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*J Immunol* 2009; 183:2837-2850; Prepublished online 27 July 2009; doi: 10.4049/jimmunol.0804178

http://www.jimmunol.org/content/183/4/2837

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**Supplementary Material**

http://www.jimmunol.org/content/suppl/2009/07/27/jimmunol.0804178.DC1

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A Panel of Artificial APCs Expressing Prevalent HLA Alleles Permits Generation of Cytotoxic T Cells Specific for Both Dominant and Subdominant Viral Epitopes for Adoptive Therapy

Aisha N. Hasan,* Wouter J. Kollen,* Deepa Trivedi,*† Annamalai Selvakumar,† Bo Dupont,† Michel Sadelain,§ and Richard J. O’Reilly2*†‡

Adoptive transfer of virus-specific T cells can treat infections complicating allogeneic hematopoietic cell transplants. However, autologous APCs are often limited in supply. In this study, we describe a panel of artificial APCs (AAPCs) consisting of murine 3T3 cells transduced to express human B7.1, ICAM-1, and LFA-3 that each stably express one of a series of six common HLA class I alleles. In comparative analyses, T cells sensitized with AAPCs expressing a shared HLA allele or autologous APCs loaded with a pool of 15-mer spanning the sequence of CMVpp65 produced similar yields of HLA-restricted CMVpp65-specific T cells; significantly higher yields could be achieved by sensitization with AAPCs transduced to express the CMVpp65 protein. T cells generated were CD8+/H1409, New York, NY 10021. E-mail address: oreillyr@mskcc.org

Received for publication February 3, 2009. Accepted for publication June 14, 2009.

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*Department of Pediatrics and †Department of Immunology, ‡The Marrow Transplantation Program, and §Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center, New York, NY 10021

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http://www.jimmunol.org/cgi/doi/10.4049/jimmunol.0804178

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to sensitize human A*0201+ T cells against coexpressed virus-specific or tumor-selective antigenic peptides. Subsequently, Papanicolau et al. (20) demonstrated that CMV-specific T cells could be generated from seropositive HLA-A*0201 donors at high frequency in vitro by sensitization with the same 3T3-based HLA-A*0201-expressing AAPCs transduced to express either the CMVpp65 peptide NLVPMAVT presented by HLA-A*0201 or the full-length CMVpp65 protein. Since then, other studies using the human K562 leukemic cell line transduced to express HLA-A*0201 or other AAPCs expressing this allele have confirmed the potential of AAPCs to induce Ag-specific HLA-A*0201-restricted T cells (13, 21). However, to date, there have been no reports of the construction or function of AAPCs expressing other HLA alleles. Although HLA-A*0201 is the most commonly inherited class I allele and AAPCs expressing this allele have provided a useful proof of principle, at least 60% of patients lack this allele (22). Furthermore, we were concerned that the potential of AAPCs expressing this HLA allele to process and present epitopes of CMVpp65 that elicit functional cytotoxic T cells might be overestimated, since HLA-A*0201-restricted human T cells capable of killing CMV-infected cells are almost exclusively specific for a single peptide, NLVPMAVT (23).

In the present study, we established a panel of AAPCs, each expressing a single common HLA class I allele (i.e., HLA-A*0201, A*0301, A*2402, B*0702, B*0801, or C*0401) which could be used to sensitize T cells from up to 80% of our healthy hematopoietic progenitor cell transplant donors against virus-specific or tumor-selective Ags. We then evaluated each AAPC for its capacity to sensitize and stimulate the expansion of HLA-restricted T cell populations specific for peptides of CMVpp65 known to be expressed on CMV-infected human cells. We chose to evaluate responses to the CMVpp65 protein because it is the immunodominant Ag that is most frequently targeted by CD8+ cytotoxic T cells in CMV-seropositive donors (24, 25) and because a large series of epitopes of CMVpp65 have been identified that can be presented by the HLA alleles in the AAPC panel and can elicit virus-specific cytotoxic T cell responses (25–27). Our results demonstrate that this panel can be used to generate CMVpp65-specific IFN-γ+ and cytotoxic CD8+ T cells of desired HLA restriction, including T cells specific for subdominant epitopes that may be represented at low or undetectable frequencies in T cells sensitized with peptide pool-loaded (PL) autologous CAMs or dendritic cells.

Materials and Methods

Donors

Blood samples were obtained from 13 healthy CMV-seropositive consenting donors according to protocols approved by the Institutional Review Board of Memorial Sloan-Kettering Cancer Center (New York, NY); 5 leukocyte units from healthy CMV-seronegative volunteer donors were purchased from the New York Blood Center. Among these donors, eight expressed HLA-A*0201 (one coexpressed A*0301 and one coexpressed HLA-B*0801), seven expressed HLA-A*2402 (two coexpressed A*0201), five expressed HLA-B*0702 (three coexpressed A*0201), four expressed C*0401 (two coexpressed A*2402 and one coexpressed A*0301), two expressed HLA-B*0801, and one donor expressed HLA-A*0301. HLA typing was performed by the Histocompatibility Testing Laboratory at Memorial Sloan-Kettering Cancer Center by analysis of HLA allele-specific nucleotide sequences using standard high-resolution typing techniques. Donor CMV serostatus was determined by standard serologic techniques in the clinical microbiology laboratory at Memorial Sloan-Kettering Cancer Center.

