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Clinical significance of the single nucleotide polymorphism TLR2 R753Q in heart transplant recipients at risk for cytomegalovirus disease

Martina Schneider[a], Teresa Matiqi[a], Michael Kundi[b], Franz J.J. Rieder[a], Martin Andreas[c], Robert Strassl[d], Andreas Zuckermann[c], Christof Jungbauer[e], Christoph Steininger (MD)[a,*]

[a] Department of Medicine I, Div. of Infectious Diseases and Tropical Medicine, Medical University of Vienna, Austria
[b] Institutes of Environmental Health, Center for Public Health, Medical University of Vienna, Austria
[c] Department of Surgery, Division of Cardiac Surgery, Medical University of Vienna, Austria
[d] Department of Laboratory Medicines, Division of Clinical Virology, Medical University of Vienna, Austria
[e] Blood Service for Vienna, Lower Austria and Burgenland, Austrian Red Cross, Vienna, Austria

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ABSTRACT

Background: The toll-like receptor 2 (TLR2) is a significant component of innate immunity against cytomegalovirus (CMV) infection but information on the clinical significance of the single nucleotide polymorphism (SNP) (R753Q) is conflicting.
Objectives: The inconsistent observations of the immunological and clinical significance of the TLR2 R753Q polymorphism for CMV infection indicates the influence of confounders.
Study design: The presence of the TLR2 polymorphism was determined by a genotyping assay of 175 HTX patients and 281 healthy blood donors and evaluated in relation to selected virological and clinical parameters.
Results: Relative frequency of TLR2 polymorphism was similar in HTX patients and blood donors (homozygous wild-type, 94.3% vs. 94.0%; heterozygous, 5.1% vs. 5.7%; homozygous mutated, <1%). CMV viremia was detectable in 108 (61.7%) of HTX patients. The TLR2 polymorphism was neither associated with occurrence or level of CMV infection nor with survival, graft failure or rejection, or CMV serostatus of patient before transplantation. Nevertheless, CMV viremia occurred in 83.1% of R+/D+, 77.1% of R+/D-, and 64.3% of R-/D+ patients. Time of first CMV viremia was in R-/D+ patients later than in CMV-seropositive patients (median, 182 days versus 23 days; $P < 0.001$) corresponding to the duration of antiviral prophylaxis in R-/D+ patients.
Conclusions: The TLR2 R753Q polymorphism is extremely rare in the general population and HTX patients. Screening for this risk factor of CMV disease may not be cost-effective in contrast to testing for CMV viremia.

1. Background

Innate immunity plays an important role in the prevention and control of opportunistic pathogens in solid-organ transplantation. Cytomegalovirus (CMV) is one of the most significant viral opportunistic pathogens in solid-organ and bone-marrow transplantation [1]. The innate immune response to CMV is initiated by recognition of the viral envelope glycoproteins B (gB) and H (gH) by TLR2. This TLR2-CMV interaction initiates a cascade of intracellular signaling events that lead to the production of antiviral peptides and cytokines and ultimately to the generation of protective adaptive immunity [2]. Single-nucleotide polymorphism (SNPs) in genes coding for TLRs may reduce the recognition capacity of receptors by changes to their structure. For example, the substitution of arginine for glutamine in position 753 of TLR2 (R753Q) (rs5743708)

Abbreviations: CMV, cytomegalovirus; D, donors; gB, glycoprotein B; gH, glycoprotein H; HTX, heart transplant recipients; IQR, interquartile ranges; MGB, minor groove binder; Mut, mutated; R, recipient; SD, standard deviation; SNP, single nucleotide polymorphism; TLR2, toll-like receptor 2.

* Corresponding author at: Department of Medicine I, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
E-mail address: christoph.steininger@meduniwien.ac.at (C. Steininger).

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was associated in several studies with a predisposition to several infections and diseases [3–8].

Recently, homozygosity for the TLR2 R753Q polymorphism was identified as significant risk factor for CMV replication and disease in liver transplant recipients with chronic hepatitis C virus infection [9,10]. In contrast, the TLR R753Q polymorphism was not associated with an increased risk for CMV replication and disease in kidney transplant recipients [11]. Moreover, Jablonska et al. observed a significantly lower frequency of heterozygosity for the TLR2 R753Q polymorphism in immunocompetent patients with primary CMV infection when compared to non-infected individuals [12]. This observation may even suggest a protective effect of the TLR2 R753Q polymorphism against CMV infection.

2. Objectives

The conflicting observations of the immunological and clinical significance of the TLR2 R753Q polymorphism for CMV infection indicates the influence of confounders. To evaluate the significance of the TLR2 R753Q polymorphism together with other clinical and laboratory characteristics of solid-organ transplant patients, we evaluated a well-characterized cohort of heart transplant recipients (HTX) in relation to occurrence of CMV infection.

