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Original Article

Characterization of In Vitro Expanded Virus-Specific T cells for Adoptive Immunotherapy against Virus Infection

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SUMMARY: Adoptive transfer of virus-specific T cells has emerged as a promising therapeutic approach for treatment of virus infections in immunocompromised hosts. Characterization of virus-specific T cells provides essential information about the curative mechanism of the treatment. In this study, we developed a T cell epitope mapping system for 718 overlapping peptides spanning 6 proteins of 3 viruses (pp65 and IE1 from cytomegalovirus; LMP1, EBNA1, and BZLF1 from Epstein-Barr virus; Penton from adenovirus). Peripheral blood mononuclear cells (PBMCs) from 33 healthy Japanese donors were stimulated with these peptides and virus-specific $CD4^+$ and $CD8^+$ T cells were expanded in vitro in the presence of interleukin (IL) 4 and IL7. A median of 13 (minimum–maximum, 2–46) peptides was recognized in the cohort. Both fresh and cryopreserved PBMCs were used for in vitro expansion. The expansion and breadth of T cell responses were not significantly different between the 2 PBMC sets. We assessed viral regions frequently recognized by T cells in a Japanese cohort that could become pivotal T cell targets for immunotherapy in Japan. We tested epitope prediction for $CD8^+$ T cell responses against a common target region using a freely available online tool. Some epitopes were considered to be predictive.

INTRODUCTION

Viral infections are major causes of severe morbidity and mortality in immunocompromised hosts, such as patients undergoing hematopoietic cell transplantation (HCT) or patients with AIDS. Cytomegalovirus (CMV), Epstein-Barr virus (EBV), and adenovirus (AdV) infections, which are frequently asymptomatic in immunocompetent hosts, are potentially life-threatening. Adoptive transfer of virus-specific T cells as immunotherapy against some viral infections has become an effective preventive and therapeutic treatment following allogeneic HCT (1–4). Virus-specific T cells can be expanded in vitro from donor peripheral blood mononuclear cells (PBMCs) following stimulation with overlapping peptides for viral proteins of interest (5,6). The virus-specific T cells can be transfused safely with significant therapeutic impact to the recipient with severe viral infection (7).

The feasibility of the use of banked third-party, virus-specific T cells has recently been reported (8), with adoptive transfer showing clinical therapeutic advantage, even with a single human leucocyte antigen (HLA)-matched allele between donor and recipient (4). T cells recognize antigen through an HLA-dependent manner. Only virus-specific T cells, which recognize peptides presented by HLAs common between the donor and the recipient, eliminate virus-infected cells in the latter. In fact, virus-specific T cells achieve clearance of infected cells only if the epitopes presented by the HLA shared between the donor and the recipient are dominant (4). For this reason, it is critical to identify target peptides of each T cell response and their HLA restriction for assessment of the effectiveness of the virus-specific T cells.

Adoptive transfer of virus-specific T cells following allogeneic HCT is under consideration for clinical application in Japan (manuscript submitted). Although epitope information on HLA restriction for CMV and EBV has been compiled (9), the information is biased towards HLA alleles prevalent in Caucasian populations. In this study, we performed enzyme-linked immunospot (ELISpot)-based epitope mapping to identify T cell target regions frequently recognized in the Japanese population for CMV (pp65 and IE1), EBV (EBNA1, LMP2, and BZLF1), and AdV (Penton). We also performed epitope prediction to characterize $CD8^+$ T cell responses against common target regions in the Japanese population.

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MATERIALS AND METHODS

Study population: PBMCs were obtained from 33 Japanese healthy volunteers of unknown CMV, EBV and AdV sero-status. All participants gave written informed consent. The study was approved by the institutional review boards of the National Institute of Infectious Diseases (574), the Institution of Medical Science at the University of Tokyo (25-73-0310), and Tokyo Medical Dental University (1428).

HLA typing: HLA class I typing was performed by Luminex assays (WAKFlow HLA Typing Kit, Wakunaga Pharmaceutical, Osaka, Japan).

Peptides: We used a commercially available mixture of 15-mer overlapping-peptides (OLPs), overlapping by 11 amino acids spanning pp65 and IE-1 in CMV; EBNA1, BZLF1, and LMP1 in EBV; Penton in AdV (JPT Peptide Technologies, Berlin, Germany) for in vitro stimulation of PBMCs. The number of peptides that covers each protein was as follows: 138 (OLP1-138) for pp65, 120 (OLP139-258) for IE1, 122 (OLP259-380) for LMP2, 139 (OLP381-519) for EBNA1, 59 (OLP520-578) for BZLF1, and 140 (OLP579-718) for Penton. For epitope mapping, the same OLPs included in the peptide mix were synthesized (Eurofin Genomics, Tokyo, Japan), and working solutions were prepared for each peptide.

