Journal of Zhejiang University SCIENCE B
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.zju.edu.cn/jzus; www.springerlink.com
E-mail: jzus@zju.edu.cn

Allograft rejection-related gene expression in the endothelial cells of renal transplantation recipients after cytomegalovirus infection*

Yang LI[†§], Hang YAN[†‡§], Wu-jun XUE, Pu-xun TIAN, Xiao-ming DING, Xiao-ming PAN, Xin-shun FENG, Xiao-hui TIAN, He-li XIANG, Jun HOU

(*Department of Renal Transplantation, Center of Nephropathy, the First Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an 710061, China*)

[†]E-mail: jullian80915@126.com; yanhang2477@sina.com

Received Apr. 21, 2009; Revision accepted Oct. 14, 2009; Crosschecked Oct. 20, 2009

Abstract: Objective: To explore the effects of cytomegalovirus (CMV) infection on rejection-related gene expression in the endothelial cells of renal transplantation recipients. Methods: Endothelial cells (ECs) were cultured and stimulated by a variety of factors: A, normal control group; B, inactivated human cytomegalovirus (HCMV) infection group; C, HCMV infection group; D, HCMV supernatant infection group; and E, ganciclovir HCMV group. Expression of intercellular adhesion molecule-1 (ICAM-1) and major histocompability complex (MHC) class I and class II antigens was detected by flow cytometry (FCM) and immunohistochemistry. Results: We found characteristic CMV-infected ECs in this study. There were no significant differences among groups A, B and D ($P>0.05$). Although the expression levels of ICAM-1 were not significantly different between groups C and E ($P>0.05$), the ICAM-1 expression in these two groups was significantly higher than that in group A ($P<0.05$). ICAM-1 expression was detected in groups C and E, while there was no expression in groups A, B and D. Furthermore, there was no significant difference of ICAM-1 mRNA expression between groups C and E ($P>0.05$). Human leucocyte antigen (HLA)-ABC expression was detected in all the groups, while HLA-DR expression was only detected in groups C and E. There were no significant differences of HLA-ABC and HLA-DR expression among groups A, B and D ($P>0.05$). However, the HLA-ABC and HLA-DR expression levels in groups C and D were higher than those of the remaining groups previously reported ($P<0.05$). Meanwhile, the HLA-ABC and HLA-DR expression levels in group E were lower than those of group C ($P<0.05$). Conclusion: CMV could up-regulate the expression levels of ICAM-1 and MHC antigens, which was closely related to allograft rejection.

Key words: Renal transplantation, Cytomegalovirus (CMV), Rejection, Intercellular adhesion molecule-1 (ICAM-1), Major histocompability complex (MHC)

doi:10.1631/jzus.B0920115 **Document code:** A **CLC number:** R692

INTRODUCTION

Renal transplantation is the most desirable therapy method for the terminal-stage nephropathy. Recently, with the development of transplantation techniques, tissue matching, and application of new immunosuppressants, the success rate of transplantation has been greatly increased. However, infectious complications after transplantation still present a big challenge. Cytomegalovirus (CMV) infection is one of the most important complications of renal transplantation (Patel and Paya, 1997; Brennan, 2001). The number of patients with CMV infection increases with the development of immunodepressive agents, such as anti-CD3 monoclonal antibody (OKT3) or antihuman thymocyte globulin (ATG), which are used in steroid-resistant acute rejection or induction therapy immediately after transplantation. Furthermore, CMV infection is one of the important factors

‡ Corresponding author
§ The two authors contributed equally to this work

* Project supported by the National Natural Science Foundation of China (No. 30772096), the Clinical Key Disciplines of National Public Health Department, the Major Scientific and Technological Special Projects of Shannxi Province (No. 2007ZDKG-67), and the Natural Science Foundation of Shaanxi Province (No. 30571799), China

influencing successful renal transplantation, because it affects immune function and is closely related to acute and chronic postoperative rejection, leading to renal allograft failure (Cainelli and Vento, 2002; Tong *et al*., 2002; Lautenschlager *et al*., 1999). Some studies have revealed that CMV disease is an independent risk factor for acute rejection in kidney allograft recipients (Krogerus *et al*., 2008; Sagedal *et al*., 2002; Toupance *et al*., 2000). Conventional anti-rejection therapy has no obvious curative effects on this type of acute rejection, while it can be reversed by anti-CMV therapy (Reinke *et al*., 1994). In addition, a study of 1399 renal transplantation recipients by Humar *et al*.(1999) has shown that the incidence of chronic rejection in patients with both CMV infection and anti-rejection therapy is significantly higher than that of patients only with anti-rejection therapy, indicating that CMV infection is an important risk factor for chronic postoperative rejection. In spite of the introduction of newer antiviral medications, CMV infection is still the most common opportunistic infection associated with renal transplantation as well as a key contributing factor for higher morbidity and mortality.

