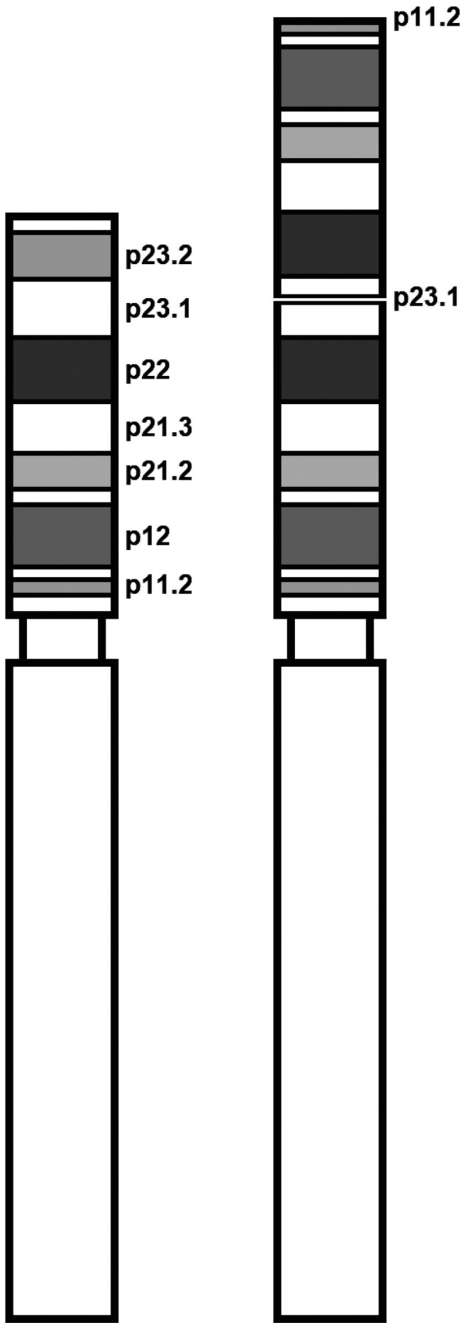

Fig. 2. A schematic diagram of homologous chromosome 8 in inv dup del(8p).
In the abnormal chromosome 8, 8p23.1-8pter is deleted and the inverted duplicated 8p11.2-8p23.1 is recombined.

in neutrophils and NK cells, whereas HNP4 exists only in neutrophils. Human α-defensin (HD)5 and 6 are isoforms derived from Paneth cells (Klotman and Chang 2006). To investigate the basal HNP1-3 level, the patient's plasma HNP1-3 concentration was quantified by enzyme-linked immunosorbent assay (ELISA) using a commercial human HNP1-3 ELISA kit (Hycult Biotechnology B.V., Uden, The Netherlands) at 11 months of age. Plasma samples from four healthy controls [median age (range), 16.5 (15-20) months] were also examined. The plasma samples were stored at −40°C after separation by centrifugation at 3,500 rpm for 5 min at room temperature. The plasma samples and standards were incubated for 60 min in microtiter wells coated with human HNP1-3 antibodies. After thorough washing six times, the second antibody containing biotinylated tracer antibody was added to capture human HNP1-3 for 60 min, and they were washed again and incubated with streptavidin-peroxidase conjugate for 60 min. The reaction was stopped with 1 mol/L sulfuric acid and the HNP1-3 concentration was measured at 450 nm using a microplate reader. The plasma α-defensin levels for this patient and the controls were 0.14 ng/mL and 1.21 ng/mL (mean; range 0.26-2.88 ng/mL), respectively (Fig. 3). Thus, the patient's plasma α-defensin level was lower than that of the controls. This result suggested that decreased NK cell activity can result from *DEF* haploinsufficiency.

Informed consent was obtained from the parents of this patient and controls. This report was approved by the Ethics Committee at Hirosaki General Medical Center (reference number: 2021-35 and 2022-01).

Discussion

Inv dup del(8p) consists of a deleted distal segment, an intact in-between segment, and a duplicated proximal segment (Shimokawa et al. 2004). The telomeric region deletion (8p23.1→pter) includes a wide range of deletion sizes from 6.09 to 11.35 Mb (García-Santiago et al. 2015; Chen et al. 2016). *ARHGEF10*, *CSMD1*, *MCPH1*, *ERI1*, and approximately 40 other genes are located in this region (Logsdon et al. 2021). In the 8p23.1 region, there are homologous low-copy repeats (LCRs), i.e., repeat-distal (REPD) and repeat-proximal (REPP), which comprise complex repeats containing olfactory receptor gene clusters (Fig. 4) (Sugawara et al. 2003). The hotspot for deletion breakpoints was approximately 7 Mb proximal to 8pter, (i.e., REPD) due to unequal recombination between REPD and REPP (Giglio et al. 2001; Shimokawa et al. 2004). The frequency of clinical symptoms in this syndrome differs depending on the deleted or duplicated range (García-Santiago et al. 2015).