Design of peptide matrices: All 718 OLPs from 6 viral proteins were tested in a peptide pool matrix to facilitate comprehensive screening of T cell target as previously described (10,11). The OLPs were included in 3 different peptide matrix systems in this study. Within a given matrix, each peptide was represented in 2 different peptide pools, allowing for the identification of the respective peptide by responses in the 2 corresponding pools.

In vitro expansion of PBMCs with overlapping peptides: Fresh or cryopreserved PBMCs were stimulated with peptide mix spanning all 6 viral proteins (n = 718 OLPs) at a final concentration of 100 ng/mL for each peptide, and cultured for 2–3 weeks in RPMI1640 supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM glutamine (R10) with 800 U/mL interleukin (IL)4 and 900 U/mL IL7 (Miltenyi Biotec, Bergisch Gladbach, Germany). The expanded PBMCs were used for functional assays following overnight incubation at 37ºC in the absence of any cytokines.

Interferon-gamma (IFN-γ) ELISpot assay: IFN-γ ELISpot assay was performed as previously described with some modifications (12). Epitope mapping was performed using 7.5×10^4–1×10^5 in vitro expanded PBMCs by incubating overnight with the matrix peptide pools. A positive response was defined as: i) at least 5 spots per well, ii) responses exceeding the mean of negative wells plus 3 standard deviations, and/or iii) responses exceeding 3 times the mean of negative wells. Reconfirmation of all positive wells in the matrix screen was performed by using the single peptide common to 2 positive pools. Peptides were used at a final concentration of 10 μg/mL for both pooled peptides and single peptides.

Intracellular cytokine staining (ICS): In vitro expanded PBMCs were stimulated with peptide mix or single peptides in the presence of costimulatory antibodies (anti-CD28 and anti-CD49d at 1 μg/mL) and monensin (GolgiStop; BD Biosciences, Franklin Lakes, NJ, USA). Following incubation for 6 h, ICS was performed as previously described (12). Dead cells were removed by LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (ThermoScientific, Tokyo, Japan). Fluorochrome-conjugated monoclonal antibodies to CD3, CD8, and IFN-γ (BioLegend, San Diego, CA, USA) were used. IFN-γ-producing cells greater than 0.05% of T cells were considered as positive responses. Data were collected on a FACSCanto II or Fortessa flow cytometer (BD Biosciences), and analyzed using FlowJo 9.9.3 softeware (TreeStar, Ashland, OR, USA).

Epitope prediction: The HLArestrictor-1.2 T cell epitope prediction tool that is available on the internet was utilized (13).

Statistical analysis: Statistical analysis was performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). Mann-Whitney test were used for all analyses. A P-value < 0.05 was considered statistically significant.

RESULTS

HLA typing: We performed HLA class I typing for all samples used in this study. The allele frequency in the current study (n = 33) and in a Japanese population (14) (n = 18,604) are shown in Fig. 1. The results indicate that the HLA distribution of our samples represents the Japanese population.

In vitro expansion of virus-specific T cells: PBMCs from 33 Japanese healthy donors were stimulated in vitro with peptides spanning 6 viral proteins from CMV (pp65, IE1), EBV (LMP2, EBNA1, BZLF1), and AdV (Penton) for 2–3 weeks (median 15 days, IQR: 13.5–16 days) to expand virus-specific T cells. Because CMV-specific $CD4^+$ T cells are important for the persistence of adoptive transfer of $CD8^+$ T cells in the recipient (15), as in the case of EBV-specific T-cell therapy (3), IL4 and IL7 were used in the expansion medium. These cytokines induce efficient expansion of polyclonal, Th1-polarized virus-specific T cells in both $CD4^+$ and $CD8^+$ T cell subsets (5). The median cell expansion (fold-increase of cell number after stimulation) was 3.8 (minimum–maximum, 1.4–9). Both fresh (n = 18) and cryopreserved PBMCs (n = 15) were used for in vitro expansion. Cell expansion was not significantly different between fresh and cryopreserved PBMCs (median, 4.1 [minimum–maximum, 1.7–9] and 3.5 [minimum–maximum, 1.4–7.7], respectively; p = 0.15; Fig. 2A).

