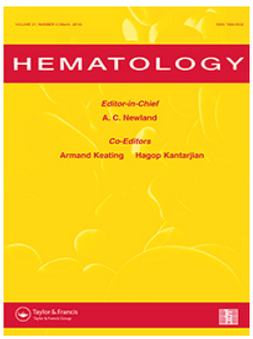

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IL-10-592 A/C polymorphisms is associated with EBV-HLH in Chinese children

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Objectives: The aim of this study is to investigate the relationship between cytokine gene polymorphisms and Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) in children, and to further reveal the possible mechanisms of EBV-HLH.

Methods: Forty-one patients with EBV-HLH, 70 patients with infectious mononucleosis (IM), and 170 EBV-seropositive healthy children were evaluated. Gene polymorphism typing was performed by a polymerase chain reaction with a sequence-specific primer of a commercially available cytokine genotyping kit. Comparison of cytokine gene polymorphisms between EBV-HLH, IM patients, and healthy controls was analyzed statistically using Chi-square test or Fisher's exact test.

Results: The frequencies of IL-10-592 C allele or IL-10-592 CC genotype were significantly higher in patients with EBV-HLH than in IM and healthy children ($P < 0.001$), but no significant difference was observed between IM and healthy children.

Conclusion: IL-10-592 locus gene polymorphism is associated with the development of EBV-HLH in Chinese children.

Keywords: Epstein–Barr virus, Hemophagocytic lymphohistiocytosis, Children, Cytokine, Interlukin, Genotype, Haplotype, Polymorphisms

Introduction

Epstein–Barr virus (EBV) is a lymphotrophic human gamma-1 herpesvirus transmitted primarily through saliva that infects over 95% of the world's population.[1] EBV is implicated in several benign and malignant conditions. Infectious mononucleosis (IM) is a clinical syndrome that is most commonly associated with primary EBV infection which is benign and self-limited. EBV establishes life-long latent infection in B lymphocyte after primary EBV infection. EBV-associated hemophagocytic lymphohistiocytosis (EBV-HLH) is a severe disease caused by primary or reactivated EBV infection characterized by persistent fever, cytopenia, liver dysfunction, hepatosplenomegaly, and hemophagocytosis in the bone marrow, lymph nodes, liver, or spleen.[2] In patients with EBV-HLH, the EBV-infected T cells or natural killer cells are most mono or oligoclonally proliferating, and hypercytokinemia plays a major role in cellular damage and dysfunction of various organs.[2] The pathophysiological mechanism of EBV-HLH is that the abnormal immune response caused by EBV infection leads to hypercytokinemia, which causes multiple organ and tissue damage. As anti-inflammatory cytokine, IL-10 plays an important role in the regulation of immune responses during infection with viruses, bacteria, fungi, protozoa, and helminths.[3] This study investigated the relationship between cytokine gene polymorphisms and EBV-HLH in children.

Materials and methods

Study population

Three subject groups were enrolled into this study, including 41 EBV-HLH cases (29 males and 12 females, aged from 11 months to 14.7 years old, median age 5.8 years old), 70 IM patients (44 males and 26 females; aged from 10 months to 16 years old, median age 6.2 years old), and 170 EBV-seropositive healthy children (109 males and 61 females, aged from 1 to 17 years old, median age 8.3 years). Informed consent was obtained from parents or guardian of each patient.

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The diagnosis of IM was proved by clinical manifestation and serologic profile of primary EBV infection: presence of antibody against EBV viral capsid antigen (VCA-IgG:Immunoglobulin G/IgM:Immunoglobulin M) concurrent with absence of EBV nuclear antigen antibody. The diagnostic criteria of EBV-HLH are that patient meets HLH diagnosis and is EBV positive. The HLH diagnosis was made according to the diagnostic guideline of HLH-2004.[4] Briefly, EBV-HLH patients had to be positive for the EBV genome in peripheral blood, bone marrow, or other tissues, and with clinical symptoms compatible with HLH, such as persistent high fever, hepatosplenomegaly, cytopenias, hypertriglyceridemia, coagulopathy with hypofibrinogenemia, liver dysfunction, elevated levels of ferritin, serum transaminases, and neurological symptoms.

