Clinical Immunology 208 (2019) 108258

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

Expression pattern of co-inhibitory molecules on CMV-specific T-cells in lung transplant patients

Vasiliki Bessa[a], Ming Sun[b], Maike Meier[c], Ye Zeng[b], Shilei Xu[b], Sebastian Dolff[c], Urte Sommerwerck[d], Johannes Korth[b], Christian Taube[a], Clemens Aigner[e], Markus Kamler[f], Andreas Kribben[b], Monika Lindemann[g], Oliver Witzke[c], Benjamin Wilde[b,*]

[a] Department of Pulmonary Medicine, University Hospital Essen – Ruhrlandklinik, West German Lung Transplantation Center, Tüschener Weg 40, 45239 Essen, Germany
[b] Department of Nephrology, University Hospital Essen, University Duisburg-Essen, Hufelandstr. 55, 45147 Essen, Germany
[c] Department of Infectious Diseases, West German Centre of Infectious Diseases, University Hospital Essen, University Duisburg-Essen, Germany
[d] Department of Pneumology, Krankenhaus der Augustinerinnen, Jakobstr. 27-31, 50678 Cologne, Germany
[e] Department of Thoracic Surgery, University Hospital Essen – Ruhrlandklinik, West German Lung Transplantation Center, Tüschener Weg 40, 45239 Essen, Germany
[f] Department of Thoracic and Cardiovascular Surgery, West German Heart Center, University Hospital Essen, University Duisburg-Essen, Hufelandstr. 55, 45147 Essen, Germany
[g] Institute for Transfusion Medicine, University Hospital Essen, University Duisburg-Essen, Virchowstr. 179, 45147 Essen, Germany

ARTICLE INFO

Keywords:
Cytomegalovirus
Lung transplant
T-cells
BTLA
PD-1
Co-inhibitors

ABSTRACT

Objectives: Cytomegalovirus infection (CMVi) occurs frequently in transplant patients. Co-inhibitory molecules on CMV-specific T-cells (TCMV) in patients after lung transplantation were investigated.
Methods: 59 lung transplant patients were stratified according to anti-CMV serostatus at time of transplantation. The co-inhibitors Programmed-Death-Receptor-1 (PD1) and B-and-T-Lymphocyte-Attenuator (BTLA) were detected on TCMV by flow cytometry (FACS).
Results: TCMV were detectable in CMV sero-positive patients (R+) and in CMV sero-negative patients with a lung graft of a CMV sero-positive donor (D+/R−); in both cases, the frequency of TCMV was higher than in healthy controls (HC). PD-1 on TCMV was increased in D+/R+ and D+/R− patients as compared to HC. BTLA was significantly enhanced on TCMV of D+/R− patients vs. HC. R+ patients with CMV reactivation in the past had an increased fraction of BTLA+ TCMV.
Conclusion: In conclusion, the expression pattern of co-inhibitory molecules on TCMV is altered in patients after lung transplantation.

1. Introduction

Human CMV is a betaherpesvirus and primary infection in healthy individuals is usually asymptomatic [1]. After primary infection, CMV persists in latency and may be reactivated. However, cytomegalovirus infection (CMVi) is a major complication in lung transplantation [2,3]. Immunosuppressive therapy to prevent organ rejection suppresses T-cell immunity essential for immunological viral control and renders the host susceptible for serious primary CMVi or reactivation. CMV interferes on multiple levels with the host immune system and has pro-inflammatory and anti-inflammatory effects at the same time [4]. Accordingly, CMVi after lung transplantation increases the risk for organ rejection, other infections and leads to increased overall mortality [3,4]. Therefore, different strategies have been developed to decrease the risk of CMVi. Patients are stratified by the donor and recipient anti-CMV-IgG serostatus at the time of transplant to estimate the risk for CMVi [3–5]. Transplant recipients lacking anti-CMV-IgG are regarded as CMV naïve and thus have a high risk of primary infection receiving

Abbreviations: CMV, Cytomegalovirus; CMVi, CMV-Infection; CsA, Cyclosporine A; GrB, Granzyme B; HC, healthy controls; IL, Interleukin; RTX, renal transplant patients

* Corresponding author.
E-mail addresses: Vasiliki.besa@rlk.uk-essen.de (V. Bessa), ming.sun@stud.uni-due.de (M. Sun), maike.meier@stud.uni-due.de (M. Meier), Sebastian.dolff@uk-essen.de (S. Dolff), USommerwerck@severinskloesterchen.de (U. Sommerwerck), johannes.korth@uk-essen.de (J. Korth), Christian.taube@rlk.uk-essen.de (C. Taube), clemens.aigner@rlk.uk-essen.de (C. Aigner), markus.kamler@uk-essen.de (M. Kamler), andreas.kribben@uk-essen.de (A. Kribben), monika.lindemann@uk-essen.de (M. Lindemann), oliver.witzke@uk-essen.de (O. Witzke), benjamin.wilde@uk-essen.de (B. Wilde).

