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Brief communication

Detection of engraftment of donor-derived antibody producing cells in a lung transplant recipient by anti-cytomegalovirus IgG avidity test

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ABSTRACT

Transplant recipients become immunocompromised through the use of immunosuppressive therapy to prevent allograft rejection. These recipients readily experience human cytomegalovirus (CMV) infection or reactivation. Therefore, CMV represents a life-threatening pathogen in transplant recipients. To demonstrate the serostatus and course of IgG maturation against CMV in transplant patients, we measured the transition of anti-CMV IgG and its affinity (avidity index; AI) as criteria for antibody maturation. Among 31 lung transplant recipients, 26 were infected with CMV before transplantation and maintained anti-CMV IgG and high AI values throughout the study period. Four of the 31 experienced primary infection with CMV through the allograft, with two of the 4 recipients presented high AI values even after 6 month post-transplantation. A significant portion of donor-derived plasma cells were detectable in one recipient. These results suggested that the plasma cells from donors are carried in through the transplanted lung and lymph nodes and produce matured high-avidity IgG from the early stage of transplantation.

Lung transplantation is an established therapeutic strategy for patients with end-stage lung diseases. Survival after lung transplantation is limited by the incidence of chronic lung allograft dysfunction (CLAD) [1] induced as a result of the infection, external environment, aspiration, or drug toxicity [2,3]. Chronic rejection is the leading cause of morbidity and mortality after lung transplantation. The balance between $CD4^+$ helper and $CD8^+$ cytotoxic T cells and Tregs can significantly affect graft survival [4]. In order to prevent allograft rejection, transplant recipients must be treated with immunosuppressive therapy.

Despite the importance of immunosuppression in preventing allograft rejection, recipients become immunocompromised and easily infected with various pathogens [5]. Human cytomegalovirus (CMV) is one of the most important and common pathogens observed after transplantation as immunosuppression leads to the reactivation of CMV. In Japan, about 70% of healthy adults have latent CMV infection [6–8]. As CMV reactivation induces host immune responses such as inflammation, CMV has been associated with morbidity including chronic allograft rejection in transplant recipients [9–11]. The monitoring of CMV reactivation is important in preventing allograft rejection. The serostatus of the donor and recipient are markers of the possible development of CMV-related disease.

In this report, we monitored the periodical anti-CMV IgG status of lung transplant recipients in Tohoku University hospital from 2014 to 2017. Informed consent was obtained from all participants prior to enrolment in the study and the study was approved by the Ethics Committee of Tohoku University Hospital. Serum specimens were collected at the time of transplantation (0 month) and at 1, 3, 6, 12, and 24 months post-transplantation. In addition, peripheral blood mononuclear cells (PBMCs) were collected with Lympholyte reagent (Cedarlane, Burlington, Ontario, Canada) and stored at −80 °C in the cryopreservation medium. All patients were administrated with triple immunosuppressive therapy (tacrolimus, mycophenolate mofetil, and a steroid or tacrolimus, sirolimus, and a steroid). The donor CMV serostatus and HLA subtype were obtained from a review of the clinical information. Anti-CMV IgG titer was measured with an anti-CMV IgG kit (Abbott Diagnostics, Santa Clara, CA). As shown in Table 1, recipients were divided into 4 groups according to pre-transplantation serostatus; D+/R+ (77.4%), D+/R− (12.9%), D−/R+ (6.5%) and D−/R− (3.2%). All recipients were treated preventively with

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Table 1
Demographic, clinical and immunological characteristics of the 31 patients pre-transplantation.

Characteristic	
Patient number	31
Age ± SD, y[a]	43.1 ± 10.5
Male sex: No. (%)	18 (58.1)
Baseline disease: No. (%)	
Idiopathic pulmonary fibrosis (IPF)	8 (25.8)
Lymphangioleiomyomatosis (LAM)	4 (12.9)
Chronic obstructive pulmonary disease (COPD)	4 (12.9)
Bronchial ectasia (BE)	4 (12.9)
Idiopathic pulmonary arterial hypertension (IPAH)	4 (12.9)
Chronic lung allograft dysfunction (CLAD)	2 (6.4)
Diffused panbrochialitis (DPB)	2 (6.4)
Proteinosis	1 (3.2)
Eisenmenger's syndrome	1 (3.2)
Pulmonary Langerhans'-cell histiocytosis (LCH)	1 (3.2)
Immunosuppressive therapy: No. (%)	
Tacrolimus/MMF[b]/Steroid	29 (93.5)
Tacrolimus/Sirolimus/Steroid	2 (6.4)
CMV serostatus: No. (%)	
D+/R+	24 (77.4)
D+/R−	4 (12.9)
D−/R+	2 (6.4)
D−/R−	1 (3.2)
Anti-CMV therapy: No. (%)	
GCV and/or VGCV	31 (100)
FOS	1 (3.2)

