Association of transforming growth factor-β1 gene polymorphism in the development of Epstein-Barr virus-related hematologic diseases

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ABSTRACT

Background and Objectives

Epstein-Barr virus (EBV) is etiologically associated with various hematologic disorders, including primary acute infectious mononucleosis (IM), hemophagocytic lymphohistiocytosis (EBV-HLH), chronic active EBV infection (CAEBV) and malignant lymphomas. Although cytokines play a central role in EBV-related immune responses, the exact mechanisms causing different clinical responses remain unclear. In this study, the pattern of cytokine gene polymorphisms was comparatively analyzed in EBV-related diseases.

Design and Methods

Eighty-nine patients with EBV-related disease were analyzed; 30 with IM, 28 with EBV-HLH and 31 with CAEBV. Eighty-one EBV-seropositive healthy adults were also used as controls. Associations with polymorphisms of various cytokines, including interleukin (IL)-1α and IL-1β were evaluated. The gene polymorphisms were typed by polymerase chain reaction with sequence-specific primers.

Results

A significant difference of polymorphisms was found for transforming growth factor (TGF)-β1; the frequency of TGF-β1 codon 10 C allele was significantly higher in patients with EBV-related diseases than in controls ($p<0.001$). The difference was significant in patients with IM or HLH ($p<0.001$), but not in those with CAEBV ($p=0.127$), compared with controls. As regards other cytokines, the frequency of the IL-1α –889 C allele was significantly lower in patients with IM than in controls ($p<0.05$).

Interpretation and Conclusions

Our results suggests that TGF-β1 codon 10 C allele plays a role in the development of EBV-related diseases and that the IL-1α –889 C allele may be involved in response failure and sequential progression into the development of HLH.

Key words: Epstein-Barr virus, cytokine gene polymorphism, IL-1, TGF-β1.

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Epstein-Barr virus (EBV) is a ubiquitous gamma herpes virus and has been etiologically associated with several disease conditions. It induces primarily subclinical infections or acute infectious mononucleosis (IM), which are generally self-limiting. EBV mainly infects B cells and multiplies in them, a process controlled by EBV-specific cytotoxic T-lymphocytes (CTL).[1] It is also known that EBV infects T and NK cells, the proliferation of which is probably uncontrollable by CTL, and leads to the development of hemophagocytic lymphohistiocytosis (EBV-HLH), chronic active EBV infection (CAEBV) or EBV-related lymphoma. The clinical features of these EBV-related diseases sometimes overlap with high mortality and morbidity except for IM.[2] The distinct manifestations of IM, HLH and CAEBV are thought to be affected by the host's different immune response to EBV, especially by cytokine production. The levels of interleukin (IL)-1α, IL-2, IL-6, and interferon (IFN)-γ have been reported to be elevated in the serum of patients with IM.[3,4] In EBV-HLH, serum levels of IFN-γ and soluble IL-2 receptor (sIL-2R) are especially high.[5,6] Patients with CAEBV were found to have abnormally high levels of circulating human IL-10 as well as EBV-encoded IL-10 (vIL-10), which may contribute to the pathogenesis of CAEBV by inhibiting host immunity.[7]

There is also increasing evidence indicating that cytokine gene polymorphisms, such as those of the IL-10[8] and IL-1α[9] genes, have an impact on susceptibility to EBV infection. These studies focused on whether resistance to EBV infection (EBV seronegativity) was influenced by the polymorphic IL-10 or IL-1β gene. By contrast, in this study we were interested to see whether, among an EBV-seropositive population, polymorphisms of these cytokine genes determine the development of distinct features between the group with subclinical infection (seropositive control, no clinical manifestation) and groups with clinical manifestations of EBV infection, such as IM, HLH and CAEBV. Regarding the development of EBV-lymphoproliferative disease (LPD) in immuno-deficient states, Dierksheide *et al.*[10] demonstrated that IFN-γ gene polymorphisms played a role in the high prevalence of LPD using human peripheral blood in SCID mice; however, no cytokine gene polymorphisms have so far been studied among patients with EBV-related hematologic diseases.

We, therefore, compared the influence of cytokine gene polymorphisms in three EBV-related diseases, IM, EBV-HLH and CAEBV, against that in EBV-seropositive healthy controls.

