Perforin gene mutations in adult-onset hemophagocytic lymphohistiocytosis

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ABSTRACT

Perforin gene (PRF1) mutations cause the primary form of hemophagocytic lymphohistiocytosis (HLH). We report a genetic defect of PRF1 in a 62-year-old Japanese man with recurrent episodes of HLH. Sequencing of PRF1 from both peripheral blood mononuclear cells and nail clippings showed compound heterozygous mutation, including deletion of two base pairs at codons 1090 and 1091 (1090–1091delCT) and guanine-to-adenine conversion at nucleotide position 916 (916G→A). Although primary HLH has been detected in infants and children, genetic mutation of PRF1 or other genes should be considered a differential diagnosis of HLH even in the elderly.

Key words: perforein mutations, hemophagocytic lymphohistiocytosis

The primary form of hemophagocytic lymphohistiocytosis (HLH),[1-3] also known as familial hemophagocytic lymphohistiocytosis (FHL), shows autosomal recessive inheritance which is fatal unless aggressively treated. Mutations in the perforin gene (PRF1) were first documented in patients with FHL by Stepp *et al*., and have been classified as FHL type2.[4] The onset of FHL typically occurs within the first year of life in 70-80% of cases, and the number of patients under 6 months of age is especially high.[5-7] We report a case of HLH at 62 years of age, referable to compound heterozygous PRF1 mutation.

A 62-year-old man was referred to us on August 18, 2004 because of persistent fever. There was no family history of unexplained fever, pancytopenia, or lymphoid malignancies. He had had tuberculosis-related pleurisy at the age of 23. He had no history of any cancer. Late in July 2004, the patient had been admitted to a regional hospital after onset of fatigue and high fever. Physical examination demonstrated marked hepatosplenomegaly. Laboratory results included hemoglobin, 10.6 g/dL; white blood cell (WBC) count, 2.9×10⁹/L; platelet count 50×10⁹/L; lactate dehydrogenase (LDH) 1,054 U/L (normal 220 to 430); ferritin 13,078 ng/mL (normal 5 to 120); and soluble interleukin-2 receptor 9,044 U/mL (normal 190 to 650). A bone marrow aspirate was reported to show normocellular marrow without hemophagocytosis. The patient was treated with broad-spectrum antibiotics without improvement. When his condition deteriorated and fever persisted, he was referred to us. Re-evaluation of the previously obtained bone marrow aspirate detected hemophagocytosis. On admission, his temperature was 37.1°C, with a gradual improvement in his general condition. The spleen was palpable 7 cm below the left costal margin. Laboratory results showed moderate pancytopenia (WBC 2.27×10⁹/L; hemoglobin, 9.9 g/dL; platelets 6.8×10⁹ /L); LDH 313 U/L (normal 119 to 229); and elevated ferritin 6,306 ng/mL. Serologic tests for herpes simplex virus (HSV), Epstein Barr virus (EBV), human herpes virus-6 (HHV-6), cytomegalovirus (CMV), and parvovirus B19 showed past infection, and PCR blood examinations were negative for HSV, EBV,
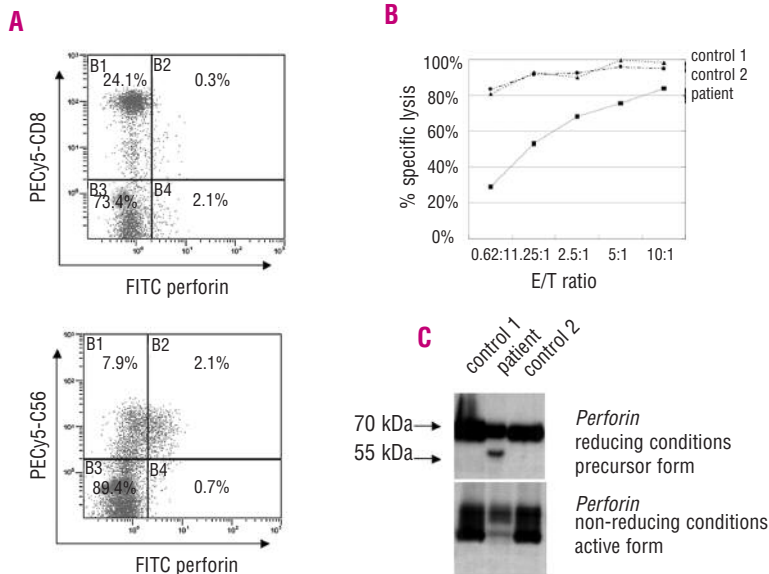
A