Generation and culture of APCs

AAPCs. The panel of AAPCs was constructed according to methods previously described (12, 20). Briefly, NIH 3T3 fibroblast cell lines (American Type Culture Collection) were first transduced sequentially with four replication-incompetent SFG retroviral vectors encoding human B7.1 (CD80), ICAM-1 (CD54), and LFA-3 (CD58) and human β2-microglobulin. After each transduction, the cells were steriley sorted by FACS (Molm, Beckman Coulter) to select populations expressing high levels of each vector-encoded human protein in the sequence. The resultant cell line was called 3T3-4. The cDNA sequences for HLA-A*0201, A*2402, A*0301, B*0702, B*0801, and C*0401 were obtained from the International Histocompatibility Working Group Workshop Cell and Gene Bank (Fred Hutchinson Cancer Center, Seattle WA). Each HLA cDNA sequence was then amplified and cloned into an SFG retroviral vector as previously described (12, 25). Separate aliquots of the 3T3-4 line were then transduced with a vector encoding a single HLA H chain. The transduced cells were then sorted and cloned to isolate AAPCs with the highest level of HLA and costimulatory molecule expression. These cells are referred to as AAPCclassI. The expression of all of the transduced costimulatory molecules as well as HLA alleles was verified by flow cytometry using FITC-labeled anti-CD80, PE-Cy5-labeled anti-CD58, allophycocyanin-labeled anti-CD54, FITC-labeled anti-β2-microglobulin, and PE- or FITC-labeled anti-human HLA class I Abs (BD Biosciences). AAPCs were maintained in DME (Invitrogen) supplemented with 10% heat-inactivated defined calf serum (DCS; HyClone).

To enhance and stabilize expression of certain HLA alleles, specifically HLA-A*2402 and B*0801, the SFG vectors encoding the HLA sequences were modified by inserting the Kosak sequence GCCGCCACC immediately before the AUG initiation codon of the HLA gene (28).

The cDNA sequence of CMVpp65 was provided by Dr. N. Cereb (Histogenetics and the Center for Genetic Polymorphism, Hawthorne, NY). This gene, linked via an internal ribosomal entry site to puromycin N-acetyltransferase, was then cloned into a SFG vector and then used to transduce aliquots of AAPCclassI, expressing each HLA allele. Cells coexpressing CMVpp65 were then selected by propagation in medium containing puromycin dihydrochloride (10 μg/ml; Sigma-Aldrich). These cells will be referred to as AAPCclassI+pp65.

Cytokine-activated monocytes (CAMs). PBMCs were isolated from whole blood using Ficoll-Hypaque gradient separation (Accurate Chemical & Scientific) and suspended in IMDM with 10% human AB serum at a concentration of 10 × 10⁶/ml. Aliquots of 2 ml/well were then plated in a 6-well tissue culture plate (Corning) to facilitate adherence of monocytes. After 1 h, nonadherent nucleated cell were removed and adherent monocytes cultured with 2 ml of serum-free IMDM per well containing 2000 IU (50 μl of GM-CSF (Immunex,) and 700 U (25 μl) of IL-4 (R&D Systems). On days 2 and 4, 2000 U of GM-CSF (50 μl) and 700 U of IL-4 (25 μl) were again added to each 2-ml culture. On day 5, TNF-α (Sigma-Aldrich) was added to achieve a final concentration of 5 ng/ml, IL-1β to 5 ng/ml, IL-6 (R&D Systems) to 150 ng/ml, and PGES (Calbiochem) to 1 μg/ml to induce final maturation of the CAMs. On day 7, the mature CAMs were harvested, characterized as to the expression of HLA class II, CD14, and costimulatory molecules by FACS immunofluorometry, counted, aliquoted, and used for sensitization of T cell lines as detailed below.

EBV-BLCLs. A panel of EBV-BLCLs of defined HLA types was generated as previously described (29, 30). The cells were maintained in RPMI 1640 (Invitrogen) supplemented with 10% FCS, l-glutamine, penicillin, and streptomycin.

CMVpp65 peptides

T cells were sensitized using APCs loaded with a pool of 138 overlapping pentadecapeptides spanning the sequence of CMVpp65 as described previously (31). Subpools of these peptides or single peptides were used to identify epitopes of CMVpp65 eliciting T cell responses observed using an epitope-mapping strategy previously described (31, 32). Each of the 138 pentadecapeptides was synthesized according to specifications of validated sequence, purity, sterility, and absence of endotoxin (Natural and Medical Sciences Institute, Tubingen, Germany).

Peptide loading of APCs (AAPCs and CAMs)

Aliquots of 0.5 × 10⁶ AAPCclassI were allowed to adhere in 6-well plates for 6–8 h in 1 ml of serum-free DMEM per well and pulsed with the pool of synthetic overlapping CMVpp65 pentadecapeptides as described previously (31). In brief, 2.5 μg of each nonamer peptide (Research Genetics) or single pentadecapeptide or a total of 24 μg of pooled pentadecapeptides (2 μg of each pentadecapeptide in the subpools and 0.18 μg of each pentadecapeptide in the complete pool) was added to 1 × 10⁶ APCs in 1 ml of serum-free IMDM for 3 h at room temperature. An additional 2 ml of IMDM was then added and the entire peptide-containing supernatant was removed. The CMVpp65 peptide PL AAPCclassI were then cultured in 2 ml of AIM V medium (Invitrogen) with 5% DCS and irradiated to 1500 Gy before addition of T cells for sensitization.

Aliquots of 1 × 10⁶ autologous CAMs were suspended in 1 ml of serum-free IMDM in 15-ml tubes and pulsed with the CMVpp65 peptide
pool (referred to as PL CAMs) using the same approach as described above.

Generation of CMV-specific T cells

Following separation of PBMCs from whole blood (Ficoll-Hypaque), T cells were enriched by depletion of CD19+, CD14+, and CD56+ cells, RBC, granulocytes, and DCs, using mAb-coated immunomagnetic beads (Pan T-Cell Isolation Kit II; Miltenyi Biotec) as previously described (29–31). Aliquots of these T cells were then sensitized by coculture with one of the following set of APCs: 1) AAPCclass I-pp65, 2) CMVpp65 peptide PL AAPCclass I (PL AAPCclass I), 3) CMVpp65 peptide PL CAMs (PL CAMs), 4) AAPCclass I-pp65 alone, or 5) autologous CAMs alone. Sensitization using APCs. In brief, 0.5 × 106 cells were cultured with 5 × 105 T cells in a 20-ml volume of IMDM supplemented with 10% heat-inactivated human AB serum (Gemini Bio-Products) in 25-cm² flasks for 7 days. T cells were restimulated with autologous PL CAMs at an effector:stimulator ratio of 10:1. T cell cultures were supplemented with IL-2 (20 U/ml) starting on day 10 and then three times per week.