*DEF*s clusters are located adjacent to REPD, and they are deleted in most of the inv dup del(8p) (Fig. 4) (Taudien et al. 2004). Human defensins are small antimicrobial peptides that are found in neutrophils, NK cells, monocytes/macrophages, and epithelial cells (Klotman and Chang 2006). Defensins have an important role particularly in innate immune systems, including the NK cell-mediated cytotoxic pathway (Ganz 2003; Chalifour et al. 2004). Chemokines secreted from airway epithelial cells recruit NK cells in the early phase of RS viral infection (Pribul et al. 2008). Microbial antigens activate NK cells through Toll-like receptors, resulting in cytokine and defensin secretion from activated NK cells (Chalifour et al. 2004). Thompson et al. (2006) reported that defensin levels were elevated in tracheal aspirates from infants with an RS viral infection during the acute phase. The RS virus is a single-

Table 1. Immunological investigation results.

Immunological investigation	Results	Reference values	Unit
Absolute leukocyte count			
Absolute neutrophil count	3,720		/μL
Absolute lymphocyte count	10,850		/μL
Absolute monocyte count	480		/μL
Lymphocyte subsets			
T cell ($CD3^+CD19^-$)	6,763	3,930 (1,900-5,900)[*1]	/μL
	67.8	65 (49-76)[*1]	%
B cell ($CD3^-CD19^+$)	2,451	1,520 (610-2,600)[*1]	/μL
	24.6	24 (14-37)[*1]	%
NK cell ($CD3^-CD16^+CD56^+$)	564	420 (170-830)[*1]	/μL
	5.6	7 (3-15)[*1]	%
Lymphocyte proliferation tests (stimulation index)			
PHA^+	1,211.30	147.5-1,251.3[*2]	
Con A^+	307.3	38.1-385.5[*2]	
Immunoglobulins			
IgG	346	371.8 (11-769)[*3]	mg/dL
IgM	55	48.8 (11-221)[*3]	mg/dL
IgA	41	22.1 (4-110)[*3]	mg/dL
Complement activity			
C3	109	72.3 (38.0-131.3)[*3]	mg/dL
C4	11	13.4 (5.1-35.5)[*3]	mg/dL
CH50	38.5		U/mL
NK cell activity	**9.9**	36.1 (± 12.3)[*4]	%

NK cell activity was evaluated at 5 months of age. Other parameters were evaluated at 11 months of age.
[*1]median (10^{th} and 90^{th} percentiles) (Shearer et al. 2003).
[*2]manufacture's standard value.
[*3]mean (95% confidence interval) (Kardar et al. 2012).
[*4]mean (± SD) (Yabuhara et al. 1990).
NK, natural killer; PHA, phytohemagglutinin; Con A, concanavalin A; Ig, immunoglobulin; SD, standard deviation.

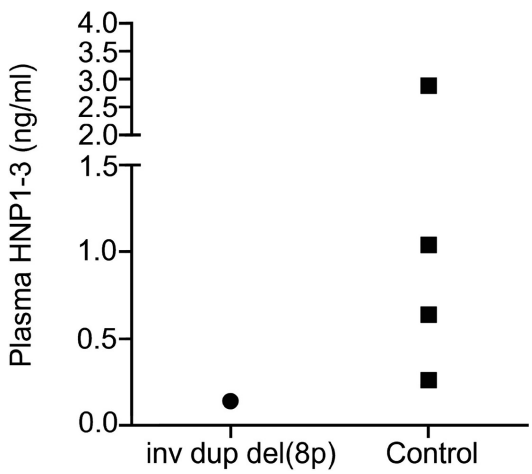

Fig. 3. Plasma α-defensin (HNP1-3) levels.
The patient's plasma α-defensin level was 0.14 ng/mL. The mean plasma α-defensin level from four healthy controls was 1.21 ng/mL (measurements were 0.26, 0.64, 1.04, and 2.88 ng/mL, and their age distribution was 17, 15, 20, and 16 months, respectively).

stranded RNA virus with an envelope (Rima et al. 2017), and defensins have antiviral activity against this envelope structure because they damage the lipid bilayer (Kota et al. 2008). Thus, in healthy individuals, recruited NK cells exert anti-RS viral effects by releasing defensins and subsequently disrupting the viral envelope. RS virus-infected airway epithelium cells also recruit neutrophils, monocytes, eosinophils, and T and B lymphocytes through cytokine and chemokine secretion (Openshaw and Tregoning 2005). They also play an important role in the anti-RS viral immune response. In the present case, the plasma defensins concentration and NK cell activity were lower compared with those of the controls, whereas other immunological investigations, including absolute lymphocyte count, lymphocyte reactivity *in vitro* after PHA/Con-A stimulation to confirm T cell-mediated immunity, and measurement of immunoglobulins to evaluate humoral immune response, were within normal limits. RS virus-specific antibodies were not assessed. Recurrent infection has been reported as a phenotype of inv dup del(8p) in the DECIPHER database