https://doi.org/10.1016/j.clim.2019.108258
Received 10 July 2019; Received in revised form 4 September 2019; Accepted 5 September 2019
Available online 06 September 2019
1521-6616/

an allograft of a CMV-positive donor [5]. Recipients being CMV-positive have an intermediate risk for CMVi regardless of donor serostatus. Seronegative recipients receiving an allograft from a seronegative donor are regarded as low risk. Patients with intermediate and high risk usually receive antiviral prophylaxis with valganciclovir which has been demonstrated to reduce the chance of symptomatic, serious CMVi [3–6]. Nevertheless, late CMV infections after cessation of antiviral prophylaxis occur frequently [3–5,7]. Previous studies have shown that inefficient cellular anti-CMV T-cell immunity might be an important factor [8]. Altered activation of virus-specific T-cells may hamper efficient immunological T-cell responses [9,10]. Co-inhibitory molecules such as PD-1 and BTLA are important checkpoints to maintain immune tolerance [11]. Ligation of these receptors on T-cells by PDL-1/2 or herpes virus entry mediator (HVEM) induces anergy and suppresses T-cell effector function. Blockade of immunoregulatory checkpoints to activate anti-tumor immunity has become a very successful therapeutic approach in oncology [11]. PD-1 and BTLA expressed on virus-specific T-cells increase the threshold for T-cell activation and have been shown to interfere with immunological viral control in other infectious diseases, e.g. HIV and Hepatitis B/C [12,13]. Co-inhibitors may also interfere with immunological control of CMV infection after solid organ transplantation. In this study, we characterized CMV-specific T-cells in patients after lung transplantation with respect to the expression pattern of co-inhibitors.

2. Material and methods

2.1. Patient cohort

Fifty-nine lung transplant patients with a mean time of 75 ± 51 months after lung transplantation were included in this study. Patients were recruited from the outpatient clinic of the department of pulmonary medicine in a consecutive way. The mean age was 57 ± 9 years (Table 1). Patients were stratified by pre-transplant recipient and donor CMV serology. Patients with detectable anti-CMV IgG titres pre-transplant were labelled as R+, patients without anti-CMV IgG were labelled as R−. Donors were labelled accordingly as D+ or D−. For nine D+/R− patients serology data was available after transplantation; all nine patients showed sero-conversion within the first year after transplantation and had detectable CMV IgG. Four D−/R− patients harbored CMV-specific T-cells; three of these patients showed detectable anti-CMV IgG levels in post-transplant sera (mean time after transplant 79 months). At the time of sampling, none of the patients had an active CMV infection or reactivation. Five D+/R− patients received anti-viral prophylaxis with valganciclovir at the time of sampling.Further details are given in Table 1. Whole blood was collected in heparinized collection tubes after peripheral venous puncture. Thirteen patients were sampleds twice over time (mean time 5 ± 1 months between the two visits). Patients were stratified into CMV risk categories according to donor and recipient anti-CMV IgG serostatus at time of transplantation. Clinical data was obtained in a retrospective manner from patient files. CMV reactivation was defined as detectable viral load of > 500 copies/ml peripheral blood or > 1000 copies/ml in the bronchoalveolar lavage and clinical manifestations of CMV infection such as pneumonia, colitis or CMV syndrome requiring antiviral treatment. Ten age-matched healthy controls being CMV IgG seropositive (3 male volunteers, seven female volunteers; mean age 53 ± 6 years) were recruited as control cohort. The study was approved by the local institutional review board. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki and all subjects provided written informed consent.

Table 1
Clinical characteristics of the patient cohort.

	Total Patients (n = 59)
Sex, n (%)	
Male	29 (46)
Female	30 (54)
Age at sampling, years	57 ± 9
Time since transplant (at sampling, months)	75 ± 51
Transplant indication, n (%)	
Cystic fibrosis	5 (8)
COPD	23 (39)
Interstitial Lung Disease	19 (32)
PAH	1 (2)
Other	11 (19)
Immunosuppressive regimen, n (%)	
Tacrolimus, MMF, prednisone	45 (76)
Sirolimus, MMF, prednisone	4 (7)
Cyclosporin A, MMF, prednisone	7 (12)
Tacrolimus, Everolimus, prednisone	3 (5)
CMV risk category according to donor (D)/recipient (R) anti-CMV IgG status at time of transplantation, n (%)	
Low (D neg/R neg)	14 (24)
Intermediate (D neg/R pos or D pos/R pos)	13 (22) and 19 (32)
High (D pos/R neg)	13 (22)
FEV1 at time of sampling, %	74.3 ± 23.6
Patients with history of CMV infection requiring anti-viral therapy, n (%)	15 (25)

Values are mean ± standard deviation and percentage of patients in parenthesis.
CMV, cytomegalovirus; COPD, chronic obstructive pulmonary disease; FEV1, forced expiratory volume in 1 s; MMF, mycophenolate mofetil; PAH, pulmonary arterial hypertension.

2.2. Whole blood stimulation

Whole blood was stimulated in presence of anti-human CD28/Cd49d (BD Biosciences, Heidelberg, Germany) with CMV lysate (10 µg/ml, Lophius Biosciences, Regensburg, Germany) to detect CMV-specific T-cells or control lysate HEL299 (10 µg/ml, Lophius Biosciences, Regensburg, Germany) to determine unspecific T-cell activation [14–16]. Stimulation with staphylococcus enterotoxin B (SEB, Sigma Aldrich, Taufkirchen, Germany) served as positive control. Whole blood was stimulated for six hours at 37 °C, 5% CO_2.