Quantitation of peptide-specific CD8+ T cells by tetrramer analysis

Tetramer analysis was performed on days 0, 7, 14, and 21 for all T cell lines using commercially available CMVpp65 MHC-peptide tetramers for HLA-A*0201, A*2402, and B*0801-bearing peptide sequences NLVPVATV, QYDVPVAAFL, and TPRVTGGGM, respectively (Beckman Coulter). Analyses performed on day 21 were used for comparisons between T cells sensitized using the different APCs. T cells were incubated with CD3 FITC-, CD8 PE-, CD4 PerCP (BD Biosciences), and an allophycocyanin-conjugated tetramer complex for 20 min on ice. The stained cells were washed and subsequently analyzed by FACS using a FACS-Calibur flow cytometer with dual laser for four-color capability. Data were analyzed using FlowJo software (Tree Star). T cells were gated on CD3- and CD8-positive cells to determine the percentage of tetramer-positive CD8+ T lymphocytes.

Functional characterization of Ag-specific T cells by intracellular IFN-γ production assay

T cell responses to specific peptides or subpools of CMVpp65 were quantitated by measuring the number of IFN-γ-positive T cells generated upon secondary stimulation with autologous APCs loaded with the peptides or peptide pool (PL) of interest according to the technique of Waldrop et al. (33) as modified by Koehne et al. (29). Peptide-loaded autologous PBMCs or autologous BLCL were used as APCs to stimulate the responding T cells.

Cytotoxicity of CMV-specific CTLs by in vitro cytotoxicity assays

All T cell lines were assessed for their capacity to lyse CMVpp65-loaded targets using a standard 51Cr release assay as previously described (29, 30). Targets used in all experiments consisted of a panel of EBV-BLCL, each sharing with T cells of a given donor a single HLA allele. These cells were pulsed, as specified for a given experiment, with the complete pool of CMVpp65 peptides or specific subpools thereof, single pentadecapeptides, or a CMVpp65 nonamer known to be presented by that allele (e.g., NLVP MVATV for HLA-A*0201, QYDVPVAAFL for HLA-A*2402, and TPRVT GGGM and RPHERNPFVT for HLA-B*0702) (31). Targets pulsed with other CMVpp65 peptides not presented by the shared HLA allele were used as controls. Absence of EBV reactivity was ascertained by lack of cytotoxicity against BLCL lines without peptide. HLA restriction was identified by cytotoxicity against targets pulsed with an identified peptide epitope presented on a specific shared HLA allele and the absence of reactivity against peptide loaded on either EBV BLCL bearing other shared alleles or fully mismatched EBV BLCL.

Characterization of CMV-specific T cells by quantitative analysis of TCRVβ repertoire

CMV peptide-HLA tetramer+ T cells were analyzed for the TCRVβ repertoire via flow cytometry using a commercially available kit containing Abs to 24 subfamilies of the Vβ region of the human TCR (Io Test β Mark; Beckman Coulter) according to procedures provided by the manufacturer (34).

Results

Construction and characterization of the panel of AAPCs

Sequential transduction and sorting of the NIH 3T3 cells with SFG vectors directing the constitutive expression of the human costimulatory molecules B7.1, ICAM-1, and LFA-3 as well as β2-microglobulin permitted the selection of a transfused line, termed 3T3-4, which exhibits stable, high expression of each of the introduced costimulatory molecules as well as β2-microglobulin (supplemental Fig. 1A). The level of expression of these transfused genes has been sustained for >2 years of reculturing.

Transduction of aliquots of 3T3-4 cells with SFG vectors encoding specific HLA class I H chains also permitted selection and cloning of AAPCs expressing each of the single HLA alleles introduced. For AAPCs selectively expressing HLA-A*0201, A*0301, B*0702, or C*0401, the level of HLA allele detected on the surface of the AAPCs, as assessed by FACS analysis with a FITC-labeled HLA class I-specific mAb, has remained stable for periods of culture exceeding 5 mo (supplemental Fig 1B). However, even when the AAPCs transduced with vectors encoding HLA-A*2402 or B*0801 were cloned for high expression of HLA, sequential analyses of these AAPCs over an additional 4 wk of culture demonstrated progressive reductions in the proportion of cells expressing these HLA alleles and in the level of each HLA expressed. In contrast, expression of β2-microglobulin by these AAPCs was sustained.

To enhance and potentially stabilize expression of HLA-A*2402 and B*0801 on transduced AAPCs, we modified the SFG vectors by inserting an initiation sequence GCCGCCACC described by Kozak (28, 35) into the 5′ end of the leader sequence of the gene encoding each of these HLA alleles. These modified vectors were then transduced into 3T3-4 cells. Thereafter, cells expressing each allele were isolated by FACS sorting and compared with 3T3-4 cells transduced with the unmodified vector for expression of the transduced HLA allele over extended periods of culture. AAPCs transduced with vectors encoding HLA-A*2402 or B*0801 lacking the Kozak sequence exhibited progressive reductions in both the number of cells expressing the vector-encoded HLA allele and the level of HLA expressed by the transduced cells (supplemental Fig. 2, A and B). AAPCs transduced with vectors including the Kozak sequence exhibited higher initial expression of the transduced HLA allele. In the AAPCs transduced to express HLA-B*0801, this high expression was sustained through the 4 wk of observation (supplemental Fig. 2B). In the HLA-A*2402-transduced AAPCs, expression of the allele decreased slightly in the first week after transduction, but was sustained thereafter (supplemental Fig. 2A).

