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Original article

Strain-specific seroepidemiology and reinfection of cytomegalovirus

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Abstract

Although there have been some reports describing the serostatus of cytomegalovirus, strain-specific antibody responses and their distribution remain unknown. In this study, ELISA using fusion proteins encompassing epitope of glycoprotein H from both AD169 and Towne strains was used to test 352 blood donors. Of these 352 donors, 207 were analyzed for strain-specific glycoprotein H antibodies. Of the 44 donors whose serum contained antibodies against both AD169 and Towne, 27 (60%) were aged 50 years or over ($p = 0.0003$). This may indicate serological evidence of reinfection with cytomegalovirus in the elder population. The nucleotide sequence analysis of cytomegalovirus glycoprotein H from the peripheral blood of the cytomegalovirus-positive renal transplant recipients showed that our strain-specific ELISA can reveal cytomegalovirus reinfection after transplantation.
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1. Introduction

Cytomegalovirus (CMV) remains a major problem, not only for immunocompromized individuals such as organ transplant recipients, but also for women of childbearing age. CMV causes serious CMV disease in immunosuppressed patients that presents as fever, leucopenia, pneumonia, retinitis and hepatitis [1,2]. Therefore, we need to use antiviral drugs as a prophylactic or preemptive therapeutic strategy for the prevention of CMV after organ transplantation [3,4]. Further, congenital CMV infection is a major public health problem, as it is also a leading cause of brain damage, sensorineural hearing loss and visual impairment [5,6]. Recent reports indicate that recurrent CMV infection of mothers who were CMV seropositive during pregnancy can cause viral transmission to infants [7,8]. This would be because the protection conferred by preconceptional immunity is limited due to its strain-dependent immune responses. Consequently reinfection with a different CMV strain can cause intrauterine transmission and symptomatic congenital infection [9]. Such reinfection can also occur during organ transplantation from a donor with preexisting immunity against one strain of CMV to a recipient with antibodies against another strain, resulting in CMV transmission. In fact, it has been reported that adverse events were more likely to occur in cases of CMV donor positive (D+)/recipient positive(R+) renal transplantation with mismatched strain-specific antibodies [10].

The glycoprotein H (gH) of CMV has been used to determine preexisting strain-specific antibodies to CMV [9–11]. The amino-terminal regions of gH show sequence heterogeneity between two laboratory strains of CMV, AD169 and Towne, and are recognized by virus-neutralizing antibodies as

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strain-specific epitopes [9]. As the gH is a major target of neutralizing antibodies [12], recombinant glycoprotein H epitopes can be used to determine neutralizing antibodies as well as strain-specific antibodies.

In individuals with past CMV infection, serological responses to the CMV glycoprotein B (gB) are also detected. Linear and conformation-dependent epitopes of neutralizing and non-neutralizing antibodies have been defined on gB [13,14]. The antigen domain 1 (AD1), which is located between amino acid (aa) positions 560 and 640 of gB, is a major neutralizing epitope [15,16]. Although AD1 is one of the major target sites for neutralizing antibodies [17], the AD1 domain is conserved between herpesviruses [18–20]. Therefore, detection of antibodies against AD1 might require careful analysis due to antigenic cross-reactivity.

The second antibody binding site on gB is the antigen domain 2 (AD2), which is located between aa 28 and 84 of gB [13]. Two antibody binding sites have been identified within the AD2 domain. Site I is located between aa 68 and 77 in the AD2 of the AD169 strain. This region is conserved between CMV isolates and is the target of neutralizing antibodies. Site II, another binding sequence in AD2, is located between aa 50 and 54. Site II binds non-neutralizing antibodies and is strain specific [13].

These glycoproteins of CMV have already been used for ELISA to detect antibodies against CMV. Recently, it was reported that an ELISA designed with antigen fragments encompassing the AD2 domains of gB from strains AD169 and Towne could be used for the sensitive detection of antibodies against CMV, whereas the AD1 domain could not provide any additional information [21].

In this study, we collected serum samples from healthy volunteers and potential donors and recipients for renal transplantation, and tested them for antibodies against strain-specific epitopes in the gH as well as the gB AD2 of CMV. The age-related distribution of strain-specific antibodies against CMV was then analyzed. Furthermore, we investigated whether we could predict reinfection of CMV from the strain-specific antibodies in organ transplantation.