2.3. Flow cytometric analysis

Following incubation, surface staining for T-cell lineage marker (anti-human CD3 Pacific Blue, Beckman Coulter or anti-human CD3 HorV450, BD Biosciences), the co-inhibitors BTLA (anti-human BTLA PE, Biolegend, San Diego, USA) or PD-1 (anti-human PD-1 PE, BD Biosciences, Heidelberg, Germany) and the activation marker CD154 (anti-human CD154 FITC, Biolegend) was performed. Appropriate isotypes were used. Then, whole blood lysis was performed with Versa Lyse (Beckman Coulter, Krefeld, Germany) and cells were analyzed on a flow cytometer (Navios, Beckman Coulter). Data was analyzed with Kaluza Software Version 1.5 (Beckman Coulter). CMV-specific T-cells were defined as $CD3^+$ T-cells expressing CD154 after stimulation with CMV lysate (Lophius Biosciences). Gating was based on fluorescence-minus-one controls. Specificity of stimulation with CMV lysate was confirmed by using the control lysate HEL299 (Lophius Biosciences). Across all patients, background staining, i.e. T-cells reactive to the control lysate, was low and an average of 0.48% (of T-cells) showed upregulation of CD154 upon stimulation with HEL299.

2.4. Statistical analysis

All values are expressed as mean ± standard deviation. Significance for the differences between unpaired groups was determined using the Mann-Whitney U test. Wilcoxon's signed rank test was used for comparison of paired data, Spearman's rank correlation was applied for correlation analysis. A p-value < .05 was considered as

statistically significant. Analysis was performed with GraphPad Prism Version 7 (Graph Pad Software Inc., San Diego, USA).

3. Results

3.1. The frequency of virus-specific T-cells is highest in patients with intermediate risk

Fifty-nine lung transplant patients with a mean time of 75 ± 51 months after lung transplantation were included in this study. Thirty-two patients were CMV sero-positive at the time of transplantation (R+ patients, "intermediate risk group"), 14 patients were sero-negative and received a transplant of a CMV sero-negative donor (D−/R− patients, "low risk group"). Thirteen patients were sero-negative at the time of transplantation and received a transplant of CMV sero-positive donor (D+/R− patients, "high-risk group"). Five out of 32 (16%) CMV sero-positive R+ patients experienced at least one episode of a clinically apparent CMV reactivation requiring anti-viral treatment. Two of these patients had CMV infection within the first year after transplantation. Ten out of 13 (77%) patients in the high-risk group had at least one episode of a clinically apparent CMV infection requiring anti-viral treatment. Six of these patients had CMV infection within the first year after transplantation. At the time of sampling, none of the patients had an active CMV infection or reactivation.

T-cells expressed CD154 after stimulation with SEB: D−/R+ 6.33 ± 4.8%; D+/R+ 8.89 ± 5.0%; D−/R−: 9.53 ± 8.2%; D+/R−: 5.63 ± 3.2%. CD154 expressing T-cells after stimulation with CMV protein lysate were defined as CMV-specific T-cells. Stratifying patients by risk category for CMV infection, R+ patients with intermediate risk showed the highest frequency of CMV-specific T-cells (Figs. 1–2, %CD154+ of CD3+ T-cells, R+ patients vs. D−/R− patients: 4.5 ± 3.5% vs. 1.2 ± 1.2%, $p < .005$). CMV-specific T-cells were also detectable in D+/R− patients (%CD154+ of CD3+ T-cells, 3.3 ± 1.5%). There was no difference comparing the frequency of CMV-specific T-cells between R+ patients versus D+/R− patients (%CD154+ of CD3+ T-cells: 4.5 ± 3.5% vs. 3.3 ± 1.5%, $p > .05$). R+ Patients and D+/R− patients harbored a higher frequency of CMV-specific T-cells than HC (2.0 ± 0.9%; vs. R+ patients $p = .03$; vs. D+/R− patients $p = .02$). Comparing D+/R+ patients versus D−/R+ patients with respect to frequency of CMV-specific T-cells revealed no significant differences (3.7 ± 3.3% vs. 5.0 ± 3.6%, $p = .25$, Fig. 2B). Interestingly, four D−/R− patients in the low risk category clearly harbored CMV-specific T-cells (frequency above 1%) with a mean frequency of 2.8 ± 0.7%. Longitudinal analysis of 13 patients sampled twice over time showed that the frequency of CMV-specific T-cells was stable over time and did not change significantly. When R+ patients were grouped by history of CMV reactivation, patients with history of reactivation showed a similar frequency of CMV-specific T-cells as compared to patients without history of reactivation (2.9 ± 3.0% vs. 4.8 ± 3.5%, $p = .2$). Likewise, D+/R− patients did not differ in CMV-specific T-cell frequency when stratified by history of CMVi. The frequency of CMV-specific T-cells did not correlate with the time since transplantation (D+/R− patients: $r = 0.1$, $p = .7$; D−/R+ patients: $r = 0.29$, $p = .33$; D+/R+ patients: $r = 0.09$, $p = .71$). There was no difference in frequency of CMV-specific T-cells when patients treated with cyclosporine A (Cyc) were compared to patients treated with tacrolimus (Tac, %CD154$^+$ of CD3$^+$ T-cells: 2.8 ± 1.2% vs. 4.4 ± 3.2%, $p = .4$).