We compared the level of HLA and costimulatory molecules expressed on the AAPCs with that expressed by T cell donor-derived autologous CAMs and EBV-BLCLs. Following maturation in vitro, the CAMs used in our studies strongly expressed both HLA class I and class II, CD40, CD86, LFA-3, and ICAM-1. They were also CD14−, but CD83+. As such, they exhibited the phenotype of monocyte-derived dendritic cells. As shown in a representative sample in Fig. 1, the AAPCs exhibited higher expression

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of B7.1, LFA-3, and ICAM-1 than CAMs and comparable expression of HLA class I.

Following establishment of this panel of AAPC<sub>class I</sub>, aliquots of cells expressing each HLA allele were further transduced with a SFG bicistronic vector encoding the full-length sequence of CMVpp65 and puromycin-N-acetyltransferase. After selection in medium containing puromycin, expression of CMVpp65 by AAPC<sub>class I</sub> was found in >90% of the cells by indirect immunofluorescence staining (supplemental Fig. 3).

AAPCs induce expansion of Ag-specific T lymphocytes that bind epitope-bearing tetramers, generate IFN-γ, and exhibit HLA-restricted Ag-specific cytotoxicity

We next comparatively assessed each AAPC for its capacity to sensitize human T lymphocytes either as AAPC<sub>class I</sub> pulsed with the CMVpp65 peptide pool (PL AAPC<sub>class I</sub>) or as AAPC<sub>class I</sub> (1-pp65). Accordingly, T cells from groups of up to eight CMV-seropositive donors, sharing a HLA allele expressed by a given AAPC, were sensitized with either PL AAPC<sub>class I</sub> or AAPC<sub>class I</sub> (1-pp65) for 21 days in vitro, and then assessed for the number of CMV peptide-specific T cells generated.

For T cells sensitized with AAPCs expressing HLA-A*0201, A*2402, and B*0702, we initially measured the number and proportion of T cells binding HLA peptide tetramers. One representative example of the increases in tetramer<sup>+</sup> T cells is presented for each of these alleles in Fig. 2A.

Quantitations of tetramer<sup>+</sup> T cells generated after sensitization with PL CAMs, PL AAPC<sub>class I</sub> sharing single HLA alleles with the donor T cell, as well as responses to AAPC<sub>class I</sub> (1-pp65) bearing the same HLA allele are shown in Fig. 2, B and C. Cultures sensitized with PL AAPC<sub>class I</sub> exhibited 50- to 200-fold increases in T cells binding tetramers containing known immunogenic epitopes presented by the HLA alleles expressed by the sensitizing AAPCs. These responses were similar to those elicited in response to the same epitopes when the T cells were sensitized with autologous PL CAMs. Strikingly, when the same T cells were sensitized with AAPC<sub>class I</sub> (1-pp65), they generated a 500- to 1000-fold increase in T cells binding the same tetramers. As shown in Fig. 2C, the absolute numbers of peptide-specific T cells generated in response to AAPC<sub>class I</sub> (1-pp65) were higher for each allele tested. For the group of HLA-A*0201<sup>+</sup> donors, this increase was statistically significant (p < 0.03).

As also shown in Fig. 2C, the absolute yields of tetramer<sup>+</sup> T cells generated in response to AAPC<sub>class I</sub> and AAPC<sub>class I</sub> (1-pp65) expressing HLA-A*0201 tended to be higher than those generated against AAPCs expressing either HLA-A*2402 or B*0702.

We also analyzed the phenotype of the tetramer<sup>+</sup> T cells at sequential times in their generation. As shown in one representative culture presented in Fig. 3A, tetramer<sup>+</sup> T cells were CD<sup>+</sup> and predominantly of a central memory phenotype at day 7 of culture. By day 10, almost half of the cells exhibited an effector memory phenotype. At days 17 and 24, the tetramer<sup>+</sup> cells were almost exclusively of an effector memory phenotype.

We also compared the TCR repertoire of T cells generated against epitopes presented by PL CAMs vs AAPC<sub>class I</sub> (1-pp65) by examining the Vβ of tetramer<sup>+</sup> T cells. Fig. 3B presents results from one representative donor and demonstrates that the Vβ chains represented in the TCRs of T cells sensitized with HLA-A*0201 AAPC<sub>class I</sub> (1-pp65) were the same as those detected among T cells sensitized with autologous PL CAMs.

When analyzed for T cells generating intracellular IFN-γ in response to secondary stimulation with PL autologous donor PBMCs, the PL AAPC<sub>class I</sub> and AAPC<sub>class I</sub> (1-pp65) expressing HLA-A*0201, A*2402, B*0702, B*0801, and C*0401 each elicited significant numbers of IFN-γ<sup>+</sup> CMVpp65-specific T cells (Fig. 4A). Again, absolute yields of CMVpp65-specific IFN-γ<sup>+</sup> T cells from cultures sensitized with the AAPC<sub>class I</sub> (1-pp65) were consistently greater than those generated from T cell cultures sensitized with PL AAPC<sub>class I</sub>. The absolute numbers of IFN-γ<sup>+</sup> T cells generated in response to any one of these AAPC<sub>class I</sub> (1-pp65) expressing a HLA allele shared by the donor were similar to the total numbers of IFN-γ<sup>+</sup> T cells generated after sensitization with PL autologous CAMs.

T cells sensitized with each of these PL AAPC<sub>class I</sub> and AAPC<sub>class I</sub> (1-pp65) also exhibited significant cytolytic activity against PL EBV-BLCL but did not lyse the same EBV-BLCL without peptide.