Design and Methods

Thirty patients with IM (16 males and 14 females; median age at onset, 4.4 years; range, 1-15 years), 28 with EBV-HLH (13 males and 15 females; median age at onset, 2.6 years; range, 0-22 years), 31 with CAEBV (13 males and 18 females; median age at onset, 8.3 years; range, 1-27 years; 17 with T-cell type and 13 with NK-cell type), and 81 EBV-seropositive healthy controls (53 males and 28 females; median age, 38 years; range, 23-65 years) were evaluated. The institutional review board of Kyoto Prefectural University School of Medicine approved the study, and informed consent was obtained from each patient or their parents.

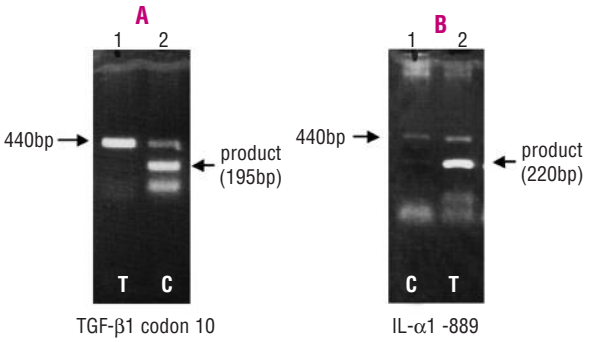
IM was diagnosed based on EBV titers, i.e. elevated titers of viral capsid antigen VCA-IgG and IgM and negativity for Epstein-Barr nuclear antigen (EBNA), with typical clinical symptoms, such as transient fever, lymphadenopathy, and mild hepatosplenomegaly. The diagnosis of EBV-HLH was made according to the criteria reported by Imashuku *et al.*,[11] modified from the HLH diagnostic criteria of the Histiocyte Society.[12] Briefly, patients had to be positive for the EBV genome in peripheral blood, bone marrow or other tissues, and/or positive for VCA-IgG in association with clinical symptoms compatible with HLH, such as persistent high fever, cytopenia, liver dysfunction and coagulopathy. CAEBV was diagnosed according to the criteria published by the Japanese Association for Research on Epstein-Barr Virus and Related Diseases.[13] Briefly, a patient was diagnosed as having CAEBV in the presence of (i) EBV-related illness continuing for more than 6 months with symptoms including fever, persistent hepatitis, extensive lymphadenopathy, hepatosplenomegaly, pancytopenia, uveitis, interstitial pneumonia, hydroa vacciniforme, or hypersensitivity to mosquito bites, (ii) increased quantities of EBV gene in either affected tissues or peripheral blood, and (iii) no evidence of any prior immunologic abnormalities or of any other recent infection, such as human immunodeficiency virus, that might explain the condition. Healthy immunocompetent adults with an elevated titer of VCA-IgG with no previous history of IM, HLH or CAEBV were used as controls.

Genomic DNA was extracted from peripheral blood using a QIAamp blood kit (Quiagen, Hilden, Germany) following the protocol recommended by the manufacturer, and 100 μL DNA were used for polymorphism studies. Gene polymorphism typing was performed by polymerase chain reaction with a sequence-specific primer (PCR-SSP) using a commercially available cytokine genotyping kit (PEL-FREEZ, USA). This kit contains specific primers to detect the following biallelic single nucleotide polymorphisms: IL-1α -889C/T, IL-1β (-511 C/T; +3942T/C), IL-1 receptor (R) pst1 1970 C/T, IL-1 receptor agonist (RA) mspa1 11,100T/C, IL-4Rα +1,902G/A, IL-12 -1β188C/A, IFN-γ +874 A/T, transforming growth factor (TGF)-β1 codon 10 C/T, tumor necrosis factor (TNF)-α (-308 G/A, -238 G/A), IL-2 (-330 T/Gβ +166 G/T), IL-4 (-1,098 T/G, -590 T/Cβ -33 T/C), IL-6 (-174 G/C, nt565 G/A), IL-10 (-1,082 G/A, -819 C/T, -592 A/C).

Statistical analysis was performed with Fisher's exact tests to compare the frequencies of cytokine alleles, haplotypes and genotypes among patients with the three types of EBV-related diseases and the controls.