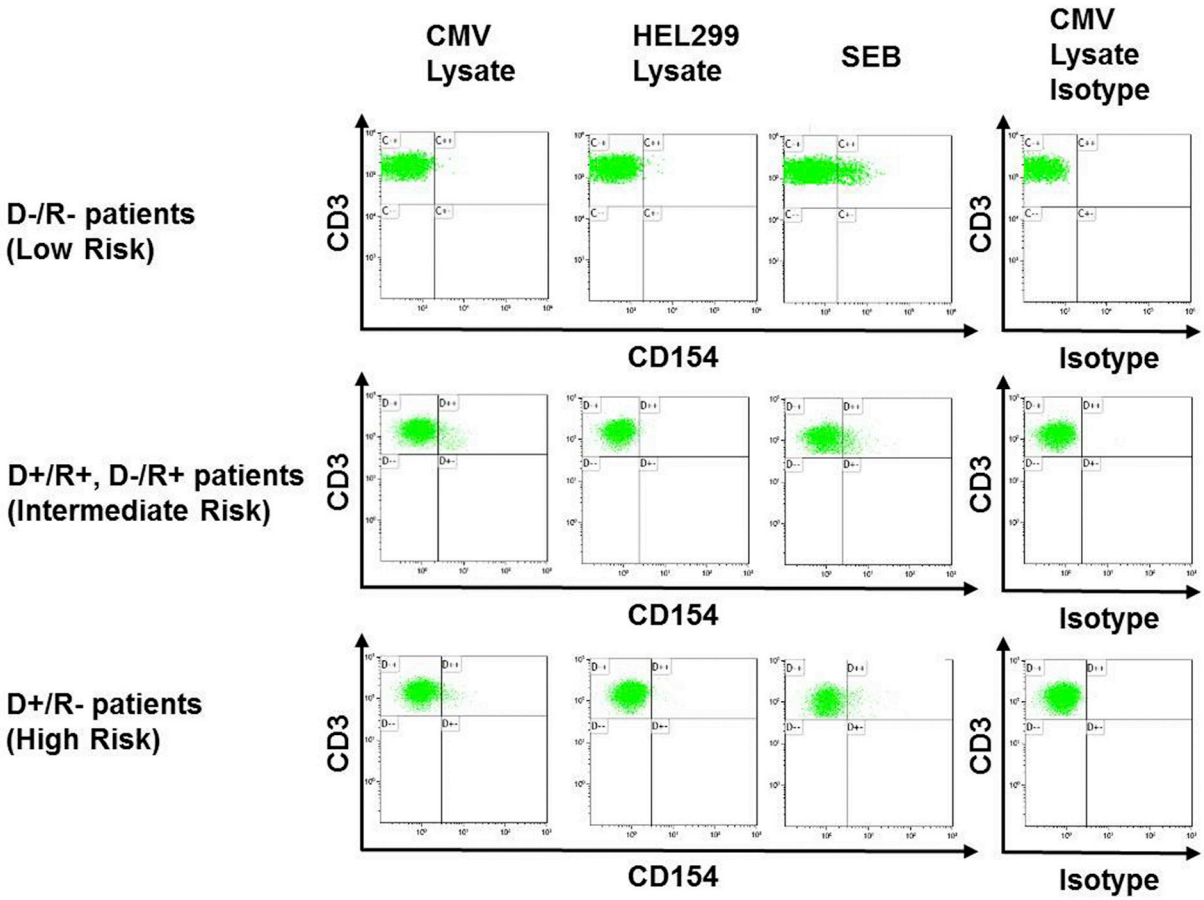

Fig. 1. Representative flow cytometric data on CMV-specific T-cells. Plots are gated on CD3$^+$ T-cells. CMV-specific T-cells were defined as CD3$^+$ T-cells expressing CD154 after stimulation with CMV lysate. Control conditions depict isotype controls, stimulation with SEB and stimulation with control lysate HEL299. Gates were adjusted according to isotype controls.