We also compared T cell cytotoxic activity against PL EBV-BLCLs matched at the HLA allele shared by the donor and sensitizing AAPCs. In these comparative assays, as shown in Fig. 4B, T cells sensitized with PL AAPC<sub>class I</sub> and AAPC<sub>class I</sub> (1-pp65) exhibited equivalent cytotoxic activity against these targets, but did not lyse unloaded HLA-sharing EBV-BLCLs or peptide-loaded targets lacking the restricting HLA allele. When T cells from the same donor were sensitized with PL CAMs and tested against PL EBV-BLCLs sharing single HLA alleles, cytotoxic responses were detected against PL EBV-BLCLs sharing A*0201, A*2402, and B*0702 but not PL EBV-BLCLs sharing C*0401 or B*0801, reflecting the fact that responses restricted by these alleles were subdominant in each of the donors studied.

We then examined to what degree the proportion of CMVpp65-specific IFN-γ<sup>+</sup>CD8<sup>+</sup> cells (TC) generated using PL CAMs or AAPC<sub>class I</sub> (1-pp65) was correlated with the level of in vitro cytotoxicity exhibited by the sensitized T cells against peptide PL autologous BLCLs. As shown in Fig. 4C, the percent cytotoxicity was significantly correlated with the proportion of IFN-γ<sup>+</sup>CD8<sup>+</sup> T cells for T cells sensitized with AAPC<sub>class I</sub> (1-pp65).
(r = 0.53, p < 0.001) as well as for T cells sensitized using PL CAMs (r = 0.44, p = 0.01). Among the T cells sensitized with PL CAMs, we usually observed T cells reactive against more than one epitope of CMVpp65. Although such epitopes might elicit IFN-γ responses, their relative capacity to lyse peptide-loaded targets may differ (23), potentially reflecting competition between cytotoxic T cells to different epitopes presented on the cell surface. In contrast, T cells sensitized with PL or transduced AAPCs are directed against specific targets. As a result, correlation between IFN-γ T cells and cytotoxicity in circumstances wherein there is target excess would be expected to be high.

FIGURE 2. Comparative HLA tetramer analysis of T cells sensitized using CMVpp65 peptide PL AAPC\textsuperscript{class I} vs AAPC\textsuperscript{class I-pp65}. A, Tetramer analysis (FACS) performed at inception (day 0) and after 21 days of sensitization is shown for a single donor from each of the three groups for which HLA CMVpp65 peptide tetramers were available (HLA-A*0201, A*2402, and B*0702). x-axis = CD8 and y-axis = CMV tetramer. Significant expansion of CMV peptide-specific T cells was seen in all cultures, while AAPC\textsuperscript{class I-pp65} induced comparable or higher proportions of tetramer-positive CD8$^+$ T cells (20% vs 22% for HLA-B*0702 donor, 6.5% vs 4% for HLA-A*2402 donor, and 91% vs 67% for HLA-A*0201 donor). B, Degree of expansion and C, absolute numbers of CD8$^+$ tetramer-positive T cells generated using autologous CMVpp65 peptide-loaded (PL) CAM and AAPC\textsuperscript{class I} \((\text{C})\) were closely correlated and were significantly lower than the number of tetramer-positive T cells generated using AAPC\textsuperscript{class I-pp65} \((\text{F})\) \((p < 0.01 \text{ for HLA-A*0201 donors})\).
FIGURE 3. Phenotype of tetramer-positive CMV-specific T cells. A, T cells from a donor bearing HLA-A*0201 were analyzed at different time points during sensitization using either PL AAPC*0201 or AAPC*0201pp65 to characterize the memory TC phenotype. CD8+ NLV-Tet+ T cells shown (x-axis = CD45 RA and y-axis = CD62L). By days 17–24, all tetramer-positive T cells sensitized using either PL AAPC or AAPC*0201pp65 were effector memory in phenotype (negative for CD45RA and CD62L).

B, TCR Vβ repertoire is shown for tetramer-positive T cells from a HLA-A*0201 donor. Each bar graph represents the percent CD8+ tetramer+ T cells in each of the 24 TCR Vβ subfamilies. T cells sensitized with both PL CAMs (upper graph) and HLA-A*0201+ AAPC*pp65 (lower graph) demonstrate predominant usage of Vβ 14 subfamily.
T cells from three HLA-A*0301 donors, when sensitized with either PL HLA-A*0301 AAPC class I or HLA-A*0301/H11001 AAPC class I-pp65, did not generate CMVpp65-specific T cells. When T cells from these donors were sensitized with PL autologous CAMs, they each generated CMVpp65-specific IFN-γ/H9253/H11001 and cytotoxic CD8/H11001 T cells. However, no HLA-A*0301-restricted CMVpp65-specific T cells were detected (data not shown).

Epitope mapping of T cells generated in response to CMVpp65 peptide-loaded and transduced AAPCs