[a] Age at transplantation.
[b] MMF: Mycophenolate mofetil.

ganciclovir (GCV) or valganciclovir (VGCV) for more than 6 months after transplantation. Although the GCV treatment of one D−/R− case was terminated at 2 months because of leukocytopenia, no CMV reactivation was observed in this case. One CLAD patient was treated with foscarnet (FOS) before secondary transplantation in order to prevent CMV reactivation but the treatment was changed to GCV after the secondary transplantation. All recipients were transfused with IgG preparations at 1, 2, 3 and 21 days post-transplantation.

In addition to the anti-CMV IgG titer, we also monitored the IgG avidity index (AI), which was defined as the strength of the IgG binding to antigenic epitopes [12,13]. Antibodies mature gradually during the 6 months following primary infection in immunocompetent individuals, while IgG AI is known to remain low for up to 3 months from primary infection in pregnant women [14,15]. On the other hand, people with a high AI can be excluded from primary infection, meaning infection within the preceding 3 months. In this report we used an anti-CMV IgG Avidity kit with thresholds of 50% or less for low, 50–60% for intermediate, and 60 or more for high (Abbott).

A representative D+/R+ case (1 of 24 cases) as well as all D−/R− (1 case) and D+/R− (4 cases) cases are shown in Fig. 1. Anti-CMV IgG titers, AI values and the amount of transfused IgG preparations are shown as closed circles with solid line, open squares with dotted line and gray bars, respectively. The amount of transfused IgG should be decreased as the mean half-life time of IgG in healthy humans, 23 days. [16].

Anti-CMV IgG titers and AI values were maintained in all CMV-positive recipients, even under immunosuppressive therapy, as shown in the results for patient 1 (Fig. 1A). According to the results for the D−/R− recipient without additional transfusion, anti-CMV antibodies with a high AI value from blood transfusion during surgery was detected at 1 month after transplantation, and then decreased over the following 3 months due to the half-life of IgG (Fig. 1B, patient 2). In addition, antibodies derived from transfusion would disappear within 4 month post-transfusion because most infants seemed to lose maternal antibodies at 4 month postnatal [17]. The changes in AI values in the D+/R− cases were classified in two groups. In one group, the anti-CMV IgG titers gradually increased after 6 months, at which time the transfused IgG is expected to have disappeared. However, the AI value remained low or intermediate even at 12 months as the maturation of IgG was delayed (Fig. 1C and D: patient 3 and 4, respectively). In the other group, the AI values did not decrease, even with the patients in an immunosuppressive state (Fig. 1E and F: patient 5 and 6, respectively).

There have only been a few reports on variations in anti-CMV IgG AI in solid organ transplant recipients to date [18,19]. Persisting CMV antigenemia might correlate with delays in antibody maturation as anti-CMV IgG with low avidity is considered to be less effective in inhibiting CMV infection [18]. However, our cases did not fit this criterion (Fig. 1C, D). In another report, the maturation of CMV-specific antibodies was shown to be delayed after primary infection in solid organ transplant patients similar to our two cases shown in Fig. 1C and D [19]. It should take more than 6 months for anti-CMV IgG to mature under immunosuppressive therapy. The alterations in IgG AI values shown in Fig. 1C and D were consistent with the findings of a previous report in which the humoral immune response was shown to be pretty poor after lung transplantation [20]. However, high anti-CMV IgG AI values were detectable over the long term in another group (Fig. 1E, F). High IgG AI values were observed even at 3 or 6 months post-transplantation in these cases. There are at least two possible explanations for this; (i) the anti-CMV IgG rapidly matured in these recipients or (ii) the allograft contained donor-derived anti-CMV IgG-producing cells.