Epitope mapping of virus-specific T cells: To assess target regions of the virus-specific T cells, peptide matrix approaches have been employed (10,11). We performed the ELISpot assay on the in vitro expanded T cells using matrices consisting of the OLPs from these viral proteins. The peptides located at the intersection of rows and columns with a positive response were subjected to the single-OLP-based ELISpot assay to determine reactive peptide(s). Among 718 OLPs from 6 viral proteins, 283 OLPs (39.4%) were recognized by PBMCs from at least single subject of the 33 donors. T cell epitopes restricted by HLA class I are 8 to 11 amino acids long (16), most of which are included in the overlapping

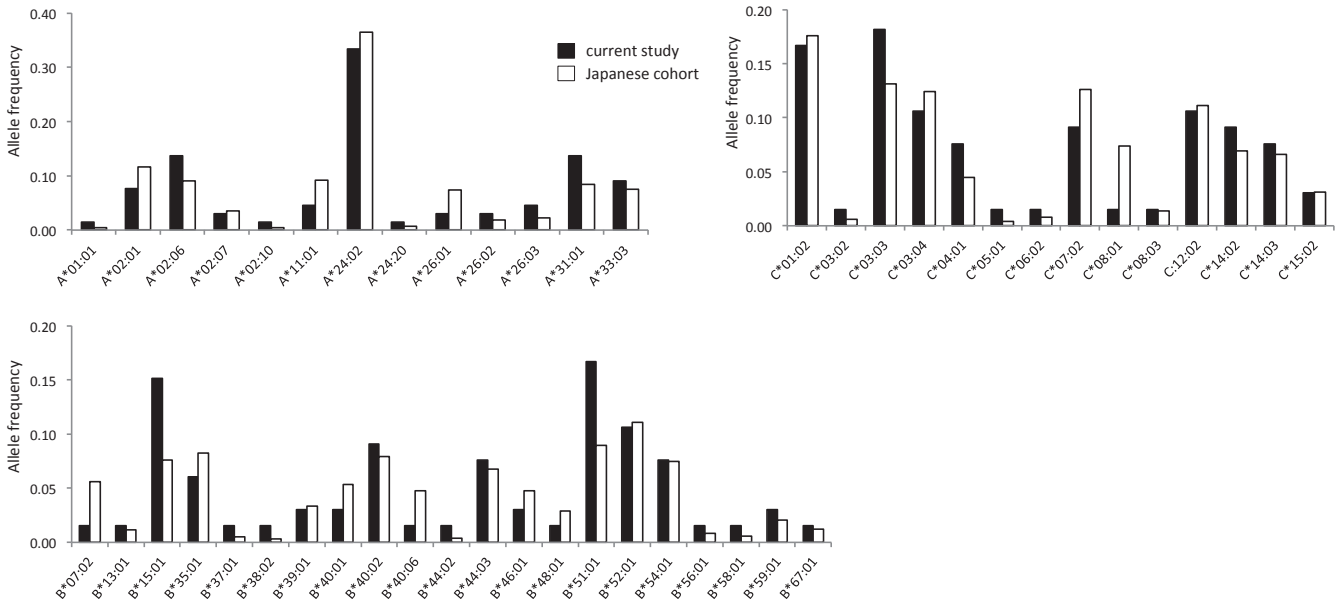


Fig. 1. Allele frequency of HLA class I in the samples used in current study and Japanese population (14). Only alleles expressing in our samples are shown.

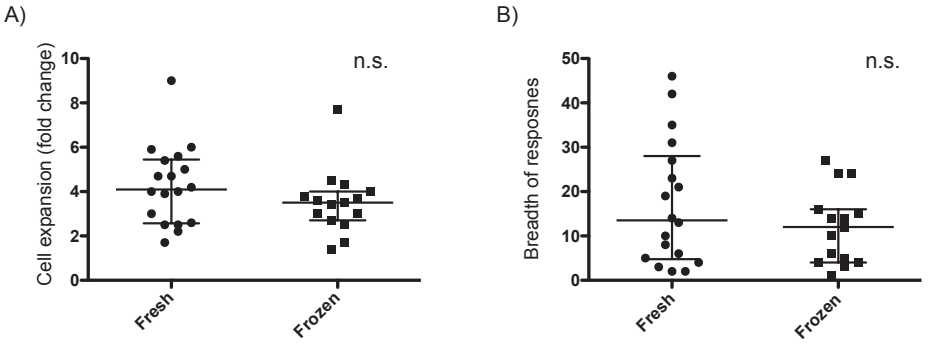


Fig. 2. Cell expansion (A) and breadth of T cell response (B) in fresh and frozen PBMCs. The horizontal bars show the median. Data were analyzed using Mann-Whitney test. n.s., not significant.

region (11 amino acids). Thus, the responses detected in adjacent OLPs were considered as the same T cell responses. Data of donor-14 is shown in Fig. 3A as representative data. The median breadth in T cell responses to all viral proteins was 13 (minimum–maximum, 2–46; Fig. 3B). The breadth of responses was not significantly different between fresh samples and frozen samples (median 13.5; minimum–maximum, 2–46) and 12 (minimum–maximum, 3–27), respectively (p = 0.5023; Fig. 2B). The broadest responses were found in CMV pp65 (median 5; minimum–maximum, 0–33).

