Cellular Immunology 336 (2019) 58–65

Contents lists available at ScienceDirect

Cellular Immunology

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

Specific T-cell receptor gene transfer enhances immune response: A potential therapeutic strategy for the control of human cytomegalovirus infection in immunocompromised patients

Runan Zhang[a], Yanyue Zhang[a], Jianhua Hu[a], Wei Wu[a], Xiaoming Chen[a], Zhongjie Lu[b], Rong Yang[a], Yaping Huang[a], Jun Fan[a,*]

[a] State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, 310003, P.R. China
[b] Department of Radiotherapy, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, 310003, P.R. China

ARTICLE INFO

Keywords:
HCMV
TCR
CD8 T cell
IFN-γ
Immune response
HSCT

ABSTRACT

Human cytomegalovirus (HCMV) infection is a leading cause of morbidity and mortality in immunocompromised patients, but no specific therapeutic strategy is effective clinically, despite recent achievements. HCMV-specific T-cell therapy was thought to be helpful for the management of HCMV infection. To conduct a deep exploration, we investigated the possibility of engineering peripheral blood mononuclear cells (PBMCs) from immunocompetent and immunocompromised subjects with specific T-cell receptor (TCR) genes. CD8-positive T cells that specifically bind to NLV pentamers could be generated by transferring TCR genes to PBMCs from immunocompetent and immunocompromised subjects. The generation of functional T cells varied among transduction of different PBMCs. The numbers of IFN-γ-secreting T cells increased significantly in immunocompetent and immunodeficient PBMCs, but were unchanged in immune-reconstituted PBMCs. TCR gene transfer is a potential therapeutic strategy for controlling HCMV infection in immunocompromised patients. The transfer of TCR genes into immunocompetent and immunodeficient PBMCs would be more meaningful in response to HCMV infection than would the transfer into immune-reconstituted PBMCs.

1. Introduction

Human cytomegalovirus (HCMV) infection remains a major cause of morbidity and mortality in immunocompromised individuals, such as allogeneic hematopoietic stem cell transplant (HSCT) recipients [1,2]. Due to the limitations of available treatments, insufficient control of HCMV recurrence affect a significant number of patients [3–5]. Approximately 95% of the population of China is infected with HCMV, and HCMV recurrence is common among immunocompromised patients [6–8]. In this study, we explored strategies for the management of HCMV infection in a high-prevalence population based on the latest technologies and achievements.

HCMV-specific T cells (VSTs) require unique T-cell receptors (TCRs) to recognize counterpart epitope peptides when responding to HCMV infection [9,10]. Hundreds of TCR CDR3β sequences have been found to be HCMV-relevant [11,12]. One sequence, CASSSANYGYFT, was selected as the research target. In this study, peripheral blood mononuclear cells (PBMCs) from HCMV-infected healthy individuals and HSCT recipients were transduced with TCR genes, and the immune responses of engineered T cells to HCMV peptide were evaluated.

2. Materials and methods

2.1. Subjects

In total, four subjects were evaluated for the study, comprised of two HCMV-seropositive immunocompetent subjects (subjects 1 and 2) and two immunocompromised subjects (subjects 3 and 4). All HSCT recipients in the First Affiliated Hospital, College of Medicine, Zhejiang University between June 2017 and July 2018 were enrolled with

Abbreviations: HCMV, human cytomegalovirus; SFFV, spleen focus-forming virus; TCR, T-cell receptor; IFN-γ, interferon-γ; HSCT, hematopoietic stem cell transplant; ELISPOT, enzyme-linked immunospot; VSTs, HCMV-specific T cells

* Corresponding author at: State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, College of Medicine, Zhejiang University, 79 Qingchun Road, Hangzhou, 310003, Zhejiang Province, P.R. China.
E-mail address: fanjun@zju.edu.cn (J. Fan).

https://doi.org/10.1016/j.cellimm.2018.12.011
Received 5 November 2018; Received in revised form 20 December 2018; Accepted 31 December 2018
Available online 02 January 2019
0008-8749/