The vascular endothelium represents the anatomical and functional interface between circulating immune components and allograft, and thus it is uniquely poised to interact with both circulating immune components and allograft. Endothelial cells (ECs) are now known to regulate leukocyte migration (Jutila *et al*., 1989), express human leucocyte antigen (HLA) molecules and present antigen (Hughes *et al*., 1990), elaborate and respond to immunomodulating cytokines as interleukin-6 (IL-6) and IL-8 (Kuldo *et al*., 2005; Viemann *et al*., 2004), and inducibly express immunoreactive cellular adhesion molecules such as E-selectin, vascular adhesion molecule (VCAM), and intercellular adhesion molecule (ICAM) (Pober and Cotran, 1990). In addition, ECs also play vital roles in angiogenesis, vascular remodeling, and tumorigenesis (Tammela *et al*., 2005; Armulik *et al*., 2005). It is also well documented that ECs are a common target for CMV infection in vivo regardless of the involved organ (Roberts *et al*., 1989).

The potential mechanisms of rejection induced by CMV infection have become one recent research focus. In support of such a role, the earliest study (von Willebrand *et al*., 1986) has shown that as a consequence of CMV infection, the expression of major histocompability complex (MHC) class II antigens on grafts may result in graft rejection on the background of CMV disease. Recently, some studies have demonstrated that CMV infection can induce allograft to promote the release of cytokines, such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and IL-8 (Cinatl *et al*., 2000). CMV infection can also up-regulate the expression of ICAM-1 and MHC in ECs, which is closely related to allograft rejection (Tajik *et al*., 2008; von Willebrand *et al*., 1986; Kloover *et al*., 2000; Ustinov *et al*., 1994; Game *et al*., 2001). However, some studies have shown the opposite results. Sedmak *et al*.(1990) reported that CMV infection cannot directly induce the expression of MHC class II antigens in human umbilical vein endothelial cells (HUVECs) but encode an MHC-like protein (Wyrwicz and Rychlewski, 2008). So far, the mechanisms of rejection reaction induced by CMV infection remain unclear. Functional studies of CMV on renal transplantation, especially about the relationship between CMV infection and graft rejection, would be helpful to improve curative effects of renal transplantation. Therefore, we investigated the related gene expression of rejection induced by CMV infection using a CMV-infected EC model.

MATERIALS AND METHODS

Cell line and virus

The HUVEC line was obtained from Cell Bank of Chinese Academy of Medicine. The cells were seeded into 12-well culture plates at 5×10^5 cells per well in 200 μl Roswell Park Memorial Institute (RPMI) 1640 culture medium (Sigma, USA) containing 100 ml/L fetal calf serum (Gibco, USA), 200 kU/L penicillin, 100 mg/L streptomycin, and 2 mmol/L L-glutamine (Gibco, USA). Then, the cells were incubated in a humidified incubator at 37 °C with 5% CO_2. Human cytomegalovirus (HCMV) line AD169 was purchased from Virus Institute of Chinese Academy of Medicine. The virus was inactivated by ultraviolet ray for 30 min.

Grouping and pretreatment

Cultured HUVECs were divided into the following five groups: A, control group (without any

treatment); B, HUVECs treated with inactivated HCMV; C, HUVECs treated with HCMV; D, HUVECs treated with HCMV supernatant; and E, HUVECs treated with HCMV and 2.0 μmol/L ganciclovir (Cymevene; Hoffman-La Roche, USA).

CMV infection in HUVECs and its identification

HUVECs were cultured on coverslips coated with 0.001% (w/v) polylysine in 12-well culture plates for 48 h to 80% confluence, and then the cells were infected with 1×10^6 plaque forming unit (pfu) of HCMV for 24 h. After fixed with cold acetone for 10 min, ECs were labelled with rat anti-human HCMV immediate early antigen (IEA) monoclonal antibody (NeuMark, USA), and then they were mounted with 60% (w/v) glycerine. HCMV IEA was detected by a light microscopy (Olympus BH-2, Japan).

ECs were harvested by brief trypsinization and fixed with 2.5% (v/v) glutaraldehyde (Sigma, USA) for 30 min, and then postfixed with repeated incubations with 1% (v/v) osmium tetraoxide/1% (v/v) tannic acid (Sigma, USA) for 1 h. Subsequently, the cells were dehydrated with gradient concentrations of acetone (70%, 80%, 90%, and 100%; v/v). After dehydration, the specimens were embedded with epoxy resin for 2 h. After ultramicrotomy, the sections were analyzed under a transmission electron microscope (Hitachi H600, Japan).

Detection of ICAM-1 expression by immunohistochemical staining

Immunohistochemical staining was performed 12 h after HCMV infection. A total of 5×10^5 cells per well were seeded into chamber slides. After fixation with cold acetone for 10 min, ECs were blocked with 10% (w/v) goat serum (Dako, Glostrup, Denmark) to eliminate non-specific binding. Cells were stained with rat anti-human ICAM-1 monoclonal antibodies (IMMUNOTECH, France) at a dilution of 1:100 at room temperature for 12 h. Then, they were washed and incubated with biotinylated rabbit anti-rat secondary antibodies (IMMUNOTECH, France) at room temperature for 30 min. Subsequently, the sections were incubated with streptavidin-horseradishperoxidase at room temperature for 60 min. Then, the sections were developed with diaminobenzadine (DAB) chromogen solution and counterstained with Mayer's hematoxylin. The expression of ICAM-1 was analyzed by colorful pathological image analysis instrument (CMAIS800, Australia).