Results

Figure 1 shows a representative electrophoresis pattern of loading PCR-SSP products. The frequency of TGF-β1 codon 10 C allele was significantly higher in patients with EBV-related diseases (IM + HLH + CAEBV) than in controls ($p<0.001$); a significant difference was also observed between patients with IM or HLH and controls (43/60 vs. 73/162 for IM; $p<0.001$ and 41/56 vs. 73/162 for HLH; $p<0.001$) (Table 1A). The frequency of the IL-1α –889 C allele was lower in patients with IM than in controls (44/60 vs. 138/162; $p=0.041$), but no difference was observed between patients with HLH (53/56 vs. 138/162; $p=0.064$) or CAEBV (55/62 vs. 138/162; $p=0.469$) and controls. (Table 2). No statistically significant differences were found in other genes including IL-1R, IL-1RA, IL-4Rα, IL-12, IFN-γ, TNF-α, IL-2 and IL-6 (*data not shown*).



Gel electrophoresis pattern of PCR-SSP products

Figure 1. Gel electrophoresis pattern of PCR-SSP products. A. Polymorphism of TGF-β1 at codon 10. A 195 bp band represents the PCR-SSP product. Lane 1 indicates the T allele and lane 2 the C allele of TGF-β1 at codon10. This gel pattern shows C/C at this position. (B) Polymorphism of IL-1α at –889. A 220bp band represents the PCR-SSP product. Lane 1 indicates the C allele and lane 2 the T allele of IL-1α at -889. This gel pattern shows T/T at this position. The asterisked band of 440 bp indicates the internal control. The blurred low molecular weight bands were non-specific.

Discussion

Our study showed that the TGF-β1 gene polymorphism differed significantly between patients with EBV-related diseases and controls; the frequency of TGF-β1 codon 10 C allele was significantly higher in patients with IM or EBV-HLH than in controls. As regards other genes, the frequency of the IL-1α –889 C allele was lower in patients with IM than in controls.

TGF-β is member of a highly pleiotropic family of growth factors involved in the regulation of numerous physiologic processes in a variety of cell types. It plays a role as a switch factor in immune suppression[14] and promotes the proliferation of cells of EBV-positive Burkitt's lymphoma cell lines as well as EBV-infected B cells.[15] TGF-β1 was found to be produced in the tonsillar tissues of patients with IM,[16] elevated in EBV-associated nasopharyngeal carcinoma,[17] and its dominant expression was demonstrated in activated T cells of CAEBV patients.[18] These previous observations show some link between high levels of TGF-β1 and the pathogenesis of EBV-related diseases. The codon 10 is an important site for the regulation of TGF-β1 gene expression and the C allele at this site was reported to be linked to high levels of TGF-β1 mRNA and its protein.[19,20] In fact, Suthanthiran *et al.* reported that both mRNA and protein levels of TGF-β1 in the population were higher among subjects genotyped as C/C or C/T at codon 10 compared with those genotyped as T/T.[19] Thus, the polymorphism causing an amino acid substitution from leucine to proline might influence TGF-β1 protein secretion, which up-regulates its own transcription via AP-1 sites located in its promoter.[19] Since our data also showed that frequency of the TGF-β1 gene codon 10 C allele was high in patients with IM and HLH, presumably, the polymorphism (C allele) at codon 10 of TGF-β1 suppresses the immune reaction to EBV that occurs at first exposure to the virus, thus failing to limit the infection at a subclinical level and enabling the development of clinically definable EBV-related diseases.

On the other hand, IL-1 is the prototype of the pro-inflammatory cytokines, which induce the expression of a variety of genes and the synthesis of several proteins that, in turn, induce acute and chronic inflammatory changes in response to injury and infection, and affects nearly every tissue and organ system.[21] IL-1 directly influences B cells by inducing their differentiation, growth and the synthesis of immunoglobulin. IL-1 also activates T cells and is involved in T-cell proliferation. It has been reported that serum levels of IL-1α were high in patients with IM.[3] The expression of IL-1β was high in the tonsils of IM patients, and that of IL-1α was high in epithelial cells.[22] By contrast, no significant increases of serum IL-1α and β levels were found in HLH patients.[23] We previously noted that serum levels of IL-1 were high in a limited number of patients with HLH in spite of extremely high levels of IFN-γ, sIL-2R, IL-6 and TNF in many such patients.[24,25] Higher IL-α protein levels were associated with the T allele at -889, but not with the C allele in the promoter region of the IL-1α gene.[26,27] Our findings that the frequency of the IL-1α –889 C allele was lower in IM and relatively higher in EBV-HLH patients than in con-

Table 1. Comparison of the frequencies of TGF-β1 alleles among patients with EBV-related diseases and controls.