The first possibility is less likely because the maturation of anti-CMV IgG basically takes 6 months even in immunocompetent individuals [12]. In addition, the maturation of antibodies should be delayed in these recipients because of the immunosuppressive therapy. In the second possibility, plasma cells or related cells are thought to be contained in the lymph nodes of allografts. To examine the existence of donor-derived plasma cells, recipient-derived PBMCs were analyzed by flow cytometer and sorted with $CD19^-/CD138^+$, a marker of plasma cells (anti-CD19: Clone HIB19; anti-CD138: clone MI15, BioLegend, San Diego, CA). As HLA-A2 (anti-HLA-A2: clone BB7.2, MBL, Nagoya, Japan) was displayed by the donors but not by either patient 5 or 6, the expression of HLA-A2 on $CD19^-/CD138^+$ cells was examined.

Because the pre-transplanted PBMC of patient 5 was not suitable for FACS analysis because of some effects of pre-treatments, we only analyzed the HLA-A2 expression level of patient 6 as shown in Fig. 2. A significant fraction of $HLA\text{-}A2^+/CD19^-/CD138^+$ cells was detectable in PBMCs of patient 6 at 6 and 12 months post-transplantation (Fig.2). These cells are thought to be derived from the lymph nodes of allografts and expressed anti-CMV IgG with a high AI. Although we could not able to analyze the carry-in of donor-derived antibody expressing cells in patient 5, it is possible that the high AI IgG would be derived from transfused immunoglobulins. As shown in Fig. 1E, Patient 5 was treated with 15 g of immunoglobulin preparations at 3 months post-transplantation (Fig. 1E gray bars). These IgG would detected with high AI at 6 month specimen.

The differentiation of plasma cells from B cells occurs in the germinal centers of secondary lymphoid organs. Donor-derived plasma cells are expected to be carried into the recipient through the lymphoid organs attached to the lung allograft. Although plasma cells are short-lived, a small proportion of plasma cells can survive and secrete antibodies for more than 1 year [21,22]. Actually, long-term allergen-specific IgE production was detected in non-allergic individuals after bone marrow transplantation from allergic individuals [23]. Most long-lived plasma cells are found in the bone marrow [24,25], but a small proportion of these cells can persist in the secondary lymphoid organs (spleen or lymph nodes) where they were generated as well as in the intestine [26–30]. However, it remains to be elucidated whether these cells are contained in the lymph nodes of solid organs such as the lungs.

To understand the effects of the carry-in of antibody-producing cells on recipient health, it is necessary to carry out further clinical studies with a larger sample size and/or recruit other organ(s) transplant recipients.

