Association Between Cytotoxic T-Lymphocyte Antigen 4 Gene Polymorphisms and Torque Teno Virus Infection After Hematopoietic Stem Cell Transplantation

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Acknowledgements: The authors have no sources of funding for this study and have no conflicts of interest to declare.

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Experimental and Clinical Transplantation (2017)

Abstract

Objectives: An association between costimulatory molecule gene polymorphisms and viral infection after hematopoietic stem cell transplantation may be related to clinical outcomes, especially acute graft-versus-host disease. Cytotoxic T-lymphocyte antigen 4 has been suggested as a crucial negative regulator of the immune system. In this study, our objective was to investigate the association between cytotoxic T-lymphocyte antigen-4 gene polymorphisms (including -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G) and torque teno virus infection after hematopoietic stem cell transplantation in patients with and without acute graft-versus-host disease.

Materials and Methods: Our study included 71 recipients. We evaluated cytotoxic T-lymphocyte antigen 4 gene polymorphisms using the polymerase chain reaction-restriction fragment length polymorphism method.

Results: Our results showed that the GG genotype of the cytotoxic T-lymphocyte antigen 4 +49 A/G was significantly more frequent in transplanted patients infected with torque teno virus, whereas the AG genotype was more common in transplanted patients who did not have this infection. In addition, the -1661 AA and GA genotypes and -318 TC genotypes were significantly more frequent in transplanted patients infected with the virus and who had low-grade (grades I and II) acute graft-versus-host disease. Among those with grade I graft-versus-host disease, the GG genotype of the cytotoxic T-lymphocyte antigen 4 +49 A/G was more frequent in transplanted patients with torque teno virus infection, whereas the AG genotype was higher in transplanted patients who did not have this infection.

Conclusions: This is the first report indicating that cytotoxic T-lymphocyte antigen 4 gene polymorphism may be implicated in prevalence of torque teno virus infection after stem cell transplant. Further larger studies and evaluation of other costimulatory molecules are suggested.

Key words: Graft-versus-host disease, Single nucleotide polymorphisms, Stem cell transplantation, Viral infections

Introduction

Transfusion-transmitted viruses or TT viruses, classified into the family Circoviridae, genus Anellovirus, were originally isolated in 1997 from the serum of a Japanese patient with posttransfusion hepatitis of unknown cause. Torque teno virus (TTV) is a nonenveloped, circular, and single-stranded DNA virus with genome of negative polarity.1,2 Torque teno virus infection is common among hepatitis patients and is presented worldwide in blood donors. Because TTV sequences can be detected in sera and liver tissues from patients with liver disease, it has been suggested that TTV would be responsible for some acute and chronic liver disorders and thereby many autoimmune diseases such as idiopathic hepatitis, idiopathic pulmonary fibrosis, aplastic anemia, systemic lupus erythematosus, and multiple sclerosis. However, the biologic nature of TTV has not yet been clearly defined.3-5

The costimulatory molecules, including programmed cell death-1 (PD-1), cluster differentiation 28 (CD28), inducible T-cell costimulator (ICOS), and cytotoxic T-lymphocyte antigen 4 (CTLA-4, also known as CD152), are expressed on the T-lymphocyte surface and take part in regulation of immune responses.6 Cytotoxic T-lymphocyte antigen 4 is an inhibitory receptor expressed on activated T cells and acts as an essential negative regulator of T-cell-mediated
immune responses that down-regulates the immune system. The function and expression of CTLA-4 may be affected by gene polymorphisms. Several polymorphisms in the CTLA-4 gene have been reported at positions -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G and in the 3’ untranslated region at position +6230, which is generally known as CT60. However, the association between CTLA-4 gene polymorphism and TTV infection after HSCT has not yet been reported.

The aim of this study was to evaluate the association between CTLA-4 gene polymorphisms, including -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G, and TTV infection after HSCT in patients with and without acute graft-versus-host disease (aGVHD).

Materials and Methods

Study group
In this cross-sectional study, 71 post-HSCT patients with and without aGVHD were recruited from South of Iran who were referred to Namazi Hospital (affiliated to Shiraz University of Medical Sciences) between 2013 and 2015. All patients were transplanted from related HLA-matched donors and subgrouped to HSCT patients who had aGVHD or did not have aGVHD. The aGVHD grade was defined according to the classic Glucksberg-Seattle criteria and the International Bone Marrow Transplant Registry. In our total group of 71 transplanted patients, 28 had acute myelogenous leukemia, 10 had chronic myelogenous leukemia, 20 had acute lymphogenous leukemia, and 13 had thalassemia.

Conditioning chemotherapy regimens included busulfan 16 mg/kg or intravenous busulfan (80% of oral dose) and cyclophosphamide (120-200 mg/kg) in leukemia patients. Graft-versus-host disease prophylaxis consisted of cyclosporine and methotrexate. Prophylactic antibiotic, antifungal, and antiviral drugs were prescribed for all patients. All blood products were irradiated with gamma rays to prevent posttransfusion GVHD. This study was approved by the Ethics Committee of Shiraz University of Medical Sciences. Witten informed consent was obtained from all patients.