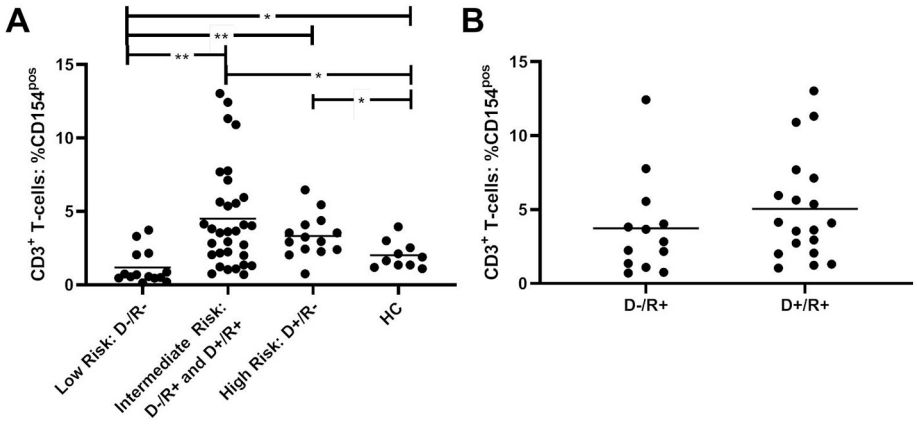

Fig. 2. CMV-specific T-cells are detectable in lung transplant patients. (A) Patients were stratified according to donor/recipient serostatus at time of transplantation. R+ Patients with intermediate risk showed the highest frequency of CMV-specific T-cells (%CD154$^+$ of CD3$^+$ T-cells, D−/R+,D+/R+ patients vs. D−/R− patients: 4.5 ± 3.5% vs. 1.2 ± 1.2%, $p < .005$; D+/R− patients vs. D−/R− patients: 3.3 ± 1.5% vs. 1.2 ± 1.2%, $p < .005$). D−/R+, D+/R+ Patients with intermediate risk and D+/R− patients with high risk harbored a higher frequency of CMV-specific T-cells than HC (2.0 ± 0.9%; vs. intermediate risk $p = .03$; vs. high risk $p = .02$). There was no difference comparing the frequency of CMV-specific T-cells between R+ patients with intermediate risk versus D+/R− patients with high risk (%CD154$^+$ of CD3$^+$ T-cells: 4.5 ± 3.5% vs. 3.3 ± 1.5%, $p > .05$). (B) D−/R+ patients showed a similar frequency of CMV-specific T-cells as compared to D+/R+ patients (D−/R+ vs. D+/R+, %CD154$^+$ of CD3$^+$ T-cells: 3.7 ± 3.3% vs. 5.0 ± 3.6%, $p = .25$). **p-value < .005; *p-value < .05.

3.2. Expression of the co-inhibitors PD-1 and BTLA on CMV-specific T-cells

CMV-specific T-cells were further analyzed for the expression of co-inhibitory molecules. When patients were stratified by risk category, D−/R− patients with low risk for CMV infection and presence of CMV-reactive T-cells showed a similar expression of BTLA on CMV-specific T-cells compared to R+ patients with intermediate risk or D+/R− patients (%BTLA$^+$ of CD3$^+$ CD154$^+$ T-cells, D−/R− patients vs. D−/R+, D+/R+ patients: 74.4 ± 17.2% vs. 61.4 ± 18.8%, p = .2; D−/R− vs. D+/R−: 74.4 ± 17.2% vs. 65.4 ± 11.1%, p = .3). BTLA expression was significantly enhanced on CMV-specific T-cells of D+/R−patients vs. HC (%BTLA$^+$ of CD3$^+$ CD154$^+$ T-cells: 65.4 ± 11.1% vs. 52.5 ± 12.4%, $p = .03$, Fig. 3). BTLA expression did not differ between R+ patients versus D+/R− patients.

Likewise, PD-1 expression was similar in D−/R−patients with low risk for CMV infection compared to D+/R− patients with high risk (%PD-1$^+$ of CD3$^+$ CD154$^+$ T-cells, D−/R− vs. D+/R−: 52.0 ± 18.8% vs. 55.7 ± 18.2%, $p = .9$). No differences were detected comparing D−/R− patients with low risk versus R+ patients and D+/R− patients versus R+ patients with intermediate risk (%PD-1$^+$ of CD3$^+$ CD154$^+$ T-cells, D−/R− patients vs. R+ patients: 52.0 ± 18.8% vs. 51.7 ± 20.8%, p = .9; high risk vs. intermediate risk: 55.7 ± 18.2% vs. 51.7 ± 20.8%, $p = .5$). PD-1 expression on CMV-specific T-cells was increased in D+/R+ and D+/R− patients as compared to HC (%PD-1$^+$ of CD3$^+$ CD154$^+$ T-cells, D+/R+ vs. HC: 55.0 ± 18.3% vs. 35.0 ± 19.6%, $p = .01$; D+/R− vs. HC: 55.7 ± 18.2% vs. 35.0 ± 19.6%, p = .03, Fig. 3).

For further analysis, R+ patients with intermediate risk were grouped according to history of CMV reactivation. PD-1 expression on CMV-specific T-cells was not different comparing patients with versus without history of CMV reactivation (%PD-1$^+$ of CD3$^+$ CD154$^+$ T-cells, reactivation vs. no reactivation: 39.37 ± 15.6% vs. 53.98 ± 21.1%, $p = .1$). BTLA expression was significantly enhanced on CMV-specific T-cells of R+ patients with CMV reactivation versus no CMV reactivation (%BTLA$^+$ of CD3$^+$ CD154$^+$ T-cells, reactivation vs. no reactivation: 81.6 ± 8.7% vs. 57.7 ± 17.8%, $p = .005$). However, the MFI of BTLA on T-cells was not significantly different (3.68 ± 2.0 vs. 4.64 ± 2.2, $p = .2$). Expression of co-inhibitors was not associated with the time since transplantation (PD1: D+/R− patients: $r = 0.46$, $p = .12$; D−/R+ patients: $r = -0.25$, $p = .41$; D+/R+ patients: $r = -0.27$, $p = .26$; BTLA: D+/R− patients: $r = 0.15$, $p = .63$; D−/R+ patients: $r = -0.05$, $p = .87$; D+/R+ patients: $r = 0.11$, $p = .64$).