	IM (n=30)	HLH (n=28)	CAEBV (n=31)	Control (n=81)
CC (Pro-Pro)	19	16	11	18
CT (Leu-Pro)	5	9	13	37
TT (Leu-Leu)	6	3	7	26
	IM+HLH+CAEBV vs control: <0.001,I M vs control: <0.001, HLH vs control: <0.001, CAEBV vs control: 0.151, IM vs HLH: 0.311, IM vs CAEBV: 0.056, HLH vs CAEBV: 0.211, HLH+CAEBV vs control: <0.009, HLH+CAEBV vs IM: 0.130			
C allele	43	41	35	73
T allele	17	15	27	89
	IM+HLH+CAEBV vs control: <0.001, IM vs control: <0.001, HLH vs control:<0.001, CAEBV vs control: 0.127, IM vs HLH: 0.85, IMHJ vs CAEBV: 0.029, HLH vs CAEBV: 0.058, HLH+CAEBV vs control: <0.001, HLH+CAEBV vs IM: 0.33			

Table 2. Comparison of the frequencies of IL-α -889 alleles among patients with EBV-related diseases and controls.

	IM (n=30)	HLH (n=28)	CAEBV (n=31)	Control (n=81)
CC	14	25	25	59
CT	16	3	5	20
TT	0	0	1	2
	IM+HLH+CAEBV vs control: 0.769, IM vs control: 0.014, HLH vs control: 0.187, CAEBV vs control: 0.616, IM vs HLH: <0.001, IM vs CAEBV: 0.007, HLH vs CAEBV: 0.508, HLH+CAEBV vs control: 0.243, HLH+CAEBV vs IM: <0.001			
C allele	44	53	55	138
T allele	16	3	7	24
	IM+HLH+CAEBV vs control: 0.95,IM vs control: 0.041, HLH vs control: 0.064, CAEBV vs control: 0.469, IM vs HLH: 0.002, IM vs CAEBV: 0.006, HLH vs CAEBV: 0.248, HLH+CAEBV vs control: 0.108, HLH+CAEBV vs IM: <0.001			

trols are probably compatible with previous observations,[3,23-25] suggesting excessive secretion of IL-1 in IM patients, which may play a role in the prompt eradication of primary EBV infection. On the other hand, insufficient production of IL-1 could lead to failure of the immune response and to sequential progression of the primary infection into HLH.

With the use of diagnostic criteria, IM, HLH and CAEBV can be differentiated into distinct disease entities, although some overlapping of the clinical features does occur; however, to date, no clear mechanism has been clarified to explain the differential development of these subtypes of EBV-related hematologic diseases. Interestingly, high EBV copy numbers in the blood, suggesting EBV reactivation in recipients of allogeneic hematopoietic stem cell transplantation, were found to be prevalent among patients with a certain IFN-γ genotype.[33] Although previous reports also showed that serum levels of EBV copy numbers may differentiate the clinical features of IM and HLH,[34,35] in our study, we were unable to demonstrate a difference in IFN-γ polymorphisms among patients with IM, HLH and CAEBV. Apart from cytokine gene polymorphisms, Zaitsu *et al.*[36] previously identified a higher prevalence of the QPY haplotype of granzyme B in patients with EBV-HLH than in either patients with IM or healthy controls. Most of the reported cases of CAEBV have occurred in Japan and the pathophysiology of this condition has been clarified.[37] However, it remains unclear how the status of CAEBV develops; does it occur as a progression from IM or HLH, or through a totally independent and different mechanism? To our surprise, in this study, the frequencies of both TGF-β1 and IL-1α gene polymorphisms in CAEBV patients were different from those in IM or HLH patients, suggesting the latter mechanism may be involved.

In summary, we demonstrated that the polymorphism of TGF-β1 at codon 10 is associated with the development of EBV-related hematologic diseases, such as IM or HLH, among Japanese subjects. According to some earlier studies on various diseases, the frequencies of polymorphisms in the TGF-β1 and IL-1α genes were not different between Caucasian and Asian ethnic groups.[28-32] However, no data are available for patients with EBV-related diseases. A future, larger study is required to determine whether our findings on EBV-related diseases are specific to Asian/Japanese patients or are common to patients of all ethnic groups.

Authors' contributions

KH, AM and SI co-designed the study, collected patients' samples, analyzed results, and wrote the manuscript; KH performed gene polymorphism typing and the statistical analysis. EI, HK, and IU collected patients' samples and contributed to data registration and quality control. SH, ST and TS provided comments, useful advice and refined the manuscript. All authors approved the final version of the manuscript.