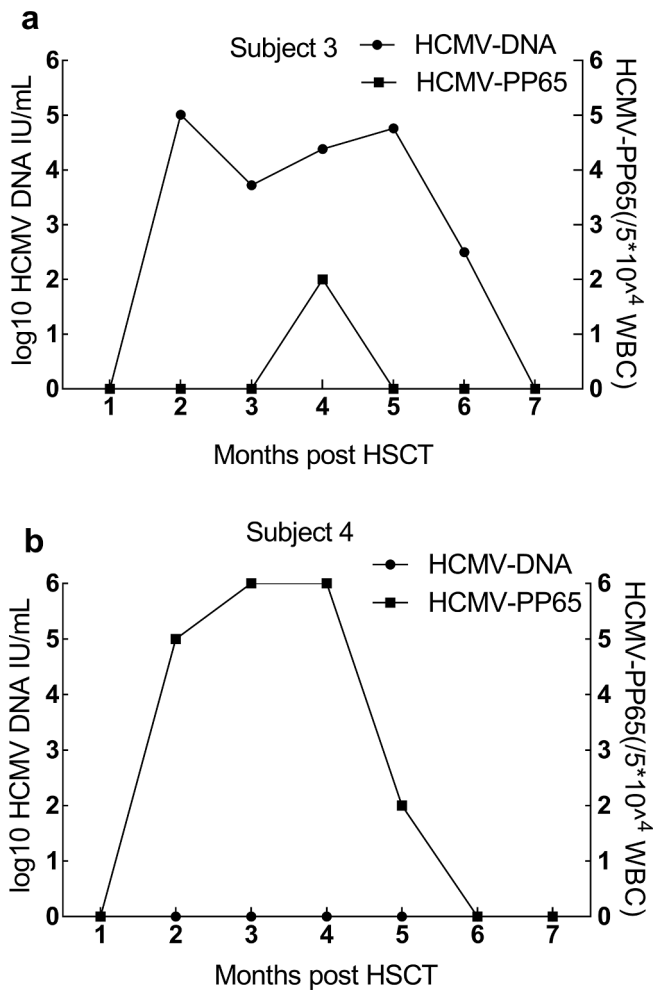

Fig. 1. Monitoring of HCMV activation in HSCT recipients. a) HCMV DNAemia was consistently positive, whereas PP65 antigenemia was detected only at month 4 post-HSCT. b) HCMV pp65 antigenemia was detected from months 2 to 5 post-HSCT; HCMV DNAemia was not detected during the first 6 months after HSCT. The detection of HCMV PP65 or HCMV DNA indicated HCMV recurrence [1]. WBC: white blood cells.

informed consent in accordance with the 1975 Declaration of Helsinki and following the requirements of the ethics committee of the First Affiliated Hospital, College of Medicine, Zhejiang University. All subjects met the following criteria: HLA-A0201 type and no Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, Hepatitis D virus, Hepatitis E virus, Herpes simplex virus, or Human Immunodeficiency Virus infection. The two HSCT recipients had persistent HCMV infection (Fig. 1) and limited numbers of VSTs (Table 1) during the first 6 months after HSCT. The methods for detecting HCMV DNA and HCMV-PP65 were described previously [13,14]. Detailed information on the four subjects is provided in Table 1.

2.2. Cells, cell lines, and peptides

The post-transplant period is usually divided into three phases: the early phase, the intermediate phase (months 1–3, characterized by cellular immunodeficiency), and the late phase (months 3–12, characterized by slow immune reconstitution) [15]. Month-3 PBMCs were defined as immunodeficient PBMCs, and were collected from HSCT recipients in the third month post-HSCT. Month-6 PBMCs were defined as immune-reconstituted PBMCs, and were collected from HSCT recipients in the sixth month post-HSCT. PBMCs collected from immunocompetent subjects were defined as immunocompetent PBMCs.

EDTA-anticoagulated whole blood was collected. PBMCs were obtained by centrifugation in Pancoll human (PAN-Biotech, Germany). TAP-deficient HLA-A2 transgenic T2 cells (174 × CEM.T2) were purchased from the FuHeng Cell Center (Shanghai, China). PBMCs and T2 cells were cultured in RPMI 1640 medium (PAN-Biotech) supplemented with 10% FBS (Gibco, USA) and 1% penicillin-streptomycin (PAN-Biotech) at 37 °C with 5% CO_2 [16]. HLA-A2–restricted HCMV $PP65_{495-503}$ NLVPMVATA (NLV) and HIV-1 pol476–484 ILKEPVHGV (ILK) peptides were synthesized by Angtai Biotechnology Co. (Hangzhou, China). T2 cells were incubated with NLV peptide or irrelevant ILK peptide for 2 h at 37 °C, then used as specific and non-specific stimulators or target cells [16–18].

2.3. Cloning of NLV-specific TCR genes and lentivirus production

NLV-specific TCR α (NCBI:FJ795368) and β (NCBI:FJ795367) chains that contain our target TCR CDR3β sequence were referenced [11,12,19]. TCR-expressing vectors were designed as described previously by Hillerdals (Fig. 2) [16,18]. A mock plasmid with no target

Table 1
Clinical details of the four subjects.

	Immunocompetent subjects		Immunocompromised subjects	
	Subject 1	Subject 2	Subject 3	Subject 4
Age	25	28	18	46
Sex	Male	Male	Male	Female
HLA type	HLA-A0201	HLA-A0201	HLA-A0201	HLA-A0201
HCMV-IgG	Positive	Positive	Positive	Positive
Underlying disease	–	–	NHL	AML
Conditioning regimen	–	–	ARC/BUCY/MeCCNU	ARC/BUCY/MeCCNU
Immunosuppressive agents	–	–	CAS/MTX/MMF	CAS/MTX/MMF/ATG
Antiviral treatment	–	–	ACV/GCV	ACV/GCV
aGVHD	–	–	YSE	YSE
IFN-γ–secreting T cell counts*	23	21	168 (Month 3) 329 (Month 6)	130 (Month 3) 319 (Month 6)

Abbreviations: NHL, non-Hodgkin lymphoma; AML, acute myeloblastic leukemia; ARC, cytosine arabinoside; BU, busulfan; CY, cyclophosphamide; MeCCNU, methyl-cyclohexyl-nitrosamine; MTX, methotrexate; MMF, mycophenolate mofetil; ATG, anti-human thymocyte globulin; ACV, acyclovir; GCV, ganciclovir; aGVHD, acute graft-versus-host disease; month 3/6: third/sixth month post-HSCT.
*Before TCR transduction, an ELISPOT assay was used to determine the frequency of IFN-γ–secreting T cells in freshly isolated PBMCs. PBMCs that secreted IFN-γ when challenged with NLV peptide were defined as IFN-γ–secreting T cells.