The subset of the responding T cells ($CD4^+$ and/or $CD8^+$) was assessed by ICS using the targeted peptides when a sufficient amount of sample was available. ICS was performed only for the OLPs with specific responses $\geq$ 500 spot forming cells per million cells in the single-OLP-based ELISpot assay, due to the lower sensitivity of the ICS compared to ELISpot assay. Both $CD8^+$ T cell and $CD8^-$ (interpreted as $CD4^+$) T cell responses were detected in donor-14 when CMV pp65 peptide mix was used as antigen (Fig. 3C). However, only $CD4^+$ T cell responses were detected against OLP15 and 71/72. $CD8^+$ T cell responses were detected against OLP85/86. A specific T cell response was not detected against OLP87 in the ICS analysis, probably due to low sensitivity of this assay (low positive response was observed in the ELISpot as well). Of 33 Japanese healthy donors, both $CD4^+$ and $CD8^+$ T cell responses were detected against all 6 viral proteins tested (Fig. 3D). The most common target peptide was OLP123 AGILARNLVPMVATV, located at amino acid position 489–503 in CMV pp65. The in vitro expanded T cells from 14 of 33 (42%) donors targeted OLP123 (Fig. 3D). Of 12 out of 14 responders for which ICS analysis was carried out, 3 of 12 and 6 of 12 donors showed $CD4^+$ and $CD8^+$ T cell responses, respectively. The other 3 donors had both $CD4^+$ and $CD8^+$ T cell responses, indicating that this peptide includes both HLA class I- and II-restricted epitopes. The well-known pp65 epitope, NLVPMVATV, which is restricted by HLA-A*02:01/06 (17), is incorporated in OLP123. Eight of 9 donors with $CD8^+$ T cell responses expressed HLA-A*02:01 or A*02:06, suggesting that a part of the $CD8^+$ T cell responses are specific for the epitope. Although some HLA-DR-restricted epitopes have also been reported in this peptide (9), these alleles are rare in the Japanese population (14). In addition, no