Detection of ICAM-1 expression by reverse transcriptase-polymerase chain reaction

Reverse transcriptase-polymerase chain reaction (RT-PCR) was also performed 12 h after HCMV infection. Total RNA was extracted from ECs with Trizol reagent (Invitrogen, USA). Genomic DNA contamination was eliminated by RNase-Free DNase Set kit (Qiagen, Germany). Then, 1 μg of the total RNA was reversely transcribed into cDNA within a 20-μl reaction volume according to the manufacturer's instructions (RNA LA PCR™ Kit, TaKaRa, Japan). A total of 10 μl cDNA was amplified by PCR with RNA LA PCR™ Kit (TaKaRa, Japan) with 0.5 mmol/L sense (5′-GGCAAGAACCTTACCCTACG-3′) and antisense (5′-GAGACCTCTGGCTTCGTCAG-3′) primers. A housekeeping gene, GAPDH, was used as internal control (the sense primer 5′-CCATCACCATCTTCCAGGAG-3′ and the antisense primer 5′-CCTGCTTCACCACCTTCTTG-3′). Briefly, PCR amplification was carried out with 30 cycles at a melting temperature of 94 °C for 1 min, an annealing temperature of 60 °C for 30 s, and an extension temperature of 72 °C for 30 s. The PCR products were determined by electrophoresis on 1.8% (w/v) agarose gel containing 0.05 μg/ml ethidium bromide, and the PCR products were quantified by Kodak 1D software (EDAS290, TAIYO, Japan).

Detection of HLA-ABC and HLA-DR expression by flow cytometry

Flow cytometry was performed 12 h after HCMV infection as described previously (Jiang *et al*., 2008). The ECs were harvested by brief trypsinization and incubated with optimal fluorescein isothiocyanate (FITC)-conjugated rat anti-HLA-ABC antibodies or FITC-conjugated rat anti-HLA-DR antibodies at 4 °C for 30 min in dark, respectively. The isotype FITC-conjugated rat IgG_{2A} for HLA-ABC and FITC-conjugated rat IgG_1 for HLA-DR (all from IMMUOTECH, France) were used as control. Cells were washed for three times and then re-suspended with phosphate buffered sodium (PBS) containing 0.1% (w/v) bovine serum albumin (BSA) and 0.1% (w/v) NaN_3. At least 1×10^4 cells were counted by an EPICS ELITE flow cytometry (BECKMAN

COULTER, USA), and the data were analyzed by EXPO 32 software (BECKMAN COULTER, USA). Propidium iodide (PI) staining was performed to exclude non-viable cells, and the expression of HLA-ABC and HLA-DR was analyzed by relative fluorescence intensity.

Statistical analysis

Statistical analyses were performed by SPSS statistical software (version 13.0, SPSS Inc., Chicago, IL, USA). The data were expressed as mean±standard deviation (SD), and the differences were evaluated by independent-sample t test. $P<0.05$ was considered statistically significant.

RESULTS

Evidence of HUVEC infection with HCMV

We found tans positive granules in the cytoplasm of HUVEC 12 h after the HCMV infection, and they became more visible at 24 h. Fig.1b shows that the granules were mainly around the nucleus, indicating that HCMV not only infected ECs but also was assembled in ECs. The typical owl's eye-like virus granule was visible in the cytoplasm under an electron microscope (Fig.2). Fig.1a shows that CMV IEA was not detected by immunohistochemistry in ECs before HCMV infection.

Effect of HCMV on ICAM-1 expression on the surface of ECs

Fig.3a shows that the ICAM-1 expression on the surface of ECs in group A was weakly positive. The expression of ICAM-1 was still weakly positive in groups B and D when treated with inactivated virus or

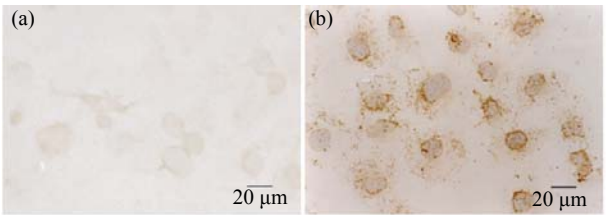

Fig.1 Observation of CMV IEA in HCMV-infected ECs. Compared with ECs before HCMV infection (a), CMV IEA was positive in ECs 24 h after HCMV infection (b) by immunohistochemical staining

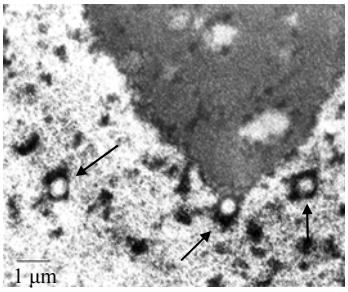

Fig.2 Observation of ECs 24 h after HCMV infection under an electron microscope. The owl's eye-like virus granules (arrow) were seen in cytoplasm