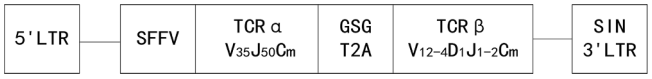

Fig. 2. Construction of the TCR-expressing vector. NLV-specific TCR α and β chains were separated by a self-cleaving GSGT2A sequence to express both TCR chains simultaneously. To improve pairing between the exogenous TCR chains and reduce mispairing with endogenous chains, the constant domains were replaced with mouse counterparts [32]. The modified sequence was sub-cloned into a plasmid PGMLV-SFFV-MCS-WPRE (Genomeditech, China) under the transcriptional control of spleen focus-forming virus (SFFV) promoter.

gene was used as a control. GFP-encoding plasmid was used to estimate transduction efficiency. TCR gene sequences, plasmid vectors, and vesicular stomatitis virus-G pseudotyped lentivirus were synthesized, constructed, and packaged by Genomeditech Co., Ltd. (Shanghai, China). Lentiviral vectors were stored at −80 °C.

2.4. PBMC transduction and enrichment of TCR-transduced T cells

Transduction and enrichment were performed as described previously [8,12]. Freshly isolated PBMCs were activated with 50 U/ml IL-2 (Abcam, UK) and 50 ng/ml OKT3 antibody (Abcam) 48 h before transduction. Activated PBMCs (5×10^5 in 500 µl) were seeded in 24-well plates precoated with 5 µg/well RetroNectin (Takara, Japan), then transduced with concentrated TCR lentiviral vectors (marked as the TCR-V group or TCR-T cells) or mock lentiviral vectors (marked as the TCR_m-V group or TCR_m-T cells) in the presence of 100 U/ml IL-2 and 4 µg/ml protamine sulfate (Sigma, USA). The plates were spinoculated for 2 h at 800 × *g* and 32 °C. The medium was replaced after 6 h of transduction.

For specific enrichment, 1.5×10^6 transduced T cells were co-cultured with irradiated (30 GY) NLV-loaded T2 cells (3.75×10^5) with 100 U/ml IL-2 in 24-well plates. For non-specific enrichment, transduced T cells (1.5×10^6) were co-cultured with irradiated ILK-loaded T2 cells at a ratio of 4:1 in the presence of 100 U/ml IL-2 and 30 ng/ml OKT3 in 24-well plates.

2.5. IFN-γ enzyme-linked immunospot assay

The functional activity of specific T cells was detected using IFN-γ enzyme-linked immunospot (ELISPOT) assay kits (MABTECH, Sweden) according to the manufacturer's instructions. In total, 3×10^4/well PBMCs were seeded on a 96-well ELISPOT plate pre-coated with anti–IFN-γ antibody, then stimulated with NLV-loaded or ILK-loaded T2 cells (3×10^4/well), mAb-CD3-2 (1:1000 diluted, 100 µl/well), or fresh medium (100 µl/well) for 18–21 h at 37 °C in 5% CO_2. After incubation and washing, 100 µl/well ALP-conjugated secondary antibody (1:200 diluted) was added and incubated at room temperature for 2 h. Then, 200 µl/well filtered (0.45 µm) substrate solution was added to develop distinct spots and stop color development, and the plate was left to dry. Spots were captured and counted by an automated reader (CTL ImmunoSpot S5 UV Analyzer; CTL Europe, Germany). PBMCs that secreted IFN-γ cytokine when challenged with NLV peptide were described as IFN-γ–secreting T cells.

2.6. Flow cytometric analysis

Flow cytometric analysis was performed to determine the percentage of NLV-specific TCR-expressed T cells after transduction and/or enrichment. Cell staining was performed according to the manufacturer's instructions (PROIMMUNE, Oxford, UK). Briefly, cells were incubated with PE-labeled NLV/HLA-A0201 pentamers (PROIMMUNE) for 10 min at room temperature, then counterstained with anti–CD8-FITC and anti-CD3-PerCp5 (Abcam) for 20–30 min on ice, shielded from light. After washing, stained cell counts were performed on a Beckman Cytomics FS 500 MPL device (Beckman Coulter Diagnostics, USA) and analyzed with FlowJo v10.3 software (Tree Star, USA). $CD3^+CD8^+$ T cells that specifically bound to NLV pentamers were described as $CD8^+/PEN^+$ T cells.

2.7. Statistical analysis

Statistical analysis was performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 7.0 (GraphPad Software, USA). Differences in IFN-γ–secreting T-cell numbers were evaluated using Student's *t* test. *P* values < 0.05 were considered statistically significant.