B

C

Figure 1. Perforin expression and function. A. Flow cytometric analysis of intracellular perforin protein expression. Peripheral blood mononuclear cells were stained with R-phy-coerythrin Cy-Chrome (PECy5)-conjugated anti-CD8 or anti-CD56 monoclonal antibody (mAb) followed by intracellular staining with fluorescein isothiocyanate (FITC)-conjugated anti-perforin mAb after lysing and permeabilizing procedures. B. Cytotoxic activity of alloantigen-specific CD8+ T-cell lines generated from peripheral blood mononuclear cells of the patient and healthy controls by stimulation with allogeneic B-LCL (KIN-LCL). Cytotoxicity against allogeneic KIN-LCLs was compared between the patient and controls. C. Perforin expression by Western analysis under reducing and non-reducing conditions. Alloantigen-specific CD8+ T-cell lines from the patient and healthy controls were cultured. Extracts of these cells (20 mg each) were analyzed by Western blot with anti-perforin antibody.

HHV-6, CMV and parvovirus B19. Eleven days after admission his general condition recovered and palpable splenomegaly had resolved. He was discharged with a diagnosis of HLH of undetermined etiology.

Late in September 2005, fever developed following symptoms of an upper respiratory infection and the patient consulted our hospital on October 18. On physical examination, body temperature was 38.5°C and the spleen was palpable 6 cm below the left costal margin. On readmission, laboratory data were WBC 3.34×10^9/L; hemoglobin 11.4 g/dL; platelets 8.6×10^9/L and LDH 510 U/L. A bone marrow examination again showed hemophagocytosis. Symptoms resolved in a week and he was discharged on November 11. However, he soon developed another upper respiratory infection and again developed HLH in mid-November. When the patient was readmitted on November 20, his body temperature was 39.5°C and the spleen was palpable at 15 cm. Hydrocortisone 100 mg was administered for 2 days, and the symptoms resolved completely. He was discharged on December 1. At the time of this report, in December 2006, he remains asymptomatic. Laboratory values are within normal limits.

Design and Methods

Flow cytometric analysis

We obtained peripheral blood mononuclear cells (PBMNC) and performed flow cytometric analysis as previously described.[8]

Assay for NK cell activity

NK cell activity among PBMNC was measured by incubating the cells with K562 targets for 4 hours at an effector-target (E/T) cell ratio of 20:1 (normal >18%).[9]

Generation of alloantigen-specific cytotoxic T cell (CTL) lines and analysis of CTL cytotoxicity

Alloantigen-specific CD8+ CTL lines were generated and analyzed as previously described.[10]

Sequencing of PRF1

This analysis was approved by the Institutional Review Board, and written informed consent was obtained from the patient. Genomic DNA was extracted from the patient's PBMNC and nail clippings. The coding region of PRF1 in exons 2 and 3 was sequenced as previously described.[4,9]

Western analysis

CTL lines were subjected to Western analysis as previously described.[11]

Results and Discussion

Flow cytometry showed markedly decreased intracellular perforin expression in both CD8+ and CD56+ cells. CD8+/perforin+ cells were 0.3% (normal 16.8%±6.0%) and CD56+/perforin+ cells 2.1% (normal 20.0%±7.2%) (Figure 1A).