Conflicts of Interest

The authors reported no potential conflicts of interest.

References

1. Seeley J, Svedmyr E, Weiland O, Klein G, Moller E, Eriksson E, et al. Epstein Barr virus selective T cells in infectious mononucleosis are not restricted to HLA-A and B antigens. J Immunol 1981;127:293-300.
2. Kasahara Y, Yachie A. Cell type specific infection of Epstein-Barr virus (EBV) in EBV-associated hemophagocytic lymphohistiocytosis and chronic active EBV infection. Crit Rev Oncol Hematol 2002;44:283-94.
3. Linde A, Andersson B, Svenson SB, Ahrne H, Carlsson M, Forsberg P, et al. Serum levels of lymphokines and soluble cellular receptors in primary Epstein-Barr virus infection and in patients with chronic fatigue syndrome. J Infect Dis 1992;165:994-1000.
4. Hornef MW, Wagner HJ, Kruse A, Kirchner H. Cytokine production in a whole-blood assay after Epstein-Barr virus infection in vivo. Clin Diagn Lab Immunol 1995;2:209-13.
5. Imashuku S, Hibi S, Tabata Y, Sako M, Sekine Y, Hirayama K, et al. Biomarker and morphological characteristics of Epstein-Barr virus-related hemophagocytic lymphohistiocytosis. Med Pediatr Oncol 1998; 31:131-7.
6. Imashuku S. Advances in the management of hemophagocytic lymphohistiocytosis. Int J Hematol 2000;72:1-11.
7. Kanegane H, Wakiguchi H, Kanegane C, Kurashige T, Tosato G. Viral interleukin-10 in chronic active Epstein-Barr virus infection. J Infect Dis 1997;176:254-7.
8. Helminen ME, Kilpinen S, Virta M, Hurme M. Susceptibility to primary Epstein-Barr virus infection is associated with interleukin-10 gene promoter polymorphism. J Infect Dis 2001;184:777-80.
9. Hurme M, Helminen M. Polymorphism of the IL-1 gene complex in Epstein-Barr virus seronegative and seropositive adult blood donors. Scand J Immunol 1998;48: 219-22.
10. Dierksheide JE, Baiocchi RA, Ferketich AK, Roychowdhury S, Pelletier RP, Eisenbeis CF, et al. IFN-gamma gene polymorphisms associated with development of EBV+ lymphoproliferative disease in hu PBL-SCID mice. Blood 2005;105: 1558-65.
11. Imashuku S. Clinical features and treatment strategies of Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis. Crit Rev Oncol Hematol 2002;44:259-72.
12. Henter JI, Elinder G, Ost A. Diagnostic guidelines for hemophagocytic lymphohistiocytosis. The FHL Study Group of the Histiocyte Society. Semin Oncol 1991;18:29-33.
13. Okano M, Kawa K, Kimura H, Yachie A, Wakiguchi H, Maeda A, et al. Proposed guidelines for diagnosing chronic active Epstein-Barr virus infection. Am J Hematol 2005;80: 64-9.
14. Wahl SM, Wen J, Moutsopoulos N. TGF-β: a mobile purveyor of immune privilege. Immunol Rev 2006;213:213-27.
15. Blomhoff HK, Smeland E, Mustafa AS, Godal T, Ohlsson R. Epstein-Barr virus mediates a switch in responsiveness to transforming growth factor, type β, in cells of the B cell lineage. Eur J Immunol 1987; 17:299-301.
16. Andersson J. Clinical and immunological considerations in Epstein-Barr virus-associated diseases. Scand J Infect Dis Suppl 1996;100:72-82.
17. Xu J, Menezes J, Prasad U, Ahmad A. Elevated serum levels of transforming growth factor β1 in Epstein-Barr virus-associated nasopharyngeal carcinoma patients. Int J Cancer 1999;84:396-9.
18. Ohga S, Nomura A, Takada H, Tanaka T, Furuno K, Takahata Y, et al. Dominant expression of interleukin-10 and transforming growth factor-β genes in activated T-cells of chronic active Epstein-Barr virus infection. J Med Virol 2004;74:449-58.
19. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, et al. Transforming growth factor-β 1 hyperexpression in African-American hypertensives: a novel mediator of hypertension and/or target organ damage. Proc Natl Acad Sci USA 2000;97:3479-84.
20. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, et al. Association of a polymorphism of the transforming growth factor-β1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. J Bone Miner Res 1998;13:1569-76.
21. Dinarello CA. Interleukin-1 and interleukin-1 antagonism. Blood 1991;15:1627-52.
22. Foss HD, Herbst, H, Hummel M, Araujo I, Latza U, Rancso C, et al. Patterns of cytokine gene expression in infectious mononucleosis. Blood 1994;83:707-12.
23. Henter JI, Andersson B, Elinder G, Jakobson A, Lubeck PO, Soder O. Elevated circulating levels of interleukin-1 receptor antagonist but not IL-1 agonists in hemophagocytic lymphohistiocytosis. Med Pediatr Oncol 1996;27: 21-5.
24. Fujiwara F, Hibi S, Imashuku S. Hypercytokinemia in hemophagocytic syndrome. Am J Pediatr Hematol Oncol 1993;15:92-8.
25. Ishii E, Ohga S, Aoki T, Yamada S, Sako M, Tasaka H, et al. Prognosis of children with virus-associated hemophagocytic syndrome and malignant histiocytosis: correlation with levels of serum interleukin-1 and tumor necrosis factor. Acta Haematol 1991;85: 93-9.
26. Shirodaria S, Smith J, McKay IJ, Kennett CN, Hughes FJ. Polymorphisms in the IL-1A gene are correlated with levels of interleukin-1α protein in gingival crevicular fluid of teeth with severe periodontal disease. J Dent Res 2000;79:1864-9.
27. Dominici R, Cattaneo M, Malferrari G, Archi D, Mariani C, Grimaldi LM, et al. Cloning and functional analysis of the allelic polymorphism in the transcription regulatory region of interleukin-1 α. Immunogenetics 2002;54:82-6.
28. Cambien F, Ricard S, Troesch A, Mallet C, Generenaz L, Evans A, et al. Polymorphisms of the transforming growth factor-β1 gene in relation to myocardial infarction and blood pressure. The Etude Cas-Temoin de l'Infarctus du Myocarde (ECTIM) Study. Hypertension 1996;28:881-7.
29. Wu L, Chau J, Young RP, Pokorny V, Mills GD, Hopkins R, et al. Transforming growth factor-β1 genotype and susceptibility to chronic obstructive pulmonary disease. Thorax 2004;59:126-9.
30. Ravindran JS, Owen P, Lagan A, Lewis J, Korendowych E, Welsh K, et al. Interleukin 1α, interleukin 1beta and interleukin 1 receptor gene polymorphisms in psoriatic arthritis. Rheumatology 2004;43:22-6.
31. Um JY, Moon KS, Lee KM, Yun JM, Cho KH, Moon BS, et al. Association of interleukin-1 α gene polymorphism with cerebral infarction. Mol Brain Res 2003; 115:50-4.
32. Cinek O, Vavrincova P, Striz I, Drevinek P, Sedlakova P, Vavrinec J, et al. Association of single nucleotide polymorphisms within cytokine genes with juvenile idiopathic arthritis in the Czech population. J Rheumatol 2004;31:1206-10.
33. Bugunia-Kubic K, Mlynarczewska A, Jaskula E, Lange A. The presence of IFNG 3/3 genotype in the recipient associates with increased risk for Epstein-Barr virus reactivation after allogeneic haematopoietic stem cell transplantation. Br J Haematol 2005; 132: 326-32.
34. Teramura T, Tabata Y, Yagi T, Morimoto A, Hibi S, Imashuku S. Quantitative analysis of cell-free Epstein-Barr virus genome copy number in patients with EBV-associated hemophagocytic lymphohistiocytosis. Leuk Lymphoma 2002;43: 173-9.
35. Kimura H, Hoshino Y, Hara S, Nishikawa K, Sako M, Hirayama M, et al. Viral load in Epstein-Barr virus-associated hemophagocytic syndrome. Microbiol Immunol 2002; 46:579-82.
36. Zaitsu M, Yamamoto K, Ishii E, Teramura T, Nakadate N, Sako M, et al. High frequency of QPY allele and linkage disequilibrium of granzyme-B in Epstein-Barr-virus-associated hemophagocytic lymphohistiocytosis. Tissue Antigens 2004;64: 611-5.
37. Kimura H. Pathogenesis of chronic active Epstein-Barr virus infection: is this an infectious disease, lymphoproliferative disorder, or immunodeficiency? Review Med Virol 2006; 16:251-61.