4. Discussion

CMV-specific T-cells were detectable in patients being sero-positive at time of transplantation and in CMV sero-negative patients having received a lung graft of a CMV sero-positive donor; in both cases, the frequency of CMV-specific T-cells was higher than in HC. The frequency of PD-1 and BTLA expressing CMV-specific T-cells was enhanced in D+/R− patients as compared to HC. Interestingly, CMV sero-positive patients with a history of CMV reactivation harbored an increased fraction of BTLA$^+$ CMV-specific T-cells as compared to CMV sero-positive patients without history of CMV reactivation. Functioning T-cell immunity is essential to bear an efficient immunological response against CMVi [4,17]. Accordingly, immunosuppressive therapy in solid organ transplantation targeting T-cell mediated immunity, may hamper efficient anti-CMV immune responses [4,17]. In our study, we found that despite standard immunosuppressive therapy, patients harbored CMV-specific T-cells. CMV-specific T-cells were even detectable in patients belonging to the high risk category and the frequency was comparable to patients with intermediate risk. This is somewhat noteworthy as high risk patients are CMV-naïve pre-transplant and must have acquired CMV-specific cellular immunity after transplantation

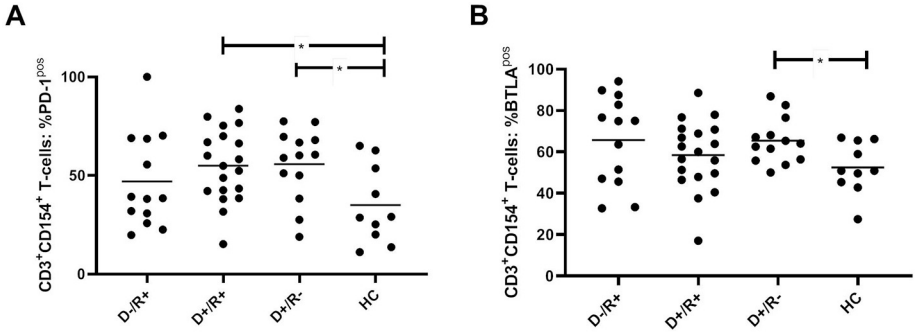

Fig. 3. Expression pattern of co-inhibitory molecules on CMV-specific T-cells. (A) PD-1 expression on CMV-specific T-cells was increased in D+/R+ and D+/R− patients as compared to HC (%PD-1$^+$ of CD3$^+$ CD154$^+$ T-cells, D+/R+ vs. HC: 55.0 ± 18.3% vs. 35.0 ± 19.6%, $p = .01$; D+/R− vs. HC: 55.7 ± 18.2% vs. 35.0 ± 19.6%, p = .03). (B) BTLA expression was significantly enhanced on CMV-specific T-cells of D+/R−patients vs. HC (% BTLA$^+$ of CD3$^+$ CD154$^+$ T-cells: 65.4 ± 11.1% vs. 52.5 ± 12.4%, p = .03). *p-value < .05.

under immunosuppressive therapy. Pipeling et al. assessed the dynamics of CMV-specific T-cell responses in a cohort of high-risk lung transplant patients before cessation of antiviral prophylaxis and during primary CMV infection [18]. In line with our results, the authors found that patients after lung transplant mount a CMV-specific T-cell response. Furthermore, Pipeling et al. demonstrated a positive correlation between frequency of CMV-specific T-cells and viral control, i.e. patients with high frequency of CMV (pp65)-specific T-cells were protected against CMV reactivation [18]. A similar observation was reported by Sester et al.; lower frequencies of CMV-specific T-cells may precede episodes of CMV reactivation in lung transplant patients [8]. Interestingly, in four patients belonging to the low risk category, CMV-specific T-cells were found. The average time since transplantation was 114 months, so that these presumably CMV-naïve patients may have contracted CMV after transplantation. Indeed, three out of four patients had detectable anti-CMV IgG levels in post-transplant sera. For the remaining patient, it has to be considered that this patient was not CMV-naïve pre-transplant and is just lacking a humoral response against CMV. Indeed, Litjens et al. found that CMV-seronegative patients may harbor cellular immunity against CMV in absence of a humoral response [19]. Similar results were reported by Terlutter et al. in a cohort of healthy controls [20].