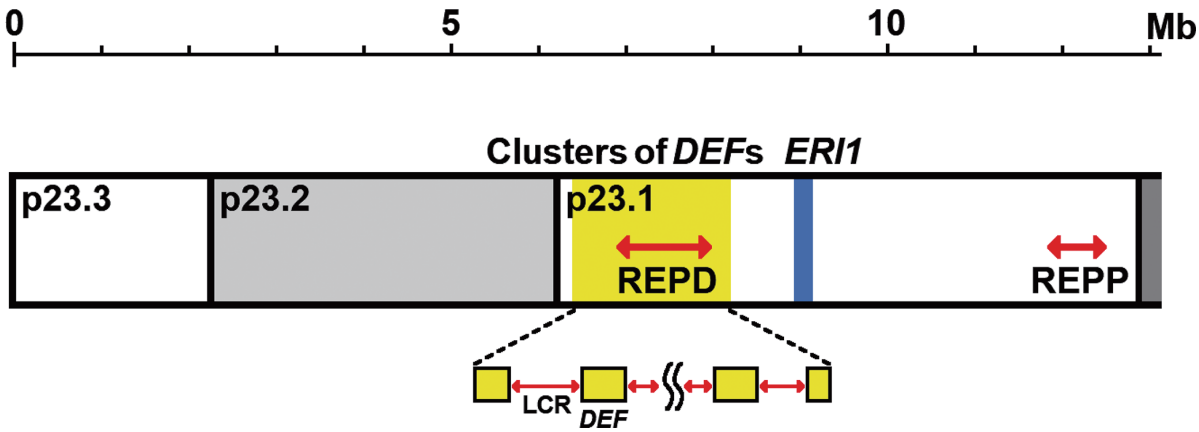

Fig. 4. A schematic diagram of around 8p23.1 deletion breakpoints.
Repeat-distal (REPD) and repeat-proximal (REPP) are sites containing homologous low-copy repeat (LCRs), wherein unequal recombination is likely to occur. REPD and REPP are located 6.9-8.0 Mb and 11.9-12.6 Mb from 8pter, respectively. Clusters of defensin genes (*DEF*s) are located 6.3-8.3 Mb from 8pter, and several LCRs are part of the REPD flank between each *DEF* (double-headed arrows). Exoribonuclease 1 gene (*ERI1*) is located 9 Mb from 8pter.

(https://decipher.sanger.ac.uk). Immune deficiency is most likely to be present in this case. The inv dup del(8p)-attributable haploinsufficiency of *DEF*s was likely to contribute to NK cell inactivation and RS virus bronchiolitis exacerbation. Although we are the first to show that the basal plasma α-defensin level was lower in this case than that in all four controls, it has been reported that plasma α-defensin levels in children were increased (median 450 ng/mL; range 194-1,031 ng/mL) under septic conditions (Thomas et al. 2002). To confirm the contribution of defensin to anti-RS viral immunity in infants, a multivariate analysis involving a large number of patients and evaluation at the acute phase of viral infection are required.

Human exoribonuclease 1 gene (*ERI1*), which encodes exoribonuclease 1, is required to promote normal NK cell immune function in mice (Thomas et al. 2012). *ERI1* is located 9 Mb from 8pter (Fig. 4) (Logsdon et al. 2021). The hotspot for deletion breakpoints was approximately 7 Mb proximal to 8pter. However, if there is a wide-range deletion over 9 Mb from 8pter, NK cell activity may be affected by *ERI1* disappearance.

NK cell activity was suppressed to approximately 30% of that of the reference data in this patient. *FCGR3A*, *DOCK2*, and *IRF8* on chromosomes 1, 5, and 16, respectively, were reported to be NK cell activity regulatory genes (Jawahar et al. 1996; Dobbs et al. 2015; Mace et al. 2017). NK cell activity can be rescued by *DEF*s clusters on a normal chromosome 8 and by other proteins that are encoded by these genes or other molecular mechanisms.

To the best of our knowledge, there are no published reports indicating that patients with inv dup del(8p) syndrome exhibit low NK cell activity and low plasma defensin concentration, resulting in RS virus bronchiolitis exacerbation. Generally, viral respiratory tract infection in patients with chromosome abnormalities is thought to be exacerbated by thorax deformity or upper airway complications. Immunological assessment is often not performed in these patients because primary immunodeficiency is not suspected (Schatorjé et al. 2016). Although our patient congenitally exhibited hypotonia and funnel chest, low defensin expression-attributable NK cell-mediated immune deficiency may partly participate in RS virus bronchiolitis exacerbation. We believe that the present report provides new findings of cell-mediated immunity in patients with inv dup del(8p) syndrome. Additional investigation is needed to clarify the pathological mechanism of cell-mediated immunity in inv dup del(8p) syndrome.

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Conflict of Interest

The authors declare no conflict of interest.

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