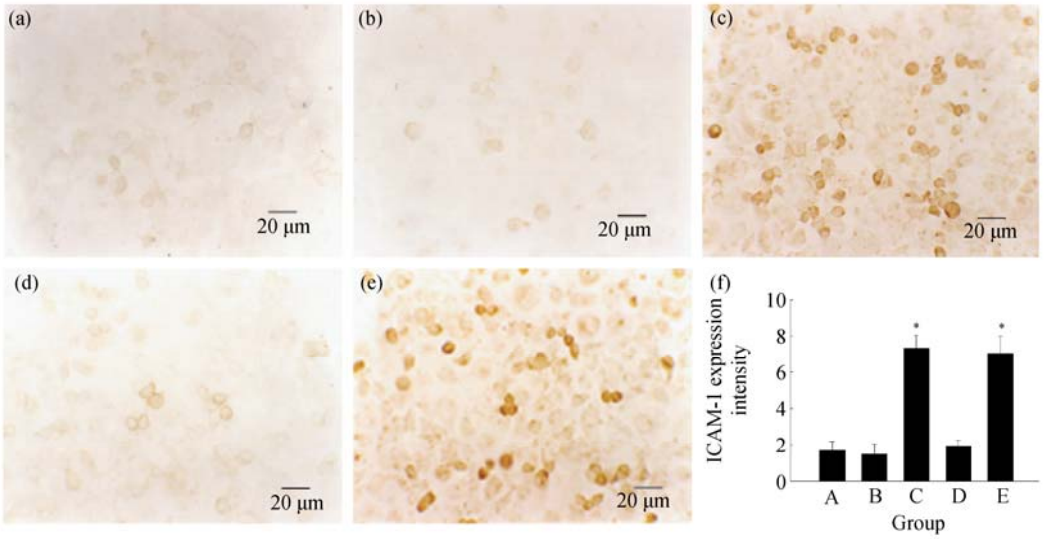

Fig.3 ICAM-1 expression in ECs in different groups. Intensity of ICAM-1 expression of groups C (c) and E (e) was significantly stronger than that in the control group (a)

(a) Control; (b) Inactivated HCMV; (c) Active HCMV; (d) HCMV supernatant; (e) HCMV with ganciclovir; (f) Comparative expression intensity of ICAM-1 in ECs of the different groups. $^{*}P<0.05$ vs. group A

virus supernatant (Figs.3b and 3d). However, ICAM-1 expression was strongly positive when ECs were infected with the active viruses (Fig.3c). ICAM-1 expression of group E treated with the active HCMV and ganciclovir was not down-regulated compared with that of group C (Fig.3e). The expression intensity of ICAM-1 determined by image analysis software was 1.70±0.48 in group A, 1.48±0.57 in group B, 7.33±0.72 in group C, 0.62±0.39 in group D, and 7.06±0.95 in group E. There was no difference among groups A, B, and D (P>0.05). Fig.3f shows that the ICAM-1 expression in groups C and E was significantly higher than that of group A (P<0.05), although there was no significant difference between groups C and E (P>0.05).

Effect of HCMV on ICAM-1 mRNA expression in ECs

ICAM-1 mRNA expression was detected in groups C and E (Fig.4), while no ICAM-1 mRNA expression was detected in group A, B, or D. Semi-quantitative analysis showed that gray scales of ICAM-1/GAPDH in groups C and E were 0.996 and 0.915, respectively. There was no significant difference between them (P>0.05).

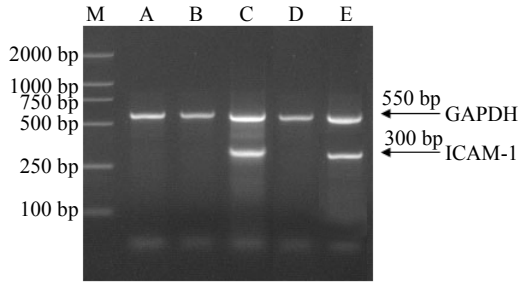

Fig.4 Expression of ICAM-1 mRNA in ECs of different groups. ICAM-1 expression could be detected in both groups C and D by PT-PCR

M: DL2000 marker; A: control; B: inactivated HCMV; C: active HCMV; D: HCMV supernatant; E: HCMV with ganciclovir

Effect of HCMV on HLA-ABC and HAL-DR expression in ECs

Fig.5 shows the detection of HLA-ABC in each group by FCM. HLA-DR expression was detected only in groups C and E, while HLA-DR nearly did not express in the remaining three groups (Fig.5). The comparative fluorescence of HLA-ABC antigen was (78±8)%, (79±6)%, (94±4)%, (76±9)%, and (89±5)% in the 5 groups, respectively, while that of HLA-DR

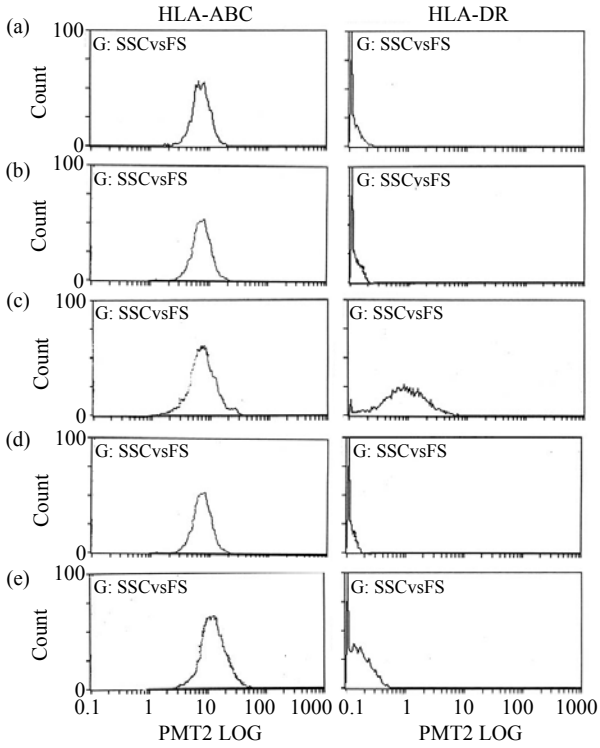

Fig.5 Expression of HLA-ABC and HLA-DR in ECs of different groups

(a) Control; (b) Inactivated HCMV; (c) Active HCMV; (d) HCMV supernatant; (e) HCMV with ganciclovir