Co-inhibitors such as PD-1, BTLA or CTLA4 are expressed on T-cells and counteract T-cell activation [21]. Co-inhibitors are important regulators of immunological tolerance [21]. However, co-inhibition can also be a mechanism of immune evasion [12,13,21,22]. This has been shown for several types of cancer and the blockade of co-inhibitory molecules has evolved as a very successful strategy to activate tumor-specific T-cells [21]. Several groups reported that hampered viral control in HIV, Hepatitis B and Hepatitis C is related to co-inhibition of virus-specific T-cells [12,13]. Thus, we assessed the expression pattern of two co-inhibitory molecules, PD-1 and BTLA, on CMV-specific T-cells. Patients in the high risk group, i.e. D+/R−, showed an increased fraction of $BTLA^{pos}$ and $PD\text{-}1^{pos}$ CMV-specific T-cells as compared to HC; in addition, CMV sero-positive R+ patients had an increased expression of PD-1 on CMV-specific T-cells in comparison to HC. Furthermore, Dirks et al. investigated the role of PD-1 on CMV-specific T-cells in renal transplant patients [9,10]. High PD-1 expression was associated with impaired cytokine secretion and proliferative capacity of CMV-specific T-cells translating to restricted viral control [10]. Blockade of the PD-1/PDL-1 axis in vitro restored functionality of virus-specific T-cells [9,10]. We also observed upregulation of another co-inhibitor, BTLA. Upregulation of BTLA has been shown on CMV-specific T-cells previously in healthy individuals during primary infection but is followed by rapid down-regulation after viral clearance [23]. Interestingly, the viral protein UL144 derived from human CMV acts as a BTLA agonist on T-cells and thus may shut down T-cell responses as an immune evasion mechanism [22,24]. We found a high expression of BTLA on CMV-specific T-cells in CMV sero-positive patients with a history of CMV reactivation; increased BTLA expression may render these CMV-specific T-cells susceptible to UL144-based immune evasion impairing immunological viral control.

So far, risk assessment for CMVi after transplantation is based on anti-CMV serostatus. This approach neglects the fact that T-cell mediated immunity against CMV is of major importance. Hence, T-cell based risk assessment may provide useful, additional information to identify lung transplant patients at risk [25,26]. Next to frequency of CMV-specific T-cells, expression pattern of co-inhibitors hampering T-cell functionality may give some indication regarding virus-specific immunocompetence. Thus, high expression of co-inhibitors such as BTLA or PD-1 on CMV-specific T-cells may indicate reduced efficacy of cellular anti-CMV immunity in lung transplant patients and could serve as biomarkers for patients at risk to develop CMVi. However, the retrospective nature of our study does not allow conclusions on the predictive value of T-cell parameters for CMV reactivation. Nevertheless, co-inhibitory molecules may provide useful, additional information in T-cell based assays and should be considered in future studies.

5. Conclusions

In conclusion, the expression pattern of co-inhibitory molecules on CMV-specific T-cells is altered in patients after lung transplantation and may hamper immunological control of CMV.

Funding information

B.W. is funded by the Dr. Werner Jackstädt-Stfitung, O.W. received funding from the Rudolf-Ackermann-Stiftung.

Declaration of Competing Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

Acknowledgement

The authors thank Mrs. Hannah Pfeng for supporting the collection of clinical data.