3. Study design

3.1. Study population

The present study was based on an ongoing observational, cross-sectional study of HTX patients with stratification for CMV status of organ donor and recipient (Medical University of Vienna Biobank Study). So far, 320 patients have been included in this study from 2009 to 2014. For the present study, only consecutive patients with archived leukocyte preparations available for DNA isolation were included (n = 175). Patients included in the present study received a heart transplant between 1991 and 2014 and were clinically followed for a mean period of 8.2 years (SD, 6.0 years). As controls, healthy blood donors were included at the Austrian Red Cross (n = 281).

3.2. Clinical investigation

Key clinical data on patient characteristics were available through a prospectively maintained data-base which includes patient demographics, donor and recipient CMV serostatus, HLA status and episodes of organ rejection. To correlate genetic and virological data with clinical outcome, additional clinical information was assessed retrospectively by review of charts and internal documentation. As prophylaxis for CMV infection, patients received 100 mg CMV hyperimmunoglobulin intravenously per week for 4 weeks. High risk patients (Donor (D)+/Recipient (R)-) received additionally Ganciclovir/Valganciclovir for 90 days. All patients received a triple-immunosuppressant therapy immediately after transplantation which included a proliferation-inhibitor, corticosteroids and a calcineurin-inhibitor. As prophylactic treatment against *Pneumocystis jiroveci* pneumonia, patients received sulfamethoxazole-trimethoprim combination treatment three times a week for 6 months. Tissue-invasive CMV disease was defined according international guidelines and as the presence of clinical signs and symptoms of tissue involvement [13].

3.3. Virological investigation

Assessment of the CMV serostatus was performed on the Diasorin Liaison system (Diasorin S.p.A., Saluggia (VC), Italy) using the Liaison CMV IgG II assay for the detection of CMV specific IgG antibodies and the Liaison CMV IgM II assay for the detection of CMV specific IgM antibodies. CMV DNA viral load in human plasma samples was screened in regular intervals in the first six months on the Abbott m2000sp/m2000rt real-time PCR platform (Abbott Laboratories, Illinois, U.S.A) using the CE/IVD approved Abbott Real-Time CMV assay for the quantification of CMV DNA. All assays were performed according to the manufacturer's manual.

3.4. Toll-like receptor polymorphism

DNA was isolated from leukocyte preparations of HTX patients with use of E.Z.N.A. Blood DNA Mini Kit (Omega Bio-tech Inc., Norcross, GA, USA) and from healthy blood donors with the QIAamp 96 DNA Blood Kit (QIAGEN, Hilden, Germany) following manufacturers' instructions. Detection of the single-nucleotide TLR2 R753Q polymorphism was performed with a TaqMan SNP Genotyping Assay that was based on two allele-specific TaqMan probes with distinct fluorescent dyes (FAM-AAGCTCGGAAGAT-NFQ-MGB for the wild-type genotype and VIC-AAGCTGCAGAAGATA-NFQ-MGB for the SNP-genotype). Upon amplification of the polymerase the probe that has annealed to the template is degraded. During the degradation the fluorophore is released and fluorescence can be detected by the cycler. Reaction mixes included 250 nM of each TaqMan probe, 150 nM of primers (TLR2 forward: 5′-CATTCCCCAGCGCTTCTG-3′ and TLR 2 reverse:5′-TCCAGGTAGGTCTTGGTGTTCA-3′), iTaq Universal Probes Supermix (BioRAD, Hercules, USA). Cycles were run under standard conditions. Positive controls for wild type detection were created from extracted DNA from human embryonic kidney cells (HEK 293), which are known to be homozygote for the wild type genotype [10]. The region of interest, was cloned into a pcDNA3.1 vector (InVitrogen, Carlsbad, CA). The positive controls for SNP detection was based on the HEK_TLRwt_pcDNA3.1 vector which was modified with QuickChange XL Site-Directed Mutagenesis Kit following manufacturers' recommendations (Agilent technologies, Santa Clara, CA) to yield the SNP TLR2 R753Q.

3.5. Statistical analysis

Descriptive analysis was done by computing means and standard deviations (SD), or medians and interquartile ranges (IQR) as appropriate. To evaluate associations between the wild-type (G/G) (wt) in contrast to the mutated genotype among HTX- transplant recipients (A/G and A/A) (mut) or CMV viremia and clinical attributes cross-tabs were calculated and Fisher's exact test or Pearson's Chi squared test performed. Associations of the time to viremia/CMV viral load and CMV area under the curve in wt and mut patient group were estimated with the Mann-Whitney *U* test. Analysis of the CMV serostatus and CMV viremia were done by Cox regression model. All analyses were performed using SPSS software version 22 (IBM Corp. USA).

4. Results

The study population included a total of 175 patients who underwent allogeneic heart transplantation. Key demographic and clinical characteristics of the 175 HTX patients are summarized in Table 1. The mean age of the patients at the time of HTX surgery was 49 years (SD, 14 years) and the majority of patients (74.9%) were male. The most common reason for heart transplantation was dilated cardiomyopathy (57.1%) and the majority of patients were still alive (93.7%) after a mean observation period of 8.2 years (SD, 6.0 years). The HLA-mismatch was positive in 45 (25.7%) cases. A majority of HTX patients was CMV-seropositive at the time of