Our studies of T cells generated in response to AAPCclass I-pp65 expressing HLA-A*0201, B*0701, or A*2402 suggested that these transduced AAPCs elicited responses to immunogenic epitopes known to be presented by human APCs expressing these alleles as reflected by the generation of high numbers of T cells binding HLA peptide tetramers bearing such epitopes. However, we wished to further examine whether these transduced AAPCs processed and presented other CMVpp65 epitopes known to be immunogenic in humans and also whether they presented other epitopes not normally presented by human APCs. Accordingly, we mapped the epitopes recognized by T cells sensitized with PL autologous CAMs or AAPCs expressing single HLA alleles. A, CMV-specific TC generated using either CAMs or AAPCs, were quantitated by an intracellular IFN-γ assay (PL CAMs or AAPCclass I (○). AAPCclass I-pp65 (●), CAMs or AAPCs without peptide (□)). Sensitization with AAPCclass I-pp65, expressing each HLA allele, resulted in higher yields of IFN-γ+ T cells compared with sensitization using PL AAPCclass I for all donors (p = 0.03 for HLA-A*0201 donors). B, Cytotoxicity against PL single HLA-sharing EBV-BLCL lines is shown after 21 days of sensitization for all cultures (E:T = 20:1). CAMs or AAPCs without peptide, □. PL CAMs or AAPCclass I – ○ and AAPCclass I-pp65, ●. PL HLA-mismatched BLCLs, ■. PL AAPCclass I or AAPCclass I-pp65-induced T cells with comparable cytotoxicity to T cells sensitized with PL CAMs when tested against EBV-BLCLs sharing the immunodominant HLA allele (A*0201, B*0702, or A*2402). Cytotoxicity against subdominant epitopes presented by C*0401 or B*0801 was higher and significant for T cells sensitized with AAPCs in comparison to TC sensitized with PL CAMs, which show minimal lysis of these targets that falls within the range of controls. C, The proportion of CD8 IFN-γ+ T cells generated using AAPCclass I-pp65 was significantly correlated with the in vitro cytotoxicity against PL autologous EBV-BLCLs for the respective T cell lines (r = 0.53, p < 0.001). The percent cytotoxicity for T cell lines sensitized with PL CAMs against PL autologous EBV-BLCLs was also correlated with the percentage of IFN-γ-producing T cells, although to a lesser degree (r = 0.44, p = 0.01).
FIGURE 5. Mapping of epitopes of CMVpp65 eliciting IFN-γ CD8⁺ T cell response after sensitization with peptide PL autologous CAMs, PL AAPC<sup>class I</sup>-pp65, or AAPC<sup>class I</sup>-pp65. IFN-γ CD8⁺ T cells generated in response to individual subpools of CMVpp65 peptides in an epitope mapping matrix (x-axis) used to identify CMV/65 epitopes. T cells were analyzed after sensitization with (left to right) PL CAMs or with AAPC<sup>class I</sup>-pp65 or PL AAPC<sup>class I</sup> expressing one HLA allele shared by the donor (y-axis = percent IFN-γ CD8⁺ T cells; first left bar, PBMCs without peptide). HLA genotype of each donor is shown above each set. A, CD8⁺ IFN-γ⁺ T cells generated using PL CAMs from a donor bearing both HLA-A*0201 and A*2402. were responsive to pools 3 and 23 which share NLVPMVATV, a HLA-A*0201 epitope but minimally responsive to pools 1, 2, and 20 (QYDPVAALF), an A*2402 epitope. PL AAPCA*2402 and AAPCA*2402 pp65 induced IFN-γ⁺ CD8⁺ T cells responses to pools 1, 2, and 20 as well as QYDPVAALF but not to pools 3 and 23 or the NLVPMVATV nonamer. B, PL CAM-sensitized T cells were responsive to pools 6, 7, and 18 containing RPHERNGFTV, a known HLA-B*0702 epitope; AAPCB*0702pp65 induced responses to pools 9 and 21 containing TPRVTGGGAM, another known HLA-B*0702 epitope; AAPCB*0702 induced IFN-γ⁺ T cells against pools 6, 7, 9, and 21 (RPHERNGFTV) and pools 7 and 18 (TPRVTGGGAM), both known epitopes of B*0702. C, PL CAM-sensitized T cells were responsive to pools 4 and 15 containing the peptide MSIYYVYALPLKMLNI, presented by HLA-A*6801. PL AAPCC*0401 and AAPCC*0401pp65 induced IFN-γ⁺ CD8⁺ T cells against pools 1, 2, and 20 (QYDPVAALF), an epitope predicted by HLA-C*0401. D, PL CAM-sensitized T cells were responsive to pools 3, 4, and 13 containing GPISGHVLK, a peptide presented by HLA-A*1101. PL AAPCB*0801 or AAPCB*0801pp65 induced IFN-γ⁺ CD8⁺ T cells against pools 3, 4, and 18 containing LTMTRNPQP, an epitope predicted to be presented by HLA-B*0801in peptides 63 and 64 and 3 and 23 containing NLVPMVATV.
T cells reacting against the same peptides as those presented by PL AAPCclass I and AAPCclass I-pp65 were also detected among T cells sensitized with PL CAMs with three exceptions: 1) T cells from donor C, sharing HLA-C*0401, when sensitized with PL CAMs, selectively responded to a peptide, VYALPLKML, that elicited responses in T cells sensitized with PL HLA-C*0401+ AAPCclass I and HLA-C*0401+ AAPCclass I-pp65 (Fig. 5C). 2) T cells from donor B, sharing HLA-B*0702, responded to RIPHERNGFTV when sensitized with either PL CAMs or HLA-B*0702+ AAPCclass I, but only responded to TPRVTGGGAM when sensitized with HLA-B*0702+ AAPCclass I-pp65 (Fig. 5B). 3) T cells from another donor (Fig. 5D), whose genotype includes HLA-B*0801 as well as HLA-A*1101, responded exclusively to pools 3, 4, and 13 that contain pentadecapeptides 3 and 4 which share the GPSGHVLK peptide known to be immunogenic when presented by the HLA-A*1101 expressed by the donor’s own CAMs, while the same donor’s T cells sensitized with PL HLA-B*0801+ AAPCclass I or HLA-B*0801+ AAPCclass I-pp65 responded to two peptides, LTMTRNPQPF and LARNLVPMV, contained in peptides 63/64 and peptide 123, respectively. The responses to the same epitope were also observed in T cells stimulated with PL autologous CAMs and HLA-C*0401+ AAPCs.

To ascertain whether we could generate Ag-specific T cell responses to subdominant epitopes of CMVpp65 using this panel of AAPCs, we compared T cell responses from selected donors with sensitization with PL CAMs to those of T cells sensitized with PL AAPCclass I or AAPCclass I-pp65 expressing different class I alleles expressed by the same donor.