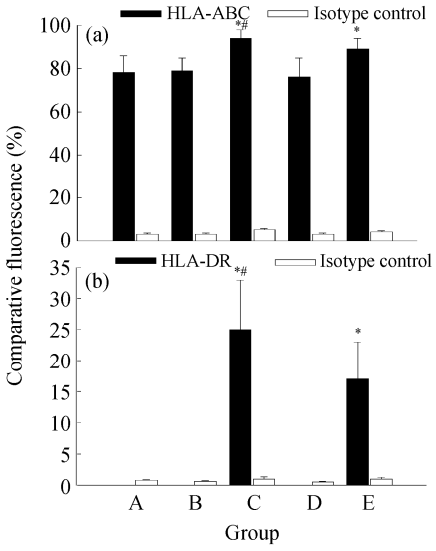

Fig.6 Relative fluorescence intensity of HLA-ABC (a) and HLA-DR (b) in different groups. CMV infection could both up-regulate expression of HLA-ABC and HLA-DR. Ganciclovir could decrease up-regulation of both HLA-ABC and HLA-DR induced by CMV infection. $^{*}P$<0.05 vs. group A; $^{\#}P$<0.05 vs. group E

Group: A, Control; B, Inactivated HCMV; C, Active HCMV; D, HCMV supernatant; E, HCMV with ganciclovir

antigen was (0±0)%, (0±0)%, (25±8)%, (0±0)%, and (17±6)%, respectively. There were no differences of HLA-ABC or HLA-DR expression among groups A, B, and E (P>0.05). However, the HLA-ABC and HLA-DR expression levels in groups C and D were higher than those of the remaining three groups previously reported (P<0.05), while the HLA-ABC and HLA-DR expression levels in group E were lower than those of group C (P<0.05) (Figs.6a and 6b).

DISCUSSION

Among many etiological agents causing infection in renal transplantation recipients, CMV, a double-helix DNA virus, is the most common and important virus, which belongs to β-subfamily of *Herpesviridae*. This virus is ubiquitously distributed in human population and often leads to a lifelong persistent infection after asymptomatic primary infection. Organ transplantation patients are the high-risk group of HCMV infection. In the last decade, there has been an increasing clinical management burden due to allograft rejection induced by CMV infection in renal transplantation recipients. Therefore, we investigated the mechanisms of rejection induced by CMV infection after renal transplantation.

As the contact surface of recipient's immune system and graft tissues, allograft vascular ECs are first recognized and attacked by recipient's immune system. They are also regarded as a bridge because immune cells chemotactically infiltrate into allograft through ECs. Thus, ECs play a key role in allograft rejection. In addition, Sinzger *et al*.(1995) showed that ECs are the major host for CMV. Therefore, we selected ECs to establish our cell model. We found characteristic images by immunohistochemistry and electron microscope, indicating successful CMV infection in ECs in vitro.

We showed that ICAM-1 of ECs expressed at a low level under normal condition by immunohistochemical staining. The expression of ICAM-1 was still low when ECs were infected by inactivated HCMV, and there was no significant difference between groups A and B, indicating that inactivated HCMV could not up-regulate ICAM-1 expression. The ICAM-1 expression was strongly positive after active HCMV infection. The intensity and gray of positive granules were significantly stronger than those of group A, indicating that HCMV could up-regulate ICAM-1 expression in ECs. A similar finding was also observed at the mRNA level. There was ICAM-1 mRNA expression when ECs were infected by active HCMV, whereas no expression in group A or B, indicating that HCMV could up-regulate ICAM-1 expression at both protein and mRNA levels.

Knight *et al*.(1999) revealed that the up-regulated ICAM-1 expression in ECs results in the accumulation of ICAM-1 mRNA. According to time sequence of CMV gene transcription and expression in cells, protein products could be divided into immediate early (IE), early (E), and late (L). An in vitro study demonstrates that synergistic action of IE expression product can activate the promoter region of ICAM-1 gene, which might be the mechanism of up-regulated ICAM-1 mRNA expression in ECs (Knight *et al*., 2000). Ganciclovir, a nucleoside analogue of guanosine, is effective in the treatment of CMV disease in humans (Martin *et al*., 1983), which is a homologue of acyclovir and the first-generation antiviral drug. In vivo, ganciclovir is transformed into ganciclovir triphosphate, which can inhibit viral DNA polymerases by competitively inhibiting the synthesis of deoxyguanosine triphosphate. In order to slow replication, ganciclovir monophosphate is inserted into the end of a growing chain of viral DNA after the release of pyrophosphate (Cheng *et al*., 1983). The transcription and expression of CMV IE gene precede the replication of CMV-DNA, and ganciclovir could only inhibit the expression of CMV E and L. In addition, our study showed that the intensity and gray of positive granules did not weaken in ganciclovir HCMV group. The ICAM-1 mRNA expression in ECs with HCMV and in the ganciclovir infection group was still up-regulated. Although the expression in group E was slightly lower than that of group C, there was no significant difference between them. This result indicates that ganciclovir was an effective anti-CMV drug, which could not inhibit the up-regulation of ICAM-1 in ECs induced by HCMV. Besides, we found that the ICAM-1 expression in ECs cultured with CMV supernatant was not up-regulated, further demonstrating that CMV IE gene could directly induce ICAM-1 expression rather than any cytokine produced by host cells. This finding may