3. Results

3.1. Efficiency and feasibility of the TCR lentivirus transfection system

GFP-expressing vectors were applied to determine general transduction efficiency. Approximately 35–40% GFP-expressing T cells was detected at 6 days post-transduction, which indicated that the transduction system was effective (Fig. 3). To confirm the feasibility of modifying PBMCs with exogenous TCR genes, PBMCs from immunocompetent subjects were transfected with TCR lentivirus. Six days later, $CD8^+/PEN^+$ T cells and INF-γ-secreting T cells were more

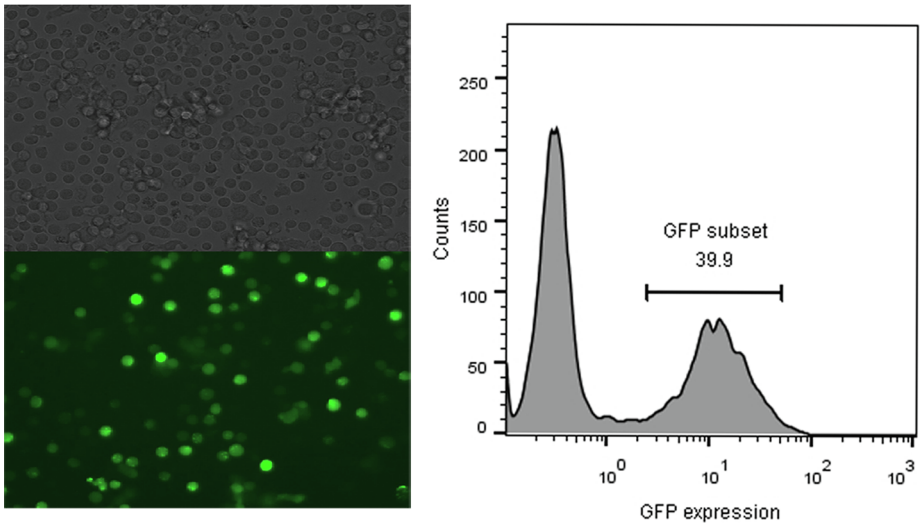

Fig. 3. Assessment of transduction efficiency. PBMCs were transfected with GFP vectors. Six days later, numbers of PBMCs expressing GFP were determined with a fluorescence microscope (left) and by flow cytometry (right). A representative transduction is shown. General transduction efficiency was represented by the proportion of GFP-positive PBMCs, which ranged from 35% to 40%.

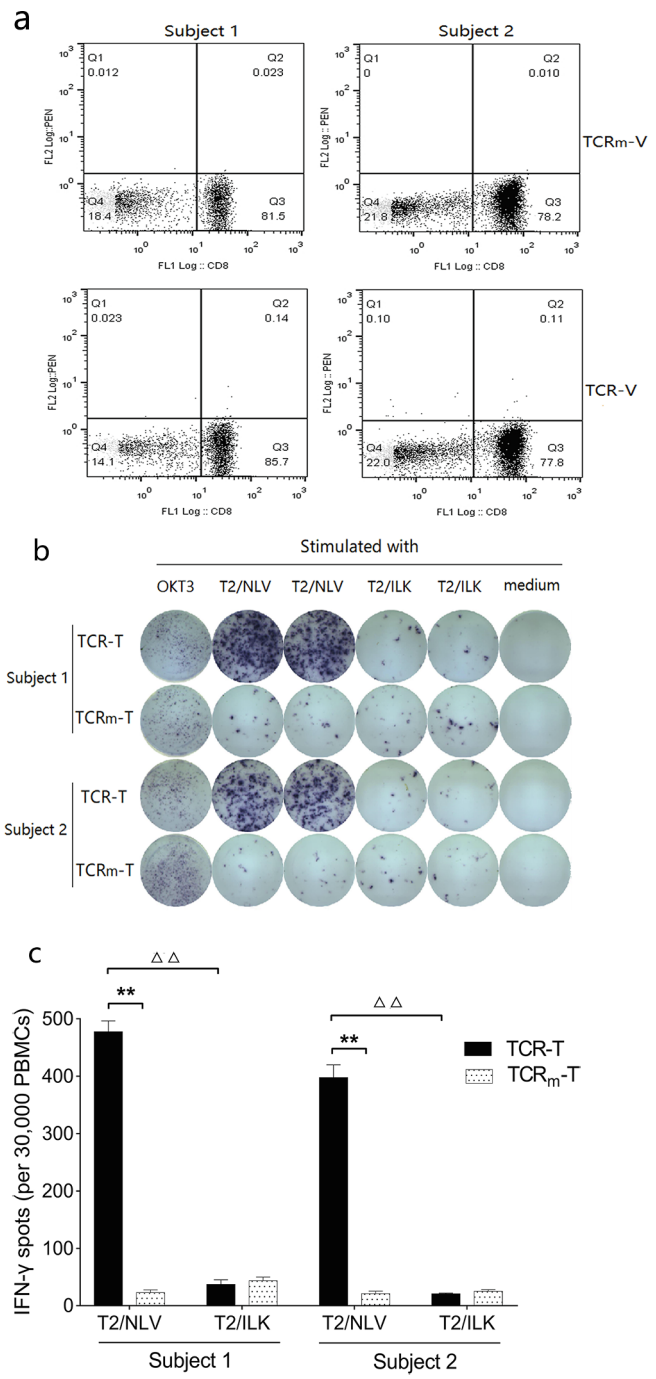

Fig. 4. Transfer of TCR genes into immunocompetent PBMCs and assessment of functional activities of engineered T cells. PBMCs from immunocompetent subjects were transduced with TCR-V and TCRm-V for 6 days, separately. a) The expression of exogenous TCR was evaluated by staining with anti-CD3 and anti-CD8 antibodies and NLV/MHC-I pentamers. b) IFN-γ secretion of TCR-T cells and TCRm-T cells in response to NLV and TIL peptide-loaded T2 cells was investigated (OKT3 and medium were used as positive and negative controls, respectively) using the IFN-γ ELISPOT assay. A representative view of the spots is shown. c) Spot counts are shown as means ± SDs, calculated from three replicates. Triangles represent significant differences between TCR-T cells in responding to T2/NLV and T2/TIL cells (ΔΔ, P < 0.001). Asterisks represent differences in IFN-γ–specific T cells between the TCR-V and TCR_m-V groups (**, P < 0.001).