NK cell activity of the patient had moderately decreased to 12.7%. Cytotoxic activities of CD8+ alloantigen-specific bulk T-cell lines generated from the patient and two healthy controls are shown in Figure 1B. Tested at an E/T ratio of 1.25:1, cytotoxicity of CTL generated from the patient was clearly low (53%) compared with CTL from two healthy controls (92% and 93%). Sequencing of PRF1 detected compound heterozygous mutation in both PBMNC and somatic nail cells. As shown in Figure 2, one mutation was a deletion of two base pairs at codons 1090 and 1091
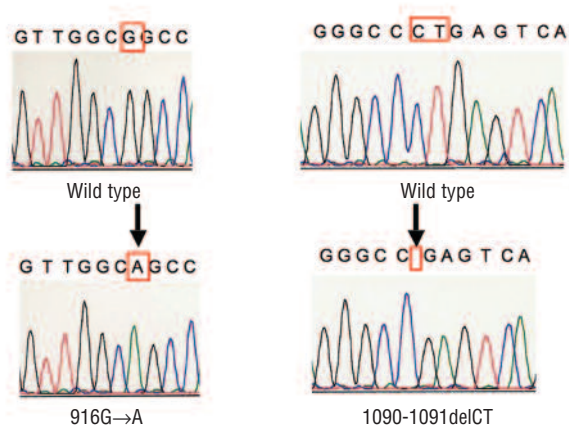
Figure 2. Sequence analysis of the perforin gene. Sequencing demonstrated compound heterozygous mutation in both the patient's peripheral blood mononuclear cells and nail clippings. One mutation was deletion of two base pairs at codon 1090 to 1091 (1090–1091delCT), while the other was a guanine-to-adenine conversion at nucleotide position 916 (916G→A).

(1090–1091delCT), while the other was a guanine-to-adenine conversion at nucleotide position 916 (916G→A). In studies on the PRF1 abnormality in Japan, the incidence of 1090-1091delCT observed in our patient was high and characteristic in Japanese people.[12]

Perforin in T-cells from healthy controls migrated to show a molecular mass of approximately 65 to 70 kDa under reducing conditions. The gene product associated with the PRF1 nonsense mutation 1090–1091delCT present in this patient has been reported to produce a band of 55 kDa under reducing conditions and no band under non-reducing condition.[8,9] Under reducing conditions, the patient's CTL showed a slightly smaller amount of normal-size perforin than controls, plus a weak band at 55 kDa. Under non-reducing conditions to detect an active form of perforin, this active mature form was markedly reduced in the patient (Figure 1C). Thus, the PRF1 missense mutation 916G→A in this patient causes the amino acid substitution V306I, inhibiting proteolytic cleavage of perforin. The marked decrease in perforin in CD8+ cells and CD56+ cells and their reduced cytotoxic activity, together with the result of Western analysis, exclude the possibility that V306I may be influential as a simple polymorphism.

The primary form of HLH is genetically determined while the secondary form is triggered by infection, malignancies, and autoimmune diseases. However, the cause of secondary HLH cannot always be determined. In fact, the causes of primary and secondary HLH are sometimes difficult to define. There are only a few reports of adult HLH patients with genetic mutation of PRF1, but most were in their twenties.[13-15] Recently, Mancebo et al. reported a 49-year-old male who developed HLH with a tuberculosis infection.[16] The PRF1 mutation has never been detected in a patient over 49 years old. Our 62-year-old patient had compound heterozygous mutation of PRF1 (1090-1091delCT and 916G→A) with decreased but residual perforin activity.[17]

Our patient raises important issues. First, even in the elderly, the differential diagnosis of HLH causation should include PRF1 mutations. HLH with genetic mutation was thought to be a disease experienced in infancy. But the introduction of molecular diagnosis has led to a recent increase in the number of confirmed cases among older children and young adults.[13-16] Thus, in the future, screening for PRF1 mutations is expected to yield more cases involving older patients among individuals presenting HLH.[18] The second issue raised is that PRF1 mutation alone might not be sufficient to cause HLH. In fact, the perforin defect in our patient is supposed to be present since birth, yet he had been asymptomatic for more than six decades. It is well known that viral infections may elicit a FHL episode in genetically predisposed individuals.[19] Although we could not detect the association of viral infection in this patient, it is still possible that a late viral infection also induces symptoms of HLH in the elderly. Alternatively, additional genetic or environmental factors may contribute importantly to the pathogenesis of HLH. A homozygous mutation of PRF1 is considered a reason for stem cell transplantation (SCT).[20] Our patient's decreased but residual perforin activity by compound heterozygous mutation of PRF1 may contribute to his relatively mild presentation of HLH. A very short course of corticosteroids treatment has maintained remission for more than 1 year. Some primary HLH with residual perforin activity may be treated without SCT.

In conclusion, since adult-onset of HLH in our patient was associated with compound heterozygous mutation of PRF1, PFR1-mutated primary HLH may occur beyond infancy and childhood.

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