explain the clinical manifestation that allograft rejection still occurs in CMV infected patients even after effective anti-CMV therapy.

So far, there are still some controversies about the effect of CMV infection on HLA expression in ECs. Although it is widely accepted that CMV infection of permissive cells causes down-regulation of MHC (Haynes *et al*., 2005), some studies report that CMV induces the regulation of MHC class I antigen expression in human aortic smooth muscle cells (Hosenpud *et al*., 1991). In our study, HLA-ABC was expressed at a low level, and HLA-DR was nearly absent in ECs under normal condition. The same finding was observed in groups B and D as well. Moreover, the HLA-ABC and HLA-DR expression in group C was significantly higher than that in groups A, B, and D. The expression of MHC class I and class II antigens enhanced antigen presentation effect of ECs and activated the resting T lymphocytes. This activation resulted in a cascade reaction of allograft rejection, indicating that CMV could up-regulate the expression of MHC class I antigen and induce the expression of MHC class II antigen through CMV gene transcription and expression. The expression levels of both HLA-ABC and HLA-DR in group E were significantly lower than those in group C, but they were still significantly higher than those in group A. This result suggests that ganciclovir could down-regulate the expression of MHC class I and class II antigens induced by CMV. However, they could not return to the initial level without CMV infection. It remains unclear how CMV infection could up-regulate the expression of MHC class I and class II antigens in ECs. A previous study showed that one kind of glycoprotein encoded by CMV is similar to MHC class I antigens (Beck and Barrell, 1988). Therefore, MHC class I antigens actually might be virus class I protein encoded by CMV genes, which are expressed on ECs and induced by CMV infection. Some studies revealed the same amino acid sequence between proteins encoded by CMV IE genes and the conservative region of HLA-DR β chain (Fujinami *et al*., 1988). Thus, MHC class II antigens expressed in CMV-infected ECs are also the products of CMV gene transcription and expression (Fujinami *et al*., 1988), which may result in up-regulated HLA antigen expression in CMV-infected ECs. Weinberg *et al*. (2000) showed that HLA antigen expression enhances antigen presentation in ECs and leads to the activation of resting T lymphocytes, and that the HLA antigen expression activates the cascade reaction of allograft rejection subsequently. We also found that the expression of MHC class I and class II antigens was down-regulated in CMV-infected ECs after the addition of ganciclovir. This could be due to that CMV genes encoding MHC class I and class II antigens in ECs were E or L gene, not IE gene. Therefore, ganciclovir could obviously down-regulate MHC antigen expression in CMV-infected ECs, which could inhibit CMV E and L gene expression.

In summary, we found that the expression of ICAM-1 and MHC antigens was up-regulated by CMV infection, which may be a key contributing factor of allograft rejection. Our findings provide a theoretical basis for further clinical research. In future, more efforts should be made to prevent allograft rejection induced by CMV infection based on the results presented here.

References

Armulik, A., Abramsson, A., Betsholtz, C., 2005. Endothelial/pericyte interactions. *Circ. Res.*, **97**(6):512-523. [doi:10.1161/01.RES.0000182903.16652.d7]

Beck, S., Barrell, B.G., 1988. Human cytomegalovirus encodes a glycoprotein homologous to MHC class I antigen. *Nature*, **331**(6153):269-276. [doi:10.1038/331269a0]

Brennan, D.C., 2001. Cytomegalovirus in renal transplantation. *J. Am. Soc. Nephrol.*, **12**(1):848-855.

Cainelli, F., Vento, S., 2002. Infections and solid organ transplant rejection: a cause-and-effect relationship? *Lancet Infect. Dis.*, **2**(9):539-549. [doi:10.1016/S1473-3099(02)00370-5]

Cheng, Y.C., Grill, S.P., Dutschman, G.E., Nakayamak, K., Bastow, K.F., 1983. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl) guanine, a new anti-herpes virus compound, in herpes simplex virus-infected cells. *J. Biol. Chem.*, **258**(20):12460-12664.