abundant in the TCR-V groups than in the TCR_m-V groups. These results imply that PBMCs can be modified with exogenous TCR chains through transfection.

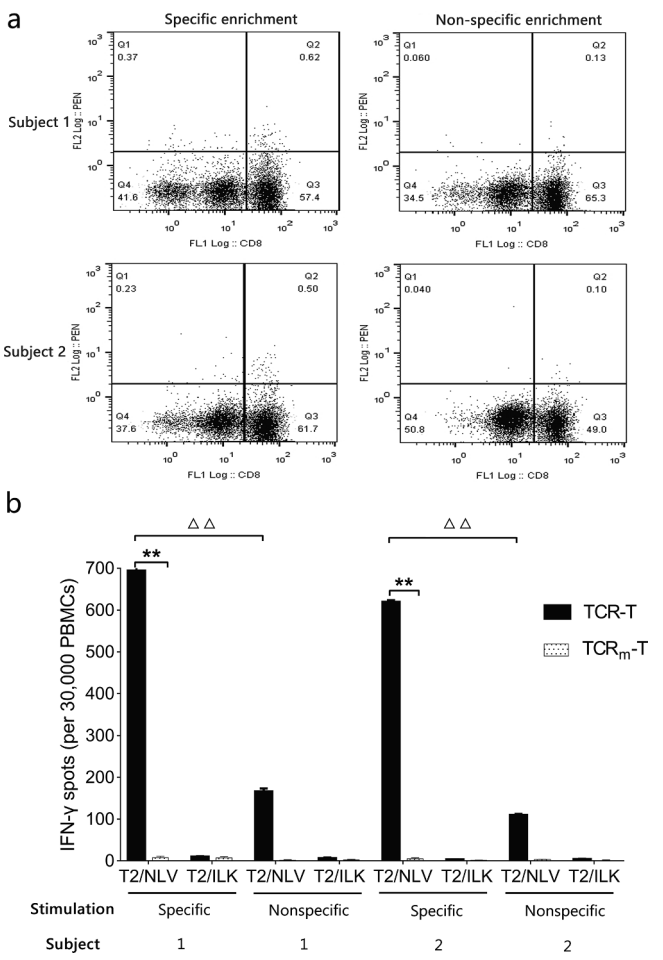

Fig. 5. Enrichment of TCR-engineered T cells. Six days after transduction, TCR-T cells and TCR_m-T cells were co-cultured with specific and non-specific stimuli. a) Five days after co-cultivation, the numbers of specific T cells were determined by flow cytometry. The percentages of $CD8^+/PEN^+$ populations in TCR-T cells is shown in Q2. Data on the stimulation of TCR_m-T cells are not shown because no enrichment was observed. b) After co-cultivation, PBMCs were collected and analyzed by the ELISPOT assay. Mean values from three replicates are shown. Differences in the responses of TCR-T cells and TCR_m-T cells to NLV peptide after specific enrichment are indicated by asterisks (**, $P < 0.001$). Specific stimulation of TCR-T cells generated more IFN-γ–secreting T cells than did non-specific stimulation (ΔΔ, $P < 0.001$).

3.2. Generation of VSTs by transfer of TCR genes into PBMCs from immunocompetent subjects

PBMCs from immunocompetent subjects were transfected with TCR lentivirus. Six days after transfer, T cells expressing TCR could be detected by NLV-peptide/HLA0201 pentamer staining (Fig. 4a), and PBMCs transduced with mock TCR vectors were almost entirely pentamer negative (Fig. 4a). The functional activity of the engineered T cells was detected through IFN-γ ELISPOT assay. TCR-transduced PBMCs produced considerable amounts of IFN-γ–secreting T cells when challenged with NLV-loaded T2 cells ($P < 0.001$; Fig. 4b, c), whereas only a few IFN-γ–positive T cells were observed in the TCR_m-T group ($P > 0.05$; Fig. 4b, c). T2 cells loaded with unrelated TIL peptides failed to induce IFN-γ secretion, indicating peptide specificity. To verify that TCR-transgenic T cells could be enriched by specific stimulation and maintain their specific function, antigen stimulation was performed (Fig. 5). Only TCR-T cells co-cultured with irradiated NLV-loaded T2 cells induced specific enrichment of $CD8^+/PEN^+$ T cells (Fig. 5a) and INF-γ-secreting T cells ($P < 0.001$; Fig. 5b). Neither specific nor non-specific stimulation enriched VSTs in TCR_m-V groups (Fig. 5b). These