As shown in Fig. 6A, when T cells from a donor coexpressing HLA-B*0702 and HLA-A*0201 were sensitized with PL CAMs, a large population of tetramer+ T cells specific for the CMVpp65 epitope TPRVTGGGAM presented by HLA-B*0702 were generated, as well as a significant (14%) population of T cells binding HLA-A*0201 tetramers containing the nonameric NLVPMVATV. Functional analysis and epitope mapping revealed that these T cells selectively generated IFN-γ in response to two epitopes presented by HLA-B*0702, the TPRVTGGGAM peptide noted above and RIPHERNGFTV; only a small number of IFN-γ+ T cells were generated in response to the NLVPMVATV presented by HLA-A*0201 (Fig. 6B). Furthermore, these T cells lysed HLA-B*0702+ EBV-BLCLs loaded with these epitopes but failed to lyse HLA-A*0201+ EBV-BLCLs loaded with the NLV peptide (Fig. 6C). In contrast, when the same donor’s T cells were sensitized with HLA-A*0201+ AAPCclass I-pp65, T cells restricted by HLA-A*0201 and specific for the NLVPMVATV peptide were generated, as demonstrated by tetramer analysis (Fig. 6A), generation of IFN-γ+ T cells in response to specific peptide containing subpools and targeted nonamers (Fig. 6B), and the capacity of these sensitized T cells to lyse HLA-A*0201+ human targets loaded with this peptide (Fig. 6C).

Table II summarizes a comparison of the responses of T cells from seven donors sensitized with PL CAMs with those of T cells sensitized with PL AAPCclass I and AAPCclass I-pp65 from the same donor, expressing a different HLA allele expressed by the donor. In all donors tested, sensitization with PL CAMs selectively induced T cells specific for one to two immunodominant CMVpp65 epitopes. Although sensitization with peptide-loaded or transduced AAPCs expressing the dominant presenting HLA alleles regularly elicited responses to the same dominant epitopes, we could also generate comparable cytotoxic T cell responses to subdominant epitopes which were either not produced or only present at low frequencies in T cells sensitized with PL CAMs (Table II).

### Discussion

We here describe a panel of murine 3T3 cell-based AAPCs, each expressing human ICAM-1, B7.1, and LFA-3 as well as β2-microglobulin and a single HLA class I H chain: HLA-A*0201, A*0301, A*2402, B*0702, B*0801, or C*0401. The potential utility of this panel is suggested by the fact that 78% of the 168 patients at our center received a HLA nonidentical HSCT between 2001 and 2005 because we could not identify a HLA-matched unrelated donor from the National Marrow Donor Program registry who inherited one or more of these HLA alleles. Planned expansion of this panel to include AAPCs expressing HLA-A*0101, A*1101, and B*4402 will cover >90% of patients for whom virus-specific T cells restricted by a HLA allele expressed by one of the AAPCs in the panel can be generated.

We chose to construct this panel of AAPCs from a murine 3T3 cell line rather than a human cell line such as the human leukemia K562 cell, which has been proposed by other groups (17, 21), primarily because of concerns that K 562 and other human cells deficient in their expression of HLA could process and present peptides of CMVpp65 to various human HLA class I alleles.

### Table I. T cell responses to known epitopes of CMVpp65

<table>
<thead>
<tr>
<th>Epitope</th>
<th>HLA</th>
<th>Donors with Shared HLA Tested</th>
<th>No. Responding to Given Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLVPMVATV</td>
<td>A*0201</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>QYDPCAALF</td>
<td>A*2402</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>RIPHERNGFTV</td>
<td>B*0702</td>
<td>4</td>
<td>1*</td>
</tr>
<tr>
<td>TPRVTGGGAM</td>
<td>B*0702</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>QYDPCAALF</td>
<td>C*0401</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* Two donors with HLA-A*2402 responded to other epitopes, QAYERTEVEL and QEPFWDAND. The responses to these two epitopes were observed both in T cells stimulated with autologous PL CAMs and PL HLA-A*2402+ AAPCs. These two epitopes are newly identified epitopes presented by this HLA.

* Only one of the four donors tested with HLA-B*0702 responded to the known B*0702 epitope RIPHERNGFTV, while all four demonstrated responses to the other known epitope TPRVTGGGAM.

* One donor with HLA-C*0401 responded to another epitope, KDVALRHVV. The responses to the same epitope were also observed in T cells stimulated with PL autologous CAMs and HLA-C*0401+ AAPCs.

** AAPCs are capable of generating Ag-specific responses against subdominant epitopes of CMVpp65**

To ascertain whether we could generate Ag-specific T cell responses to subdominant epitopes of CMVpp65 using this panel of AAPCs, we compared T cell responses from selected donors with sensitization with PL CAMs to those of T cells sensitized with PL AAPCclass I or AAPCclass I-pp65 expressing different class I alleles expressed by the same donor.

As shown in Fig. 6A, when T cells from a donor coexpressing HLA-B*0702 and HLA-A*0201 were sensitized with PL CAMs, a large population of tetramer+ T cells specific for the CMVpp65 epitope TPRVTGGGAM presented by HLA-B*0702 were generated, as well as a significant (14%) population of T cells binding HLA-A*0201 tetramers containing the nonameric NLVPMVATV. Functional analysis and epitope mapping revealed that these T cells selectively generated IFN-γ in response to two epitopes presented by HLA-B*0702, the TPRVTGGGAM peptide noted above and RIPHERNGFTV; only a small number of IFN-γ+ T cells were generated in response to the NLVPMVATV presented by HLA-A*0201 (Fig. 6B). Furthermore, these T cells lysed HLA-B*0702+ EBV-BLCLs loaded with these epitopes but failed to lyse HLA-A*0201+ EBV-BLCLs loaded with the NLV peptide (Fig. 6C). In contrast, when the same donor’s T cells were sensitized with HLA-A*0201+ AAPCclass I-pp65, T cells restricted by HLA-A*0201 and specific for the NLVPMVATV peptide were generated, as demonstrated by tetramer analysis (Fig. 6A), generation of IFN-γ+ T cells in response to specific peptide containing subpools and targeted nonamers (Fig. 6B), and the capacity of these sensitized T cells to lyse HLA-A*0201+ human targets loaded with this peptide (Fig. 6C).