References

[1] C.E. Koval, Prevention and treatment of cytomegalovirus infections in solid organ transplant recipients, Infect. Dis. Clin. N. Am. 32 (3) (2018) 581–597.
[2] C. Kurihara, R. Fernandez, N. Safaeinili, M. Akbarpour, Q. Wu, G.S. Budinger, et al., Long-term impact of cytomegalovirus serologic status on lung transplantation in the United States, Ann. Thorac. Surg. 107 (2018) 1046–1052.
[3] E. Beam, T. Lesnick, W. Kremers, C.C. Kennedy, R.R. Razonable, Cytomegalovirus disease is associated with higher all-cause mortality after lung transplantation despite extended antiviral prophylaxis, Clin. Transpl. 30 (3) (2016) 270–278.
[4] R.S. Almaghrabi, A.S. Omrani, Z.A. Memish, Cytomegalovirus infection in lung transplant recipients, Expert. Rev. Respir. Med. 11 (5) (2017) 377–383.
[5] S.M. Palmer, A.P. Limaye, M. Banks, D. Gallup, J. Chapman, E.C. Lawrence, et al., Extended valganciclovir prophylaxis to prevent cytomegalovirus after lung transplantation: a randomized, controlled trial, Ann. Intern. Med. 152 (12) (2010) 761–769.
[6] C. Mabilangan, J. Preiksaitis, C. Cervera, University of Alberta Cardiothoracic Transplant G, Impact of donor and recipient cytomegalovirus serology on long-term survival of lung transplant recipients, Transpl. Infect. Dis. 20 (5) (2018) e12964.
[7] V. Monforte, H. Sintes, C. Lopez-Gallo, M. Delgado, F. Santos, F. Zurbano, et al., Risk factors, survival, and impact of prophylaxis length in cytomegalovirus-seropositive lung transplant recipients: a prospective, observational, multicenter study, Transpl. Infect. Dis. 19 (3) (2017).
[8] U. Sester, B.C. Gartner, H. Wilkens, B. Schwaab, R. Wossner, I. Kindermann, et al., Differences in CMV-specific T-cell levels and long-term susceptibility to CMV infection after kidney, heart and lung transplantation, Am. J. Transplant. 5 (6) (2005) 1483–1489.
[9] J. Dirks, A. Egli, U. Sester, M. Sester, H.H. Hirsch, Blockade of programmed death receptor-1 signaling restores expression of mostly proinflammatory cytokines in anergic cytomegalovirus-specific T cells, Transpl. Infect. Dis. 15 (1) (2013) 79–89.
[10] U. Sester, D. Presser, J. Dirks, B.C. Gärtner, H. Köhler, M. Sester, PD-1 expression and IL-2 loss of cytomegalovirus- specific T cells correlates with viremia and reversible functional anergy, Am. J. Transplant. 8 (7) (2008) 1486–1497.
[11] A. de Sousa Linhares, J. Leitner, K. Grabmeier-Pfistershammer, P. Steinberger, Not all immune checkpoints are created equal, Front. Immunol. 9 (2018) (1909).
[12] H. Cho, H. Kang, H.H. Lee, C.W. Kim, Programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) in viral hepatitis, Int. J. Mol. Sci. 18 (7) (2017) 1517.
[13] A. Saeidi, K. Zandi, Y.Y. Cheok, H. Saeidi, W.F. Wong, C.Y.Q. Lee, et al., T-cell exhaustion in chronic infections: reversing the state of exhaustion and reinvigorating optimal protective immune responses, Front. Immunol. 9 (2018) (2569).
[14] M. Frentsch, O. Arbach, D. Kirchhoff, B. Moewes, M. Worm, M. Rothe, et al., Direct access to CD4+ T cells specific for defined antigens according to CD154 expression, Nat. Med. 11 (2005) 1118.
[15] W. Tu, S. Chen, M. Sharp, C. Dekker, A.M. Manganello, E.C. Tongson, et al., Persistent and selective deficiency of CD4 < sup > + < /sup > T cell immunity to cytomegalovirus in immunocompetent young children, J. Immunol. 172 (5) (2004) 3260–3267.
[16] P. Bacher, A. Scheffold, Flow-cytometric analysis of rare antigen-specific T cells, Cytom. Part A 83A (8) (2013) 692–701.
[17] M. Nosotti, P. Tarsia, L.C. Morlacchi, Infections after lung transplantation, J. Thorac. Dis. 10 (6) (2018) 3849–3868.
[18] M.R. Pipeling, E.R. John, J.B. Orens, N. Lechtzin, J.F. McDyer, Primary cytomegalovirus phosphoprotein 65-specific CD8+ T-cell responses and T-bet levels

predict immune control during early chronic infection in lung transplant recipients, J. Infect. Dis. 204 (11) (2011) 1663–1671.
[19] N.H.R. Litjens, L. Huang, B. Dedeoglu, R.W.J. Meijers, J. Kwekkeboom, M.G.H. Betjes, Protective cytomegalovirus (CMV)-specific T-cell immunity is frequent in kidney transplant patients without serum anti-CMV antibodies, Front. Immunol. 8 (2017) 1137.
[20] F. Terlutter, R. Caspell, T.M. Nowacki, A. Lehmann, R. Li, T. Zhang, et al., Direct detection of T- and B-memory lymphocytes by ImmunoSpot(R) assays reveals HCMV exposure that serum antibodies fail to identify, Cells 7 (5) (2018).
[21] L. Mazzarella, B.A. Duso, D. Trapani, C. Belli, P. D'Amico, E. Ferraro, G. Viale, G. Curigliano, The evolving landscape of 'next-generation' immune checkpoint Inhibitor: A Review, Eur. J. Cancer 117 (2019) 14–31.
[22] T.C. Cheung, I.R. Humphreys, K.G. Potter, P.S. Norris, H.M. Shumway, B.R. Tran, et al., Evolutionarily divergent herpesviruses modulate T cell activation by targeting the herpesvirus entry mediator cosignaling pathway, Proc. Natl. Acad. Sci. U. S. A. 102 (37) (2005) 13218–13223.
[23] N.-E. Serriari, F. Gondois-Rey, Y. Guillaume, E.B.M. Remmerswaal, S. Pastor, N. Messal, et al., B and T lymphocyte attenuator is highly expressed on CMV-specific T cells during infection and regulates their function, J. Immunol. 185 (6) (2010) 3140.
[24] J.R. Šedý, M.O. Balmert, B.C. Ware, W. Smith, I. Nemčovičova, P.S. Norris, et al., A herpesvirus entry mediator mutein with selective agonist action for the inhibitory receptor B and T lymphocyte attenuator, J. Biol. Chem. 292 (51) (2017) 21060–21070.
[25] T. Bunde, A. Kirchner, B. Hoffmeister, D. Habedank, R. Hetzer, G. Cherepnev, et al., Protection from cytomegalovirus after transplantation is correlated with immediate early 1-specific CD8 T cells, J. Exp. Med. 201 (7) (2005) 1031–1036.
[26] A. Zeevi, P. Morel, K. Spichty, J. Dauber, S. Yousem, P. Williams, et al., Clinical significance of CMV-specific T helper responses in lung transplant recipients, Hum. Immunol. 59 (12) (1998) 768–775.