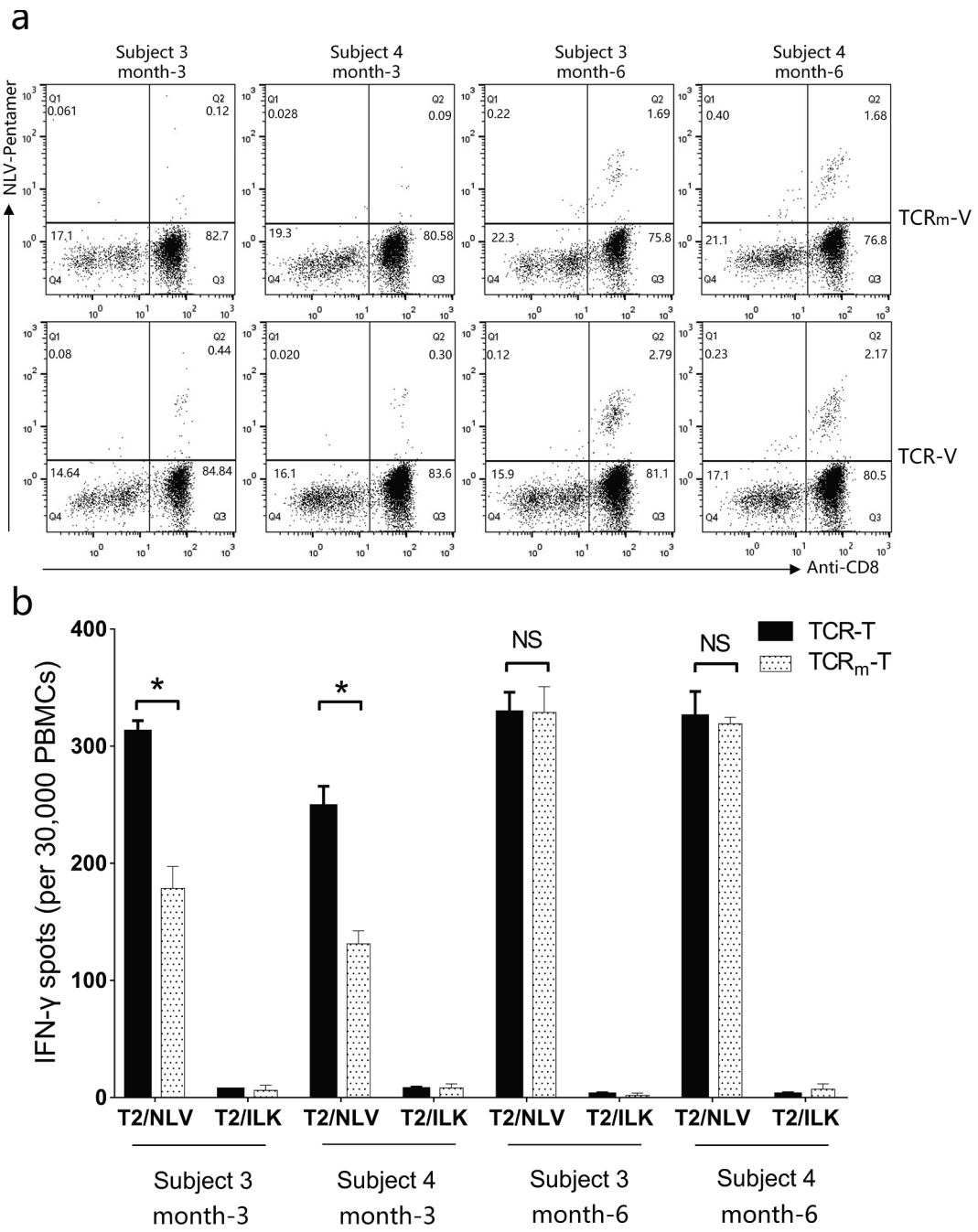

Fig. 6. Transfer of TCR genes into PBMCs from immunocompromised patients. PBMCs from the two recipients were collected and transduced with TCRm-V and TCR-V vectors. a) Six days post-transduction, VSTs were characterized by CD8 antibodies and NLV/MHC pentamer staining. The proportion of CD8 + /PEN + T cells is shown in gate Q2. b) Specific reactivity of engineered T cells was analyzed by an IFN-γ ELISPOT assay 6 days after transfer. Mean values from three replicates are shown. Asterisks indicate significant differences (*, $P < 0.05$) and NS represents no significant difference ($P > 0.05$).

results implied that VSTs can be generated from immunocompetent PBMCs through TCR gene transfer, and that the engineered T cells expand and maintain their specific function when challenged with counterpart peptides.

3.3. Generation of VSTs by transfer of TCR genes into PBMCs from immunocompromised subjects

PBMCs from immunocompetent subjects were engineered efficiently with exogenous TCR, which enabled them to stably proliferate and produce cytokines when responding to specific peptides. Next, we investigated whether TCR transduction could equip PBMCs from immunocompromised subjects with an increasing number of functional VSTs. PBMCs were collected from subjects 3 and 4 at months 3 and 6 after HSCT, and were then transduced with TCR lentivirus vectors and mock vectors.