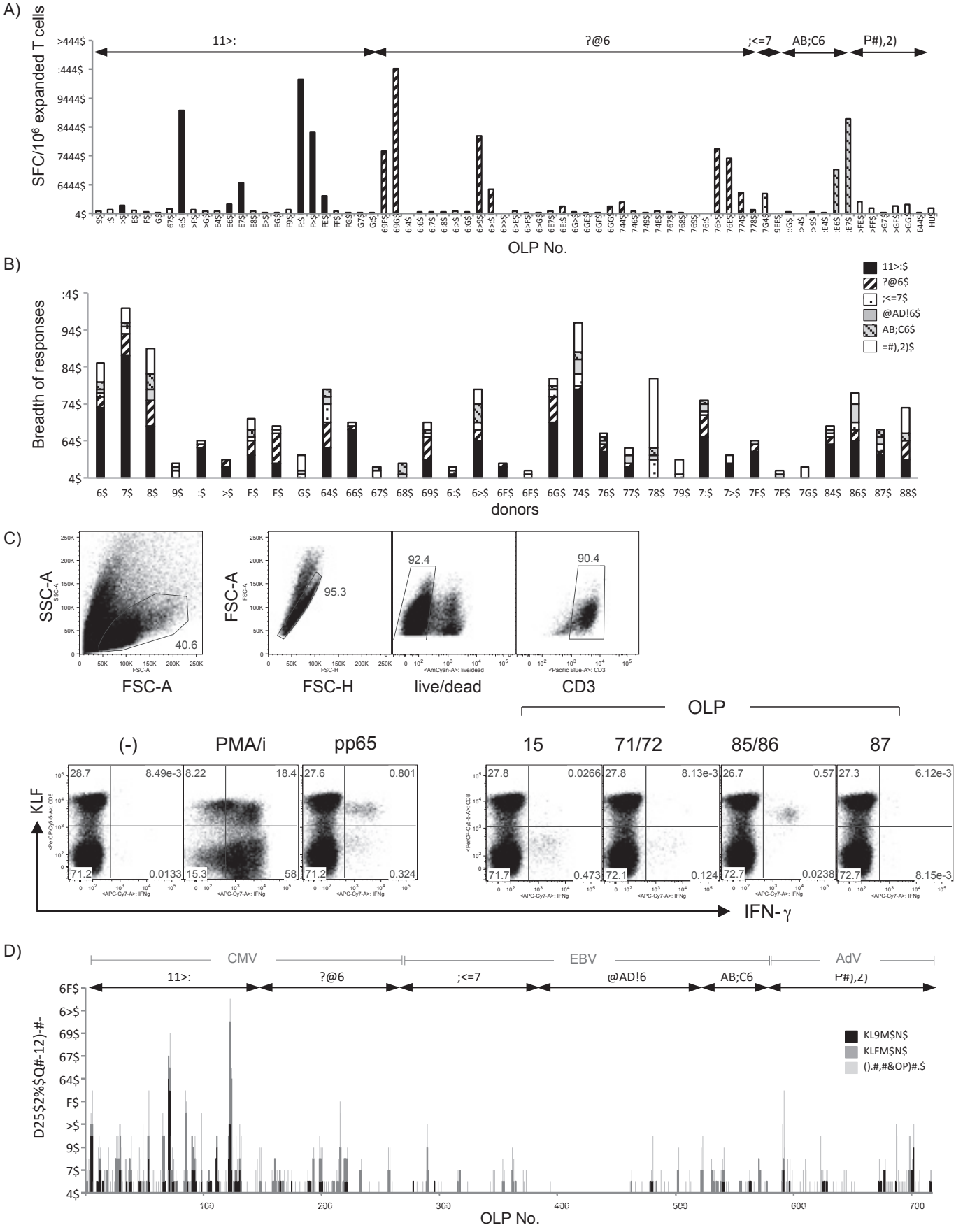


Fig. 3. Virus-specific T cell responses in in vitro expanded T cells. A) Single-OLP-based ELISpot was performed for all positive wells in the ELISpot using peptide matrices. Data from donor-14 are shown. B) Breadth of T cell responses in 33 donors. Graph legends correspond for both A and B. C) Representative FACS profile of IFN-γ intracellular staining. Data for donor-14 is shown. D) Number of responses for each OLP. $CD4^+$ and $CD8^+$ T cell responses are black and dark gray, respectively. The responses where T cell subset type could not be determined are shown in light gray.

Table 1. Common target regions of in vitro expanded virus-specific T cells in Japanese population

virus	protein	OLP	aa position	sequence	No. of response		
					CD4	CD8	U.D.[1)]
CMV	pp65	5-6	17-35	PISGHVLKAVFSRGDTPVL	5	1	3
CMV	pp65	12	45-59	GIHVRVSQPSLILVS	2	1	2
CMV	pp65	26-31	101-135	SICPSQEPMSIYVYALPLKMLNIPSINVHHYPSAA	2	10	7
CMV	pp65	38-39	149-167	IHASGKQMWQARLTVSGLA	2	2	3
CMV	pp65	49	193-207	VALRHVVCAHELVCS	0	1	5
CMV	pp65	53-55	209-231	ENTRATKMQVIGDQYVKVYLESF	3	3	7
CMV	pp65	66-67	261-279	QPFMRPHERNGFTVLCPKN	1	4	2
CMV	pp65	71-73	281-303	IIKPGKISHIMLDVAFTSHEHFG	10	4	3
CMV	pp65	85-86	337-355	VELRQYDPVAALFFFDIDL	0	7	2
CMV	pp65	91-93	361-383	PQYSEHPTFTSQYRIQGKLEYRH	4	4	2
CMV	pp65	99	393-407	AQGDDDVWTSGSDSD	1	1	5
CMV	pp65	111-112	441-459	SSATACTSGVMTRGRLKAE	4	1	1
CMV	pp65	122-124	485-507	PPWQAGILARNLVPMVATVQGQN	6	10	2
CMV	pp65	128-129	509-527	KYQEFFWDANDIYRIFAEL	2	0	3
CMV	pp65	131-132	521-539	YRIFAELEGVWQPAAQPKR	2	1	6
CMV	IE1	10-11	37-55	QTMLRKEVNSQLSLGDPLF	0	1	4
CMV	IE1	77-79	305-327	LSEFCRVLCCYVLEETSVMLAKR	0	5	5
EBV	LMP2	32-33	125-143	LPVIVAPYLFWLAAIAASC	0	4	3
EBV	EBNA1	101	477-491	KFENIAEGLRALLAR	1	0	4
EBV	BZLF1	3-4	9-27	EDVKFTPDPYQVPFVQAFD	0	2	3
EBV	BZLF1	52-53	205-223	AAKSSENDRLRLLLKQMCP	0	3	2
ADV	Penton	13-14	49-67	TGGRNSIRYSELAPLFDTT	2	1	8
ADV	Penton	45	177-191	ETMTIDLMNNAIVEH	0	0	5
ADV	Penton	109-110	433-451	GSEQVYWSLPDMMQDPVTF	1	0	5
ADV	Penton	120-121	477-495	NDQAVYSQLIRQFTSLTHV	0	2	3
ADV	Penton	124	493-507	THVFNRFPENQILAR	4	0	2

[1)]: undetermined.

responder expressed any of these HLA-DRB1 molecules (data not shown), implying that epitopes in this peptide are restricted by an as-yet-unidentified HLA class II molecule(s). Regions covered by sequential OLPs recognized by more than 5 donors are listed in Table 1.