Cinatl, J.Jr., Kotchekov, R., Weimer, E., Blaheta, R.A., Scholz, M., Vogel, J.U., Gumbel, H.O., Doerr, H.W., 2000. The antisense oligonucleotide ISIS 2922 prevents cytomegalovirus-induced upregulation of IL-8 and ICAM-1 in cultured human fibroblasts. *J. Med. Virol.*, **60**(3):313-323. [doi:10.1002/(SICI)1096-9071(200003)60:3<313::AID-JMV10>3.0.CO;2-K]

Fujinami, R.S., Nelson, J.A., Walker, L., Oldstone, M.B., 1988. Sequence homology and immunologic cross-reactivity of human cytomegalovirus with HLA-DR chain: a means for graft rejection and immunosuppression. *J. Virol.*, **62**(1):100-105.

Game, D.S., Warrens, A.N., Lechler, R.I., 2001. Rejection mechanisms in transplantation. *Wien. Klin. Wochenschr.*,

113(20):832-838.

Haynes, L.D., Waldman, W.J., Bushkin, Y., Love, R.B., Burlingham, W.J., 2005. CMV-infected allogeneic endothelial cells initiate responder and bystander donor HLA class I release via the metalloproteinase cleavage pathway. *Hum. Immunol.*, **66**(3):211-221. [doi:10.1016/j.humimm.2004.12.005]

Hosenpud, J.D., Chou, S.W., Wagner, C.R., 1991. Cytomegalovirus-induced regulation of major histocompatibility complex class I antigen expression in human aortic smooth muscle cells. *Transplantation*, **52**(5):896-902. [doi:10.1097/00007890-199111000-00027]

Hughes, C.C., Savage, C.O., Pober, J.S., 1990. The endothelial cell as a regulator of T cell function. *Immunol. Rev.*, **117**(1):85-102. [doi:10.1111/j.1600-065X.1990.tb00568.x]

Humar, A., Gillingham, K.J., Payne, W.D., Dunn, D.L., Sutherland, D.E.R., Matas, A.J., 1999. Association between cytomegalovirus disease and chronic rejection in kidney transplant recipients. *Transplantation*, **68**(12):1879-1885. [doi:10.1097/00007890-199912270-00011]

Jiang, Y.F., Zhao, F.D., Li, X.B., Ning, Y.X., Zhi, X.L., Qian, R.Z., Yin, L.H., 2008. Effects of RNA interference-induced tryptase down-regulation in P815 cells on IL-6 and TNF-alpha release of endothelial cells. *J. Zhejiang Univ. Sci. B*, **9**(8):656-661. [doi:10.1631/jzus.B0810188]

Jutila, M.A., Berg, E.L., Kishimoto, T.K., Picker, L.J., Bargatze, R.F., Bishop, D.K., Orosz, C.G., Wu, N.W., Butcher, E.C., 1989. Inflammation-induced endothelial cell adhesion to lymphocytes, neutrophils, and monocytes. Role of homing receptors and other adhesion molecules. *Transplantation*, **48**(5):727-731. [doi:10.1097/00007890-198911000-00001]

Kloover, J.S., Soots, A.P., Krogerus, L.A., Kauppinen, H.O., Loginov, R.J., Holma, K.L., Bruggeman, C.A., Ahonen, P.J., Lautenschlager, I.T., 2000. Rat cytomegalovirus infection in kidney allograft recipients is associated with increased expression of intercellular adhesion molecule-1, vascular adhesion molecule-1, and their ligands leukocyte function antigen-1 and very late antigen-4 in the graft. *Transplantation*, **69**(12):2641-3647. [doi:10.1097/00007890-200006270-00026]

Knight, D.A., Waldman, W.J., Sedmak, D.D., 1999. Cytomegalovirus-mediated modulation of adhesion molecule expression by human arterial and microvascular endothelial cells. *Transplantation*, **68**(11):1814-1818. [doi:10.1097/00007890-199912150-00030]

Knight, D.A., Briggs, B.R., Bennett, C.R., Harindranath, N., Waldman, W.J., Sedmak, D.D., 2000. Attenuation of cytomegalovirus-induced endothelial intercellular adhesion molecule-1 mRNA/protein expression and T lymphocyte adhesion by a 2′-*O*-methoxyethyl antisense oligonucleotide. *Transplantation*, **69**(3):417-426. [doi:10.1097/00007890-200002150-00019]

Krogerus, L., Soots, A., Loginov, R., Bruggeman, C., Lautenschlager, I., 2008. CMV increases tubular apoptosis through the TNF-alpha-TNF-R1 pathway in a rat model of chronic renal allograft rejection. *Transpl. Immunol.*, **18**(3):232-236. [doi:10.1016/j.trim.2007.07.004]

Kuldo, J.M., Westra, J., Asgeirsdottir, S.A., Kok, R.J., Oosterhuis, K., Rots, M.G., Schouten, J.P., Limburg, P.C., Molema, G., 2005. Differential effects of NF-{kappa}B and p38 MAPK inhibitors and combinations thereof on TNF-{alpha}- and IL-1{beta}-induced proinflammatory status of endothelial cells in vitro. *Am. J. Physiol. Cell Physiol.*, **289**(5):C1229-C1239. [doi:10.1152/ajpcell.00620.2004]

Lautenschlager, I., Soots, A., Krogerus, L., Inkinen, K., Kloover, J., Loginov, R., Holma, K., Kauppinen, H., Bruggeman, C., Ahonen, J., 1999. Time-related effects of cytomegalovirus infection on the development of chronic renal allograft rejection in a rat model. *Intervirology*, **42**(5-6):279-284. [doi:10.1159/000053961]

Martin, J.C., Dvorak, C.A., Smee, D.F., Matthews, T.R., Verheyden, J.P.H., 1983. 9-[(1,3-dihydroxy-2-propoxy) methyl]guanine: a new potent and selective antiherpes agent. *J. Med. Chem.*, **26**(5):759-761. [doi:10.1021/jm00359a023]

Patel, R., Paya, C.V., 1997. Infections in solid-organ transplant recipients. *Clin. Microbiol. Rev.*, 10(1):86-124.