The number of IFN-γ–secreting T cells in mock-transduced PBMCs was similar to that in original PBMCs at 6 days post-transduction, indicating that standard cultivation did not change the proportion of VSTs. Compared with mock transduction, transfer of TCR genes into month-3 PBMCs increased the percentage of $CD8^+/PEN^+$ T cells (Fig. 6a) and significantly enhanced the frequency of IFN-γ–secreting T cells ($P < 0.05$; Fig. 6b). For month-6 PBMCs, $CD8^+/PEN^+$ T cells were more abundant in the TCR-V groups than in the TCR_m-V groups

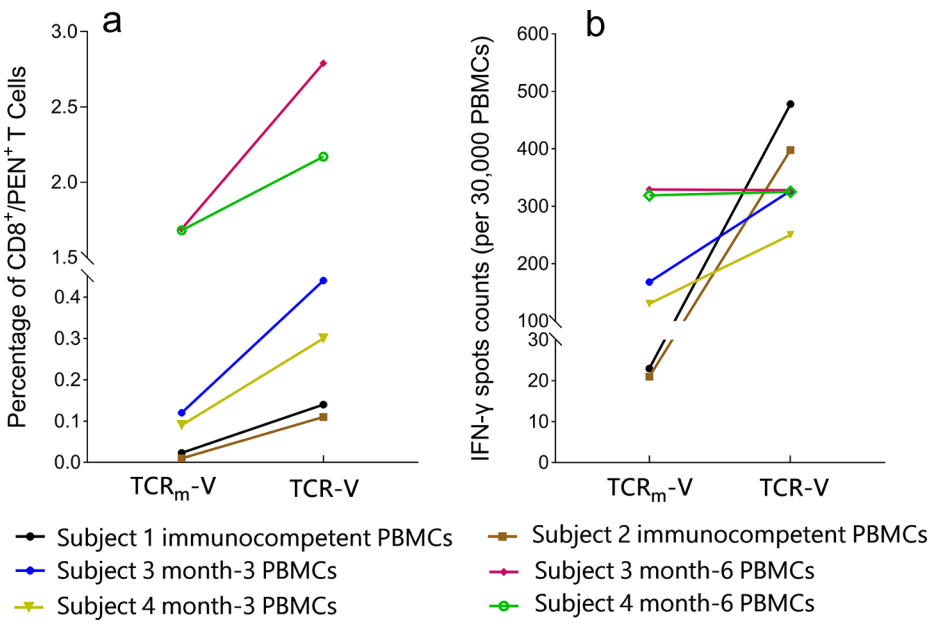

Fig. 7. Differences in the generation of VSTs after transfer of different PBMCs. a) TCR gene transfer increased the percentages of $CD8^+/PEN^+$ T cells in immunocompetent PBMCs, month-3 PBMCs, and month-6 PBMCs; the mean fold increases were 8.5, 3.5, and 1.5, respectively. b) The numbers of IFN-γ–specific-secreting T cells increased significantly in immunocompetent PBMCs ($P < 0.001$) and month-3 PBMCs ($P < 0.05$), but were unchanged in month-6 PBMCs ($P > 0.05$). The mean fold increases in immunocompetent PBMCs, month-3 PBMCs, and month-6 PBMCs were 19.9, 1.9, and 1.0, respectively. Fold increases in $CD8^+/PEN^+$ T cells were calculated by dividing the percentage of $CD8^+/PEN^+$ T cells in the TCR-V group by the percentage in the TCR_m-V group; fold increases in IFN-γ T cells were calculated by dividing the number of IFN-γ T cells in the TCR-V group by the number in the TCR_m-V group. Mean values are shown.

(Fig. 6a), whereas no difference was observed in the numbers of IFN-γ–secreting T cells between the TCR-V and TCR_m-V groups ($P > 0.05$; Fig. 6b). Thus, we concluded that TCR gene transduction increased the number of functional VSTs in PBMCs from immunocompromised subjects.

3.4. Comparison of TCR gene transfer into PBMCs from immunocompetent and immunocompromised subjects

The percentage of $CD8^+/PEN^+$ T cells and the number of IFN-γ–secreting T cells differed significantly after transfer of TCR genes into PBMCs from immunocompetent and immunocompromised subjects (Fig. 7a, b).

For immunocompetent PBMCs, $CD8^+/PEN^+$ T cells and IFN-γ–secreting T cells were 8.5- and 19.9-fold more abundant, respectively, in the TCR-V groups than in the TCR_m-V groups. For month-3 PBMCs, $CD8^+/PEN^+$ T cells and IFN-γ–secreting T cells were 3.5- and 1.9-fold more abundant, respectively, in the TCR-V groups than in the TCR_m-V groups. For month-6 PBMCs, TCR gene transfer increased the percentage of $CD8^+/PEN^+$ T cells, but did not increase the frequency of IFN-γ–secreting T cells. HCMV-specific TCR gene transfer increased the frequencies of $CD8^+/PEN^+$ T cells and IFN-γ–secreting T cells in immunocompetent and month-3 PBMCs. No difference was observed in the numbers of IFN-γ T cells between the TCR-V and TCR_m-V groups after transduction of month-6 PBMCs. Transfer of TCR genes into immunocompetent and month-3 PBMCs would be more meaningful in response to HCMV infection than would transfer into month-6 PBMCs.