Determination of cytokine genotypes

Genomic DNA was extracted from 2 ml whole blood samples by using a blood genomic DNA isolation kit (Cat# RT403, Tiangen, China) according to manufacturer's instructions, and 50 μl DNA was used for gene polymorphism studies. Gene polymorphism typing was performed by a polymerase chain reaction with a sequence-specific primer (PCR-SSP) using a commercial cytokine genotyping kit (Invitrogen™, USA). This kit contains specific primers to detect the following biallelic single nucleotide polymorphisms: interlukin (IL)-1a-889 C/T, IL-1β (−511 C/T, +3942 T/C), IL-1 receptor (R) pst1 1970 C/T, IL-1 receptor agonist (RA) mspa1 11 100 T/C, IL-4Ra +1902 G/A, IL-12-1188 C/A, IFN-γ +874 A/T, transforming growth factor-β1 codon 10 C/T, codon25 G/C, tumor necrosis factor-a (−308 G/A, −238 G/A), IL-2 (−330 T/G, +166 G/T), IL-4 (−1098 T/G, −590 T/C, −33 T/C), IL-6 (−174 G/C, nt565 G/A), IL-10 (−1082 G/A, −819 C/T, −592 A/C).

Statistical analysis

Software package SPSS 18.0 was used to analyze the data. The Chi-square test (χ^2 test) and Fisher's exact test were used to compare the frequencies of cytokine alleles, haplotypes, and genotypes between patients with IM, EBV-HLH, and the controls. In this study, the result was considered to be significant if there were significant differences of frequencies of gene polymorphism between EBV-HLH and IM patients or EBV-seropositive healthy children respectively, but no significant differences between IM patients and EBV-seropositive healthy children.

Table 1 Comparison of the frequencies of IL-10-592 alleles between patients with EBV-HLH, IM, and healthy controls

Allele	EBV-HLH ($n = 41$)	IM ($n = 70$)	Healthy control ($n = 170$)
C	56	44	106
A	26	96	234

P value: IM vs. HLH: 0.000; HLH vs. control: 0.000; IM vs. control: >0.05.
IM: infectious mononucleosis; EBV-HLH: Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis.

Results

The frequency of IL-10-592 C allele was significantly higher in patients with EBV-HLH than in IM and healthy control subjects ($P < 0.001$), but no significant difference was observed between IM and healthy control subjects (shown in Table 1). The frequency of IL-10-592 CC genotype was significantly higher in patients with EBV-HLH than in IM and healthy control subjects ($P < 0.001$), but no significant difference was observed between IM and healthy control subjects (shown in Table 2). No statistically significant differences were observed in other alleles and genotypes between the patients with EBV-HLH, IM, and the healthy control subjects (data not shown).

Discussion

The relationship between IL-10 gene polymorphism and EBV-HLH was studied in this research. The results showed that the frequencies of IL-10-592 C allele and IL-10-592 C/C genotype were significantly higher in patients with EBV-HLH than in IM and healthy control subjects.

IL-10 is the most important cytokine to decrease the inflammatory functions of the immune cells, including Th1 and 2 lymphocytes, B lymphocytes, NK cells, macrophages, and dendritic cells.[5,6] The three promoter polymorphisms of IL-10, namely −1082 A/G, −819 C/T, and −592 C/A, lie in a putative transcription factor-binding site,[7] making them important loci to be studied in relation to disorders affected by levels of this particular cytokine.