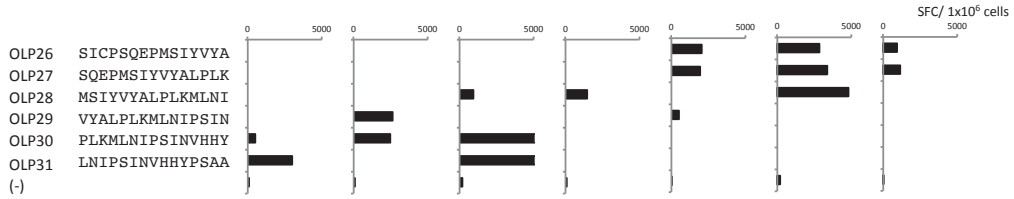
Epitope prediction: Accumulating information on epitopes and their HLA restriction has enabled the development of epitope prediction tools (13,18,19). The regions covered by OLP26-31, SICPSQEPMSIYVYALPLKMLNIPSINVHHYPSAA, located at 101–135 in pp65, were targeted by 19 T cell responses in 12 donors, in which 10 of 19 were $CD8^+$ T cell responses (Table 1). All $CD8^+$ T cell responses from 7 donors are shown in Fig. 4A. We performed epitope prediction of peptides within the OLP26-31 region by HLArestrictor-1.2 (13). Predicted epitopes restricted by the HLA class I alleles expressing in the responders are shown in Fig. 4B. Donor-3, -7, and -16, all expressing HLA-B*51:01, had $CD8^+$ T cell responses against OLP28, in which the HLA-B*51-restricted epitope LPLKMLNI has been reported (20). Similarly, OLP30/31 targeted by PBMCs from donor-1 and donor-3 expressing HLA-B*35:01 included HLA-B*35:01-restricted epitope, IPSINVHHY, which has also been previously reported in the literature (21). Both epitopes were predicted as a strong binder by HLArestrictor (Fig. 4B). These data suggest that the responses to OLP28 and OLP30/31 are expected to be against the reported epitopes, though we cannot exclude possibility that the responses targeted other unknown peptides. $CD8^+$ T cell responses against OLP26/27 were detected in 3 donors (donor-10, -16, and -19) sharing HLA-B*54:01 and HLA-C*01:02 (Fig. 4A). The epitopes restricted by HLA-B*54:01 and -C*01:02 were predicted in either OLP26 or OLP27 (EPMSIYVYA and EPMSIYVYAL for HLA-B*54:01, MSIYVYALPL and SIYVYALPL for HLA-C*01:02) (Fig. 4B). Other $CD8^+$ T cell responses detected in this study, responses against OLP29 in donor-2 and donor-10, also had predictive epitopes restricted by HLA expressing the responders in the target OLP (HLA-A*24:02-restricted VYALPLKMLNI and HLA-B*54:01-restricted LPLKMLNI, LPLKMLNIPS, and LPLKMLNIPSI).

DISCUSSION

Adoptive transfer of donor-derived, virus-specific T cells has been demonstrated to be an effective strategy to control CMV, EBV, and AdV infections after allogeneic HCT (4, 22–25). Peptide mix spanning multiple viral proteins has the advantages of stimulating virus-specific T cells and eliciting broad virus-specific T cell responses regardless of HLA phenotype. However, there are some difficulties in characterizing these responses with respect to quality, breadth of the responses, and HLA restriction. Cellular immune response by cytotoxic T lymphocytes (CTL) is a critical defense mechanism against virus infection. Both the magnitude and breadth of T cell responses are important for virus control (26,27). In HIV infection, broader T cell responses against Gag, a highly conserved region, but not other viral proteins that tolerate amino acid change, are associated with viral control (26), suggesting that target region of virus-specific T cells is critical for T cell mediated anti-viral activity. Although the genetic stability of the viral genome is much higher in DNA viruses compared to RNA viruses, such as HIV and HCV, escape mutants from CTL recognition by amino acid substitutions in CTL epitopes or dele-

A)

	donor-1	donor-2	donor-3	donor-7	donor-10	donor-16	donor-19
A	24:02/24:02	24:02/26:02	24:20/31:01	02:07/31:01	24:02/33:03	31:01/31:01	24:02/31:01
B	07:02/35:01	40:02/48:01	35:01/51:01	46:01/51:01	54:01/58:01	51:01/54:01	40:02/54:01
C	03:03/07:02	03:03/08:03	03:03/14:02	01:02/14:02	01:02/03:02	01:02/15:02	01:02/03:04