2. Materials and methods

2.1. Serum samples

In the initial seroepidemiological analysis, which was approved by the institutional ethics committee, blood samples were obtained from a total of 352 subjects (aged 15–75), consisting of healthy volunteers and consecutive potential donors and recipients for renal transplantation both at the Fukushima Medical University and at the Tokyo Women's Medical University. No subjects undergoing immunosuppressive therapy or with infectious or malignant disease were included in the study, and written informed consent was obtained from all subjects.

For the analysis of the correlation between the strain-specific antibody response and the nucleotide sequence encoding the CMV glycoprotein H epitope from the peripheral blood of renal transplant recipients, blood samples were collected from routine diagnostic laboratory samples. The blood samples from the transplant recipients who had progressed CMV disease after transplantation were subjected to nucleotide sequence analysis of the CMV glycoprotein H.

2.2. Cloning and expression of recombinant antigens of CMV glycoprotein

To evaluate CMV strain-specific antibodies, the gH epitopes (aa 32–42 of gH) from the AD169 and Towne strains were used for ELISA as previously reported [10]. Briefly, DNA fragments encoding the epitopes were prepared by annealing two synthetic oligonucleotides: 5′-GAT CCC CGA AGC GCT GGA CCC TCA CGC ATT TCA CCT ACT ACT CG-3′ and 5′-AAT TCG AGT AGT AGG TGA AAT GCG TGA GGG TCC AGC GCT TCG GG-3′ for AD169; and 5′-GAT CCC CGA ACC GCT GGA CAA AGC GTT TCA CCT ACT GCT CG-3′ and 5′-AAT TCG AGC AGT AGG TGA AAC GCT TTG TCC AGC GGT TCG GG-3′ for Towne. The constructed double-strand DNA cassettes were then cloned into the pGEX-5x plasmid vector to produce GST-fusion proteins. AD2 site I and site II were employed for the detection of antibodies against gB. Viral DNA fragments encoding the conserved epitope in CMV AD2 site I were constructed using the oligonucleotide strands 5′-TTT *GGA TCC* GTC ATA GAG CCA ACG AGA CTA TCT ACA ACA CTA CCC TCA AGT ACG GA-3′ and 5′-CGG *GAA TTC* AGT GTT GAC TCC CAC CAC ATC TCC GTA CTT GAG GGT AGT GTT GTA GAT-3′. The first oligonucleotide strand was composed of a three-nucleotide additional sequence, the recognition site of the restriction enzyme BamHI and the AD2 site I-specific sequence from aa 191 to 237. The second strand consisted of the AD2 complementary sequence from aa 211 to 258, followed by the EcoRI recognition motif and an additional sequence. The DNA strand for the AD2 site I region was synthesized by Taq DNA polymerase after annealing the two oligonucleotides. The fragment was digested with restriction enzymes BamHI and EcoRI, purified using a Minielute PCR purification kit (QIAGEN) and then cloned into the expression vector pGEX-5x (Amersham Bioscience).

For the strain-specific AD2 site II epitope, DNA was constructed by annealing two synthetic complementary oligonucleotides; 5′-*GAT CC*C TGC TCA AAC CCG GTC AGT CTA TTC TCA ACA CGT AG-3′ and 5′-*AAT TC*T ACG TGT TGA GAA TAG ACT GAC CGG GTT TGA GCA GG-3′ for the AD169 strain; and 5′-*GAT CC*C TGC TCA TTC TCG ATC CGG TTC AGT CTC TCA ACG CGT AG-3′ and 5′-*AAT TC*T ACG CGT TGA GAG ACT GAA CCG GAT CGA GAA TGA GCA GG-3′ for the Towne strain. These two complementary single-strand oligonucleotides were annealed and directly cloned into the EcoRI and BamHI sites of expression vector pGEX-5x.

Each epitope was expressed in *E. coli* DH5α as a fusion protein with GST, and purified using GSTrap FF (Amersham Bioscience) according to the manufacturer's protocol.

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