So far, the IL-10 promoter polymorphism at position −592 was studied in immune-related diseases, such as type 2 diabetes with and without nephropathy, multiple sclerosis and asthma,[8] tuberculosis,[9] occult HBV infection,[10] and EBV infection,[11] but except in EBV-HLH. The research in South-Eastern Iranian patients suggested that the frequency of the C allele

Table 2 Comparison of the frequencies of IL-10-592 genotypes between patients with EBV-HLH, IM, and healthy controls

Genotype	EBV-HLH ($n = 41$)	IM ($n = 70$)	Healthy control ($n = 170$)
C/C	17	5	12
C/A	22	34	82
A/A	2	31	76

P value: IM vs. HLH: 0.000; HLH vs. control: 0.000; IM vs. control: >0.05.
IM: infectious mononucleosis; EBV-HLH: Epstein–Barr virus-associated hemophagocytic lymphohistiocytosis.

at position −592 of the IL-10 promoter is significantly higher in asthma patients and they hypothesized that this mutation influences transcriptional activity of the IL-10 promoter, causing a reduction in IL-10 expression in patients.[12] Karjalainen *et al.* showed that the C allele is associated with lower expression of IL-10 than the A allele.[13] In this study, IL-10-592 C allele were significantly higher in patients with EBV-HLH. Hence, it would be speculated that this mutation may also decrease the expression of IL-10 in EBV-HLH. The speculation is in accordance with Helminen's research.[11] They suggest that high IL-10 levels protect against EBV infection and, conversely, that low IL-10-producing capability makes individuals more susceptible to a severe EBV infection.

However, hypercytokinemia is a hallmark of HLH. Tang *et al.* showed that the significant increase of IFN-gamma and IL-10 and a slightly increased level of IL-6 is an early, specific, and prognostic cytokine pattern for childhood hemophagocytic syndrome.[14] Xu *et al.* suggested that the specific cytokine pattern of markedly elevated levels of IFN-gamma and IL-10 with only modestly elevated IL-6 levels has high diagnostic accuracy for HLH.[15] Wada *et al.* showed that IL-10 was markedly elevated in patients with EBV-HLH.[16] It seems that there was contradiction in the IL-10 expression in EBV-HLH. Previous study showed that there were two ELISAs for IL-10 detection.[17] One ELISA recognized both human and viral IL-10 and the other specific for viral IL-10 only. It is reported that viral IL-10 is a unique product of the late EBV gene BCRF1 that is transcribed during viral replication. And the viral IL-10 shares extensive structural homology with the human IL-10.[18] Thus, the high levels of IL-10 detected in EBV-HLH may be the amount of the human and viral IL-10. Additionally, the mechanism of IL-10 expression is unclear. The signaling pathway regulating the IL-10 expression is complicated. Different factors may regulate the IL-10 expression though different pathways in different cells.[19,20] And the cytokines could form a complex regulatory network for interaction. So the higher expression of IL-10 in EBV-HLH aforementioned may also be compensatory increase.

There is no conclusion whether the higher frequency of the C allele and C/C genotype at position −592 of the IL-10 promoter can cause a reduction in IL-10 expression in EBV-HLH patients. Because the IL-10-592 C/A lie in a putative transcription factor-binding site, the future research about this cytokine in EBV-HLH should be carried out at mRNA and protein levels.

Conclusions

The frequencies of IL-10-592 C allele and IL-10-592 C/C genotype were significantly higher in EBV-HLH in Chinese children. IL-10-592 A/C gene polymorphism is associated with the development of EBV-HLH.

Acknowledgments

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Disclaimer statements

Contributors Z.X. and K.S. conceived and designed the study. K.S. obtained funding and ethics approval. Q.Q., L.W., and Y.L. collected the data. Y.W. and C.L. analyzed the data. J.A. interpreted the data. Y.W. wrote the article. Z.X. revised the article.

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Conflicts of interest The authors declare that there were no potential conflicts of interest.

Ethics approval This study was approved by the ethics committee of Beijing Children's Hospital.

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