Pober, J.S., Cotran, R.S., 1990. Cytokines and endothelial cell biology. *Physiol. Rev.*, **70**(2):427-451.

Reinke, P., Fietze, E., Odehakim, S., Prosch, S., Lippert, J., Ewert, R., Volk, H.D., 1994. Late acute renal allograft rejection and symptomless cytomegalovirus infection. *Lancet*, **344**(8939-8940):1737-1738. [doi:10.1016/S0140-6736(94)92887-8]

Roberts, W.H., Sneddon, J.M., Waldman, W.J., Stephens, R.E., 1989. Cytomegalovirus infection of gastrointestinal endothelium demonstrated by simultaneous nucleic acid hybridization and immunohistochemistry. *Arch. Pathol. Lab. Med.*, **113**(5):461-464.

Sagedal, S., Nordal, K.P., Hartmann, A., Sund, S., Scott, H., Degre, M., Foss, A., Leivestad, T., Osnes, K., Fauchald, P., Rollag, H., 2002. The impact of cytomegalovirus infection and disease on rejection episodes in renal allograft recipients. *Am. J. Transplant.*, **2**(9):850-856. [doi:10.1034/j.1600-6143.2002.20907.x]

Sedmak, D.D., Roberts, W.H., Stephens, R.E., Buesching, W.J., Morgan, L.A., Davis, D.H., Waldman, W.J., 1990. Inability of cytomegalovirus infection of cultured endothelial cells to induce HLA class II antigen expression. *Transplantation*, **49**(2):458-462. [doi:10.1097/00007890-199002000-00043]

Sinzger, C., Grefte, A., Plachter, B., Gouwash, A.S.H., The, T.H., Jahn, G., 1995. Fibroblasts, epithelial cells, endothelial cells and smooth muscle cells are major targets of human cytomegalovirus infection in lung and gastrointestinal tissues. *J. Gen. Virol.*, **76**(4):741-745. [doi:10.1099/0022-1317-76-4-741]

Tajik, N., Salari, F., Ghods, A.J., Hajilooi, M., Radjabzadeh, M.F., Mousavi, T., 2008. Association between recipient ICAM-1 K469 allele and renal allograft acute rejection. *Int. J. Immunoqenet.*, **35**(1):9-13. [doi:10.1111/j.1744-313X.2007.00727.x]

Tammela, T., Enholm, B., Alitalo, K., Paavonen, K., 2005. The biology of vascular endothelial growth factors. *Cardiovasc. Res.*, **65**(3):550-563. [doi:10.1016/j.cardiores.2004.12.002]

Tong, C.Y.W., Bakran, A., Peiris, J.S.M., Muir, P., Herrington, C.S., 2002. The association of viral infection and chronic allograft nephropathy with graft dysfunction after renal transplantation. *Transplantation*, **74**(4):576-578. [doi:10.1097/00007890-200208270-00026]

Toupance, O., Bouedjoro, M.C., Carquin, J., Carquin, J., Novella, J.L., Lavaud, S., Wynckel, A., Jolly, D., Chanard, J., 2000. Cytomegalovirus-related disease and risk of acute rejection in renal transplant recipients: a cohort study with case-control analyses. *Transpl. Int.*, **13**(6):413-419. [doi:10.1111/j.1432-2277.2000.tb01019.x]

Ustinov, J., Bruggeman, C., Hayry, P., Lautenschlager, I., 1994. Cytomegalovirus-induced class II expression in rat kidney. *Transplant. Proc.*, **26**(3):1729-1736.

Viemann, D., Goebeler, M., Schmid, S., Klimmek, K., Sorg, C., Ludwig, S., Roth, J., 2004. Transcriptional profiling of IKK2/NF-kappa B- and p38 MAP kinase-dependent gene expression in TNF-alpha-stimulated primary human endothelial cells. *Blood*, **103**(9):3365-3373. [doi:10.1182/blood-2003-09-3296]

von Willebrand, E., Pettersson, E., Ahonen, J., Ahonen, J., Hayry, P., 1986. CMV infection, class-II antigen expression, and human kidney allograft-rejection. *Transplantation*, **42**(4):364-367. [doi:10.1097/00007890-198610000-00006]

Weinberg, A., Zhang, L., Hayward, A.R., 2000. Alloreactive cytotoxic $CD4^+$ responses elicited by cytomegalovirus-infected endothelial cells: role of MHC class I antigens. *Viral. Immunol.*, **13**(1):37-47. [doi:10.1089/vim.2000.13.37]

Wyrwicz, L.S., Rychlewski, L., 2008. Cytomegalovirus immediate early gene UL37 encodes a novel MHC-like protein. *Acta Biochim. Pol.*, **55**(1):67-73.