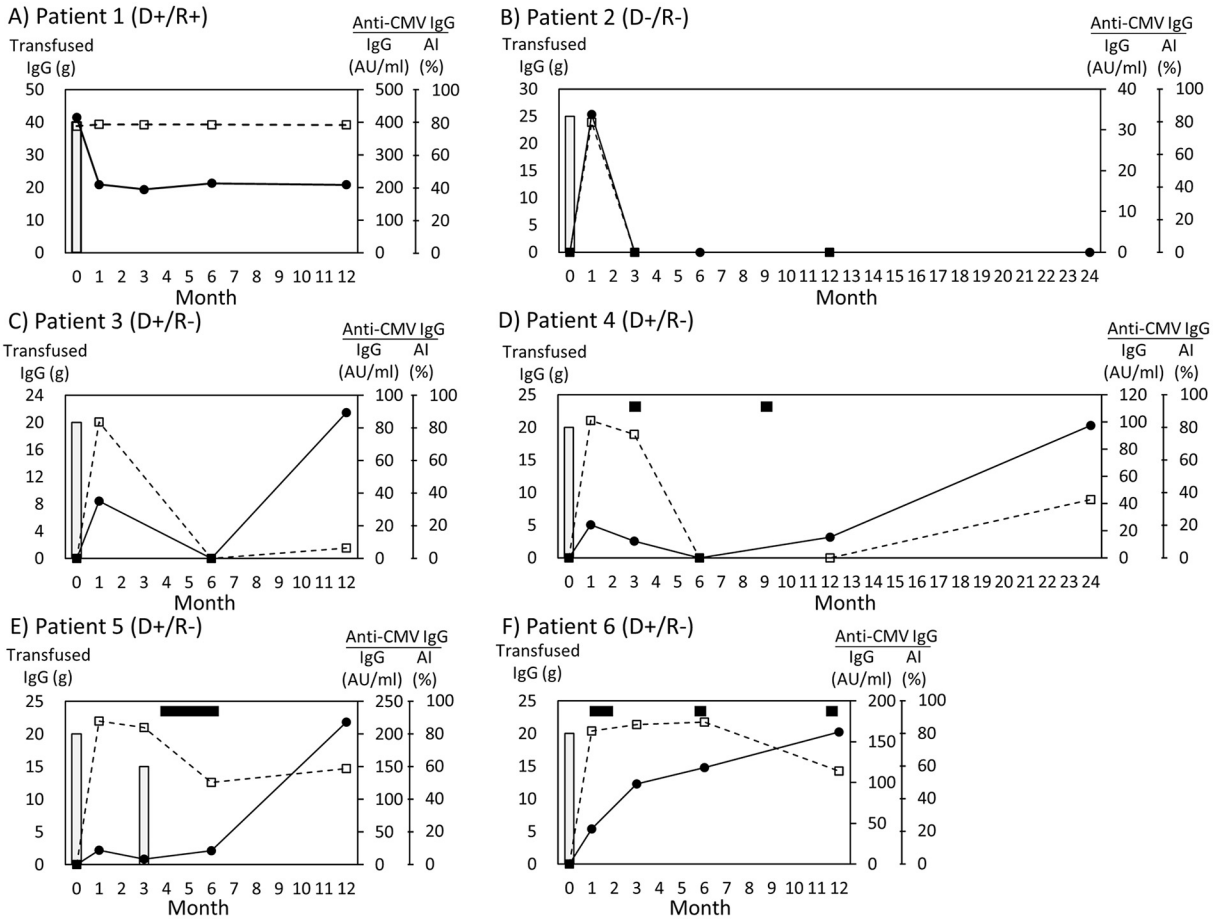

Fig. 1. Anti-CMV IgG titer and avidity index kinetics in the recipients. A representative of D+/R+ case (A) as well as one D−/R− (B) and four D+/R− (C to F) cases are shown. The amount of transfused IgG preparations (g) is shown as gray bars, anti-CMV IgG titer (AU/ml) as closed circles with a solid line, and avidity index (%) as open squares with a dotted line. The horizontal black bars indicate periods during which antigenemia was detected. The horizontal axis indicates months post-transplantation.

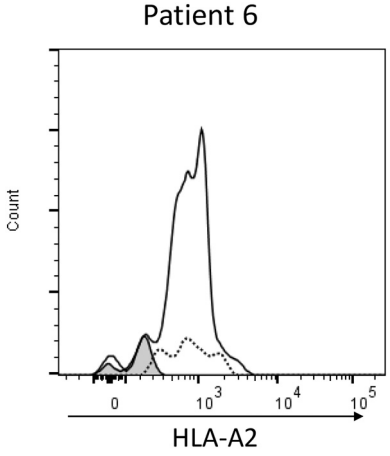

Fig. 2. The existence of donor-derived HLA-A2-positive $CD19^{-}/CD138^{+}$ in HLA-A2-negative recipient PBMCs. PBMCs were collected at pre-transplantation (gray area with solid line) and 6 (dotted line) and 12 (solid line) months post-transplantation, and then stored at −80 °C in cryopreservation medium. After reconstitution, cells were analyzed by flow cytometry with anti-HLA-A2, anti-CD19 and anti-CD138 antibodies. The histograms indicated the expression level of HLA-A2 on $CD19^{-}/CD138^{+}$ cells from patient 6.

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Contributions

T. Suzutani, Y. Okada and T. Kondo were involved in the study conception and design of this study. Y. Matsuda and H. Suzuki participated in patient identification, clinical care and discussion of clinical events. T. Koshizuka, K. Ikuta, T. Kobayashi and R. Kanno participated in acquisition and analysis of data. T. Koshizuka, K. Ikuta, and T. Suzutani participated in the interpretation of results and the drafting of the article. All authors participated in the critical revision of article.

Disclosure

The authors have no conflicts of interest to declare in association with this manuscript.

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