Molecular detection of torque teno virus infection
The TTV infection was detected using the polymerase chain reaction (PCR)-based method. Briefly, the TTV genomic DNA was extracted from blood using a dinitrophenol kit (Cinna Gen Inc., Tehran, Iran) according to the manufacturer’s instructions. The presentation of TTV genomic DNA was analyzed in patients who had HSCT using an in-house semi-nested PCR protocol, as previously described.

Screening for cytotoxic T-lymphocyte antigen 4 gene polymorphisms using the polymerase chain reaction- restriction fragment length polymorphism method
Genomic DNA was extracted from EDTA-treated blood samples using a QIAamp DNA mini-kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Cytotoxic T-lymphocyte antigen 4 gene polymorphisms (including -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G) were analyzed in all patients using PCR-restriction fragment-length polymorphism method as previously described.

Statistical analyses
Statistical evaluation was carried out using SPSS software (SPSS: An IBM Company, version 18.0, IBM Corporation, Armonk, NY, USA). The frequencies of alleles/genotypes and the relation between CTLA-4 single nucleotide polymorphisms (SNPs) and active TTV infection were analyzed in both groups of HSCT patients by chi-square test and Fisher exact test. The odds ratios (ORs) and 95% confidence intervals (95% CIs) for relative risks were calculated. A two-tailed P < .05 was considered statistically significant.

Results

Patient characteristics
In this study, 71 post-HSCT patients, including 44 male (62%) and 27 female patients (38%), were genotyped for CTLA-4 -1722T/C, -1661 A/G, -318 C/T, and +49 A/G SNPs. The mean age of patients was 23.47 ± 1.2 years (ranging from 20 to 30 years old). Forty patients (56.3%) had aGVHD, including 22 patients (55%) with grade I, 11 (27.5%) with grade II, 4 (10%) with grade III, and 3 (7.5%) with grade IV. Of 71 patients, 24 (33.8%) were infected with TTV and 47 (66.2%) had no TTV infection after HSCT.
Cytotoxic T-lymphocyte antigen 4 polymorphisms and torque teno virus infection

The frequency of all SNPs was compared in TTV-infected and noninfected HSCT patients. As indicated in Table 1, the GG genotype of the CTLA-4 +49 A/G was significantly more frequent in TTV-infected HSCT patients than in HSCT patients who were not infected (P = .02; OR = 9.20, 95% CI, 0.85-23). On the other hand, the AG genotype of the CTLA-4 +49 A/G had significantly higher frequency in non-TTV-infected HSCT patients than in TTV-infected ones (P = .018; OR = 0.28, 95% CI, 0.08-0.94; Table 1).

In addition, the frequency of all SNPs was compared in TTV-infected and noninfected HSCT patients according to the grade of aGVHD. Our analyses showed that the AA and GA genotypes of the CTLA-4 -318 were significantly more frequent in TTV-infected HSCT patients who had low-grade (grades I and II) aGVHD (P = .03 and P = .01, OR = 0.00, 95% CI, 0.00-3;73; Table 2).

For CTLA-4 -318, our results showed that the TC genotype of the CTLA-4 -318 was more frequent in TTV-infected HSCT patients who experienced low-grade aGVHD (P = .02, OR = 0.00, 95% CI, 0.00-3.73; Table 2).

Among HSCT patients who had experienced grade I aGVHD, presence of the GG genotype of the CTLA-4 +49 A/G was significantly higher in TTV-infected HSCT patients than in noninfected patients (P = .025, OR = 9.11, 95% CI, 0.85-23.25; Table 3). In addition, the AG genotype was significantly more frequent in non-TTV-infected HSCT patients than in patients with TTV (P = .02, OR = 9.28, 95% CI, 0.85-101; Table 3).

When the allogeneic HSCT patients were classified according to male versus female patients, we observed that the A allele of the CTLA-4 -1661 was significantly more frequent in TTV-infected male

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>Low-Grade aGVHD, No. (%)</th>
<th>High-Grade aGVHD, No. (%)</th>
<th>P Value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1661 (A/G)</td>
<td>AA</td>
<td>16 (72.7)</td>
<td>31 (73.8)</td>
<td>0.95</td>
<td>1.20</td>
<td>0.40-4.95</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>4 (18.2)</td>
<td>7 (16.7)</td>
<td>87</td>
<td>1.11</td>
<td>0.33-2.66</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>2 (9.1)</td>
<td>3 (7.0)</td>
<td>0.97</td>
<td>0.97</td>
<td>0.33-2.66</td>
</tr>
</tbody>
</table>

| Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphisms; TTV, torque teno virus; P ≤ .05 was considered significant. |
patients than in female patients \((P = .04, \text{OR} = 7.29, 95\% \text{ CI}, 0.78-169.02)\). Furthermore, the CC \((P = .01)\), CT \((P = .01)\), and C \((P = .01)\) alleles of the CTLA-4 -318 were significantly more frequent in non-TTV-infected male patients than in corresponding female patients \((OR = 0.00, 95\% \text{ CI}, 0.00-0.92)\).