4. Discussion

China has a large population of patients with HCMV infection, and many immunocompromised patients have a high risk of HCMV recurrence [6,20]. Currently, antiviral prophylaxis and pre-emptive therapy with antiviral drugs are two predominant strategies for the treatment of HCMV infection in HSCT recipients [1]. Specific T-cell therapy is thought to be an effective alternative [21–23]. In this study, PBMCs from immunocompetent and immunocompromised subjects were transfected with TCR-expressing vectors. Pentamer staining and ELISPOT assay were applied to evaluate the quantity and specific function of the TCR-engineered T cells. We demonstrated that transfer of TCR genes into immunocompetent and immunodeficient PBMCs increased the numbers of VSTs, which potentially contribute to the prevention and treatment of HCMV infection.

In immunocompromised patients, VSTs are thought to be required for the elimination of active HCMV infection and protection from HCMV-related complications [24,25]. Transduction of TCR genes into immunocompetent PBMCs increased the number of VSTs. Additionally, engineered T cells stably proliferated and maintained specific function when responding to specific peptides. The increment of $CD8^+/PEN^+$ T cells and IFN-γ–secreting T cells was also observed in immunodeficient PBMCs after TCR transduction. Although many studies have focused on HCMV-specific TCR transduction [12,26], reports describing the transfer of TCR genes into PBMCs from seropositive donors and immunocompromised recipients are rare. The findings of this study may provide new strategies for TCR therapy for HCMV infection.

$CD8^+/PEN^+$ T cells were generated from immunodeficient and immune-reconstituted PBMCs through TCR gene transfer. The production of functional T cells differed. PBMCs were collected at months 3 and 6 post-HSCT. Month 6 post-HSCT is characterized by immune reconstitution [2]. HSCT recipients with effective immune recovery may be able to respond to HCMV infection [10,27]. Furthermore, transfer of TCR genes into immuno-reconstituted PBMCs did not increase the frequency of IFN-γ–secreting T cells. TCR therapy for HCMV infection at month 6 post-transplant might be unnecessary. Month 3 post-HSCT is characterized by cellular immunodeficiency [1,2]. It is an important clinical period for the prevention and treatment of HCMV infection after HSCT [28]. Transfer of TCR genes into immunodeficient PBMCs significantly increased the number of IFN-γ–secreting T cells. Transfer of HCMV-specific TCR genes into immunodeficient PBMCs, rather than into immune-reconstituted PBMCs, may be more effective for the control of HCMV infection.

The frequency of IFN-γ–secreting T cells did not differ between the TCR-V and TCR_m-V groups after transduction of immune-reconstituted PBMCs. One probable reason for this response is that specific T cells were reconstituted sufficiently to respond to HCMV infection in HCMV-infected recipients. Although the numbers of specific T cells increased after transduction, the engineered T cells would not exhibit an additional specific function. TCR-engineered T cells generated from immune-reconstituted PBMCs may exhibit functional defects temporarily, but respond effectively to HCMV re-infection. Another probable reason is that the stimulus was not sufficient to trigger the specific immune response that might be elicited by a higher concentration or multiple

antigen peptides in HCMV infection. The immune response mechanism between T cells and HCMV activation in immunodeficient patients remains unclear. Specific T cells seem to be inactive in seropositive recipients in terms of preventing HCMV infection during immune recovery [29]. This inactivity might explain the common HCMV recurrence in HSCT recipients. Thus, TCR therapy is more meaningful in patients with severe immunodeficiency than in those with effective immune reconstitution.

Two main approaches are used to generate HCMV-specific T cells in research on cellular immune responses against HCMV. One is to purify and expand VSTs from seropositive donor PBMCs [30], and the other is to modify seronegative donor PBMCs with specific TCRs [12]. The innovation of this study is its investigation of the possibility of engineering PBMCs from seropositive immunocompetent donors and immunocompromised patients with HCMV-specific TCR genes. This strategy has rarely been suggested as a way to address HCMV infection.

Although the absolute numbers of pentamer-positive T cells were limited in this study, the quantity was greatly enhanced after TCR transfer, which was essential for conducting this research. Additionally, TCR transduction yields a considerable number of IFN-γ–secreting T cells, and the engineered T cells were able to expand and maintain their specific function. It has been reported that HCMV control is related to the recovery of functional T cells rather than the number of specific T cells [2,31]. Thus, this strategy would be potentially applicable to the control of HCMV infection.

5. Conclusions

TCR gene transfer is a potential therapeutic strategy for controlling HCMV infection in immunocompromised patients. Transfer of TCR genes into immunocompetent and immunodeficient PBMCs would be more meaningful in response to HCMV infection than would transfer into immune-reconstituted PBMCs.

Acknowledgements

RN.Z. and YY.Z. were responsible for investigation, formal analysis and writing. JH.H. was responsible for project administration. W.W., XM.C., and ZJ.L. were responsible for methodology and software. YP.H. and R.Y. were responsible for data curation and validation. J.F. was responsible for resources, funding acquisition and writing – review & editing.

Funding

This work was funded by the Zhejiang Provincial Natural Science Foundation of China (LY14H190002).

Competing interests

None declared.

Ethical approval

This study was approved by the Ethics Committee of the First Affiliated Hospital, College of Medicine, Zhejiang University.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellimm.2018.12.011.

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