B)

aa position in pp65	predicted epitope sequence*	HLA restriction	Affinity(nM)	%Rank	ref
103-111	CPSQEPMSI	B51:01	NA	0.3	-
103-113	CPSQEPMSIYV	B51:01	NA	0.3	-
107-115	EPMSIYVYA	B54:01	21	0.12	-
107-116	EPMSIYVYAL	B54:01	119	0.5	-
109-116	MSIYVYAL	C03:04	44	0.3	-
109-118	MSIYVYALPL	C03:03	27	0.1	-
109-118	MSIYVYALPL	C03:04	27	0.1	-
109-118	MSIYVYALPL	C01:02	477	0.4	-
110-118	SIYVYALPL	C01:02	229	0.12	-
111-118	IYVYALPL	C14:02	11	0.25	-
111-118	IYVYALPL	A24:02	37	0.4	-
112-120	YVYALPLKM	C14:02	18	0.4	-
113-123	VYALPLKMLNI	A24:02	47	0.4	-
116-123	LPLKMLNI	B51:01	NA	0.05	20
116-123	LPLKMLNI	B54:01	138	0.5	-
116-125	LPLKMLNIPS	B54:01	127	0.5	-
116-126	LPLKMLNIPSI	B51:01	NA	0.03	-
116-126	LPLKMLNIPSI	B54:01	77	0.4	-
123-131	IPSINVHHY	B35:01	11	0.1	21

Fig. 4. Epitope prediction. A) $CD8^+$ T cell responses, measured by ELISpot, against OLP26-31. All T cell responses that were confirmed as $CD8^+$ T cell responses by IFN-γ–ICS are shown. Alleles in HLA-A, B, and C loci for each donor are shown. B) Epitopes predicted by HLArestrictor-1.2. The peptides predicted as "strong binder (% Rank is less than 0.5) to the cognate HLA" are shown. "Affinity" shows predicted binding affinity (IC50 [nM]) to the cognate HLA molecule. NA, not assessed. The references are shown if the epitope has been reported.

tion mutants have been reported for gamma herpesvirus (28,29). Elucidation of target regions and their HLA restriction of virus-specific T cells, and quantification of the number of T cells for each target region would provide us with essential information for quality control for cellular therapy products.

Although single-HLA-expressing cell lines are useful for determination of HLA restriction in each T cell response, it is time-consuming to prepare multiple single-HLA-expressing cell lines to cover even dominant HLA molecules in the population. We used the online epitope prediction tool, HLArestrictor, to obtain supplementary information about HLA restriction of each $CD8^+$ T cell responses. In the common target region in CMV-pp65, $CD8^+$ T cell responses to the same OLP were detected in donors expressing the same HLA, e.g., HLA-B*35:01 in donor-1 and -3, and HLA-B*51:01 in donor-3, -7, and -16 (Fig. 4A), and the peptides (IPSINVHHY and LPLKMLNI) were predicted as strong binders to the shared HLA molecules (HLA-B*35:01 and -B*51:01, respectively, Fig. 4B). These peptides have already been reported as optimal epitopes with experimental data (20,21), supporting the utility of the epitope prediction tool. We also found $CD8^+$ T cell responses against OLP26/27 in PBMCs from 3 donors expressing HLA-B*54:01 and -C*01:02; and both HLA-restricted epitopes were predicted in the OLPs by HLArestrictor (Fig. 4).

Although epitope databases and prediction tools provide useful information, genes of the HLA are the most variable coding loci in the human genome, and the allele distribution is ethnically variable. These tools are less informative for rare HLA alleles in well-studied populations, such as Caucasoids. The prevalence of HLA-B*54:01 is much higher in Asia including Japan where the allele frequency is 7.63%, the 6th most prevalent HLA-B allele. However, HLA-B*54:01 is very rare in Caucasoid and African populations (less than 0.1% in both ethnicities) (14), and there is no HLA-B*54:01-restricted epitope information in CMV, EBV, and AdV in an epitope database (9). In the case of Penton in AdV, only 3 epitopes have currently been reported. Accumulation of epitope information would improve the accuracy of epitope prediction tools. The accumulation from broad geographical regions might be useful for clinical application of the T cell immunotherapy for virus infection.

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Conflict of interest None to declare.