**Discussion**

The main potential target of TTV is thought to be the liver; however, studies have shown that TTV is distributed not only in the liver but that the TTV viral load tends to be high in the bone marrow, lungs, spleen, and liver, although this can vary by infected individual.\(^{13,14}\) It has been suggested that TTV is involved in complicating the clinical outcomes of patients with malignancies, especially leukemia. In addition, it may be one of the most impressive viral infections after allogenic HSCT.\(^{15}\) In this study, the possible associations between genetic polymorphisms of CTLA-4 -1722 T/C, -1661 A/G, -318 C/T, and +49 A/G SNPs and TTV infection were evaluated in patients after HSCT who presented with and without aGVHD.

Our results showed that the GG genotype of the CTLA-4 +49 A/G was significantly more frequent in TTV-infected HSCT patients, whereas the AG genotype was more common in non-TTV-infected HSCT patients. In addition, the -1661 AA and GA genotypes and the -318 TC genotype were more frequent in TTV-infected HSCT patients who experienced low-grade aGVHD. Among HSCT patients who had experienced grade I aGVHD, the GG genotype of the CTLA-4 +49 A/G was more frequent in TTV-infected patients, whereas the AG genotype was more frequent in non-TTV-infected patients.

There are several studies indicating that CTLA-4 gene polymorphisms at position +49 A/G and -318 C/T may affect the susceptibility and chronicity of hepatitis B virus infection.\(^{16-19}\) However, few reports are available about the relation between CTLA-4 polymorphisms and susceptibility to viral infection after HSCT. In our previous studies, a significant association was found between CTLA-4 -1661 A/G and -318 C/T genotypes and CMV and hepatitis B virus infection, respectively, after HSCT.\(^{9,10}\) Consistent with our results, the former study by our group demonstrated that the CTLA-4 +49 AG genotype was significantly more frequent in HSCT patients without CMV than in HSCT patients with CMV infection.\(^9\) However, this study showed that the G allele and GG genotype of the CTLA-4 -1661 A/G polymorphism were more frequent in active CMV-infected allogeneic HSCT patients who experienced low-grade aGVHD, a result different from that shown in our present study. According to these studies, it seems that the CTLA-4 +49 AG genotype might be associated with protection against both CMV and TTV infection in our population.

Cytotoxic T-lymphocyte antigen 4 is an important negative regulator of the immune system, which is expressed mainly on activated T cells and which competes for the costimulatory molecule CD28 for binding to the B-7 molecule. Because of the indispensable function of CTLA-4 in inducing immunomodulatory and homeostatic signals in the ongoing immune response, CTLA-4 can be a strong candidate susceptibility gene in a broad range of human disorders, including autoimmunity, infection, and human malignancies. In addition, CTLA-4 is also expressed by regulatory T cells, which play a fundamental role in the suppressive function of these subsets of T cells.\(^{20}\) Cytotoxic T-lymphocyte antigen 4 has many variants, and more than 100 SNPs have been identified in the entire region of the CTLA-4 gene. Most of these SNPs in the CTLA-4 gene have been located at positions -1722, -1661, -318, and +49 and in 3' untranslated region at position +6230, known as CT60.\(^8\) The functional significance of these SNPs in the regulatory role of CTLA-4 has not been well understood. However, it has been demonstrated that, in cases of a CTLA-4 -318 C > T SNP, the T allele has higher promoter activity and increased CTLA-4 expression versus the C allele, which is believed to play an important role in promoting the suppressive properties of CTLA-4.\(^{21,22}\) The CTLA-4 +49 A > G SNP causes Thr > Ala substitution in the leading peptide of the CTLA-4 receptor. Sun and associates have recently found that the Thr > Ala change in CTLA-4 greatly enhanced its interaction with the ligand B7-1.\(^8\) They also reported that recombinant CTLA-4-Ala had a significantly stronger ability to inhibit T-cell proliferation and activation than CTLA-4-Thr and that peripheral blood mononuclear cells from individuals who carry the CTLA-4 +49 AA genotype showed a significant decrease in T-cell proliferation and activation versus peripheral blood mononuclear cells having the CTLA-4 +49 GG genotype on stimulation with phytohemagglutinin.\(^{23}\)
These results delineate that the CTLA-4 +49 A > G SNP results in stronger CTLA-4-mediated inhibition of T-cell proliferation and activation.23 However, additional studies are needed to clarify the consequences of such SNPs in CTLA-4 expression and its regulatory function. In this regard, genetic variations of other inhibitory and activatory costimulatory molecules should be considered.

Together, this is the first report indicating that CTLA-4 +49 A/G polymorphism may be implicated in prevalence of active TTV infection after stem cell transplantation. In addition, the -1661 A/G and -318 C/T could be associated with TTV infection in transplanted patients with low-grade aGVHD. Further larger studies and evaluation of other costimulatory molecules may provide additional data for the assessment of susceptibility to TTV infection after HSCT.

References