REFERENCES

1. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet. 1995;345:9-13.
2. Heslop HE, Brenner MK, Rooney CM. Donor T cells to treat EBV-associated lymphoma. N Engl J Med. 1994;331:679-80.
3. Heslop HE, Slobod KS, Pule MA, et al. Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients. Blood. 2010;115:925-35.
4. Doubrovina E, Oflaz-Sozmen B, Prockop SE, et al. Adoptive immunotherapy with unselected or EBV-specific T cells for biopsy-proven EBV$^+$ lymphomas after allogeneic hematopoietic cell transplantation. Blood. 2012;119:2644-56.
5. Gerdemann U, Keirnan JM, Katari UL, et al. Rapidly generated multivirus-specific cytotoxic T lymphocytes for the prophylaxis and treatment of viral infections. Mol Ther. 2012;20:1622-32.
6. Tzannou I, Papadopoulou A, Naik S, et al. Off-the-shelf virus-specific T cells to treat BK virus, human herpesvirus 6, cytomegalovirus, epstein-barr virus, and adenovirus infections after allogeneic hematopoietic stem-cell transplantation. J Clin Oncol. 2017;35: 3547-57.
7. Papadopoulou A, Gerdemann U, Katari UL, et al. Activity of broad-spectrum T cells as treatment for AdV, EBV, CMV, BKV, and HHV6 infections after HSCT. Sci Transl Med. 2014;6:242ra83.
8. O'Reilly RJ, Prockop S, Hasan AN, et al. Virus-specific T-cell banks for 'off the shelf' adoptive therapy of refractory infections. Bone Marrow Transplant. 2016;51:1163-72.
9. Vita R OJ, Greenbaum JA, Ponomarenko J, et al. The immune epitope database (IEDB) 3.0. Available at <www.iedb.org>. Accessed September 19, 2017.
10. Frahm N, Korber BT, Adams CM, et al. Consistent cytotoxic-T-lymphocyte targeting of immunodominant regions in human immunodeficiency virus across multiple ethnicities. J Virol. 2004;78:2187-200.
11. Addo MM, Yu XG, Rathod A, et al. Comprehensive epitope analysis of human immunodeficiency virus type 1 (HIV-1)-specific T-cell responses directed against the entire expressed HIV-1 genome demonstrate broadly directed responses, but no correlation to viral load. J Virol. 2003;77:2081-92.
12. Kawana-Tachikawa A, Llibre JM, Bravo I, et al. Effect of maraviroc intensification on HIV-1-specific T cell immunity in recently HIV-1-infected individuals. PLoS One. 2014;9:e87334.
13. DTU Bioinformatics. HLArestrictor--a tool for patient-specific predictions of HLA restriction elements and optimal epitopes within peptides. Available at <http://www.cbs.dtu.dk/services/HLArestrictor>. Accessed September 19, 2017.
14. Allele frequency net 2015 update: new features for HLA epitopes, KIR and disease and HLA adverse drug reaction associations. Available at <http://www.allelefrequencies.net/>. Accessed September 19, 2017.
15. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 1995;333:1038-44.
16. Rammensee HG, Falk K, Rotzschke O. Peptides naturally presented by MHC class I molecules. Annu Rev Immunol. 1993;11:213-44.
17. Solache A, Morgan CL, Dodi AI, et al. Identification of three HLA-A*0201-restricted cytotoxic T cell epitopes in the cytomegalovirus protein pp65 that are conserved between eight strains of the virus. J Immunol. 1999;163:5512-8.
18. SYFPEITHI: database for MHC ligands and peptide motifs. Available at <http://www.syfpeithi.de>. Accsessed September 19, 2017.
19. HLA peptide binding predictions. Available at <https://www-bimas.cit.nih.gov/molbio/hla_bind/>. Accsessed September 19, 2017.
20. Nastke MD, Herrgen L, Walter S, et al. Major contribution of codominant CD8 and CD4 T cell epitopes to the human cytomegalovirus-specific T cell repertoire. Cell Mol Life Sci. 2005;62:77-86.
21. Lidehall AK, Sund F, Lundberg T, et al. T cell control of primary and latent cytomegalovirus infections in healthy subjects. J Clin Immunol. 2005;25:473-81.
22. Feuchtinger T, Richard C, Joachim S, et al. Clinical grade generation of hexon-specific T cells for adoptive T-cell transfer as a treatment of adenovirus infection after allogeneic stem cell transplantation. J Immunother. 2008;31:199-206.
23. Vickers MA, Wilkie GM, Robinson N, et al. Establishment and operation of a good manufacturing practice-compliant allogeneic epstein-barr virus (EBV)-specific cytotoxic cell bank for the treatment of EBV-associated lymphoproliferative disease. Br J Haematol. 2014;167:402-10.
24. Leen AM, Bollard CM, Mendizabal AM, et al. Multicenter study of banked third-party virus-specific T cells to treat severe viral infections after hematopoietic stem cell transplantation. Blood. 2013;121:5113-23.
25. Qasim W, Derniame S, Gilmour K, et al. Third-party virus-specific T cells eradicate adenoviraemia but trigger bystander graft-versus-host disease. Br J Haematol. 2011;154:150-3.
26. Kiepiela P, Ngumbela K, Thobakgale C, et al. CD8$^+$ T-cell responses to different HIV proteins have discordant associations with viral load. Nat Med. 2007;13:46-53.
27. Abdel-Hakeem MS, Bédard N, Murphy D, et al. Signatures of protective memory immune responses during hepatitis C virus reinfection. Gastroenterology. 2014;147:870-81. e8.
28. Loh J, Popkin DL, Droit L, et al. Specific mutation of a gammaherpesvirus-expressed antigen in response to CD8 T cell selection in vivo. J Virol. 2012;86:2887-93.
29. Gottschalk S, Ng CY, Perez M, et al. An epstein-barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs. Blood. 2001;97:835-43.