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### Discussion

We here describe a panel of murine 3T3 cell-based AAPCs, each expressing human ICAM-1, B7.1, and LFA-3 as well as β2-microglobulin and a single HLA class I H chain: HLA-A*0201, A*0301, A*2402, B*0702, B*0801, or C*0401. The potential utility of this panel is suggested by the fact that 78% of the 168 patients at our center received a HLA nonidentical HSCT between 2001 and 2005 because we could not identify a HLA-matched unrelated donor from the National Marrow Donor Program registry who inherited one or more of these HLA alleles. Planned expansion of this panel to include AAPCs expressing HLA-A*0101, A*1101, and B*4402 will cover >90% of patients for whom virus-specific T cells restricted by a HLA allele expressed by one of the AAPCs in the panel can be generated.

We chose to construct this panel of AAPCs from a murine 3T3 cell line rather than a human cell line such as the human leukemia K562 cell, which has been proposed by other groups (17, 21), primarily because of concerns that K 562 and other human cells deficient in their expression of HLA could process and present...
FIGURE 6. AAPCs can be used to generate CMV-specific TC of desired HLA restriction. A, CMV-specific TC frequency from a donor coexpressing HLA-A*0201 and B*0702 at the inception of the TC culture (day 0) demonstrated 0.3% HLA-B*0702 tetramer$^+$CD8$^+$ TC only. The dominant T cell response induced by PL CAMs was to a peptide, TPRVTGGGAM, presented by B*0702 (35% B7 tetramer$^+$CD8$^+$ TC), with a subdominant response to the HLA-A*0201 presented peptide NLVPMVATV (14% A2 tetramer$^+$CD8$^+$ TC). AAPCB*0702pp65 stimulated TC contained only B*0702 tetramer$^+$CD8$^+$ TC (20%) and no A*0201 tetramer$^+$ TC. In contrast, AAPCA*0201pp65 induced only A*0201 tetramer$^+$ TC (7%) and no B*0702 tetramer$^+$ TC. B, IFN-$\gamma$ CD8$^+$ TC generated using (left) PL CAMs were responsive to pools 6, 7, and 18 (RPHERNGFTV), a B7 epitope, and pools 8, 9, and 21 (TPRVTGGGAM), also a B7 epitope, with minimal response to pools 3 and 23 (NLVPMVATV), an A2 epitope. (middle) AAPCB*0702pp65 were responsive only to pools 6, 7, and 18 (B7-RPHERNGFTV) and pools 9 and 21 (B7-TPRVTGGGAM), but not to pools containing A2 epitopes (pools 3 and 23) and (right) AAPCA*0201pp65 generated responses to pools 3 and 23 (A2-NLVPMVATV), and not to pools containing B7 epitopes (pools 6–9, 18, and 21). C, T cells sensitized using (left) PL CAMs lysed only B*0702-matched peptide-loaded targets, (middle) AAPCB*0702pp65 lysed only against B*0702-matched targets and not against HLA-A*0201-matched targets, and (right) AAPCA*0201pp65 lysed only HLA-A*0201-matched targets and not HLA-B*0702-matched targets.
minor alloantigens in the context of a transduced HLA allele that might stimulate donor T cells capable of inducing graft-versus-host disease following adoptive transfer. We had additional concerns regarding the use of the K 562 cells because they express the human MHC class I chain-related genes MICA and MICB, which, on the one hand, can be a significant alloantigen (38–40) and, on the other, can release soluble MICA and MICB, which, by downregulating surface expression of NKG2D on CD8+ T cells can interfere with T cell effector functions (41). In contrast, although 3T3 cells might elicit a xenogenic response, our studies have shown that T cells generated against either peptide-pulsed or CMVpp65-transduced AAPCs generate IFN-γ+ T cells and lyse targets upon secondary stimulation only against human targets bearing CMVpp65 epitopes and the T cells’ restricting the HLA allele, no alloresponses are recorded. A similar level of allosereactivity of T cells sensitized with the AAPCs coexpressing HLA-A*0201 and telomerase has been reported (42). Similarly, 3T3 cells genetically modified to express CD40L have also been safely used to provide costimulation in trans to T cells sensitized in vitro against autologous melanoma cells for adoptive therapy (43). Furthermore, clinical trials using human epithelial cells cultured on a 3T3 cell matrix for skin transplants (44–46) or corneal repair (47) have provided evidence indicating that their capacity to elicit alloantigenic responses is low.

The NIH 3T3-based AAPCs in this panel stably express each of the transduced human cositomoligene molecules as well as β2-microglobulin. Similarly, the expression of HLA-A*0201, A*0301, and B*0702 has remained constant in cultures for >5 mo. The basis for the loss of expression of HLA-A*2402 and B*0801 in AAPCs transduced with the same vector is unclear. It is unlikely that preferential outgrowth of untransduced AAPCs was the cause, since even clones selected for high expression of these alleles exhibited this fall off. Gene silencing from selective insertion of SFG vectors at susceptible sites was also thought to be unlikely since the same instability of HLA expression was exhibited in two to three different aliquots of transduced 3T3-4s. Based on the possibility that the initiation sequences of these alleles are susceptible to inhibition of translation, the vectors were modified to include the Kozak sequence (28, 35) immediately upstream of the initiator codon of the gene encoding these HLA H chains. This sequence has been shown to optimize mRNA translation when inserted proximal to AUG start codons of genes in eukaryotic cells (28, 48, 49). This permitted generation of AAPCs with sustained high expression of each of these alleles.

Our results demonstrate that AAPCs individually expressing HLA-A*0201, A*2402, B*0702, B*0801, or C*0401 can each stimulate the generation of large populations of CMVpp65-specific HLA-restricted T cells. From a starting population of 10^5 to 10^6 unselected T cells containing ~10^6 CMVpp65 peptide-reactive T cells, sensitization with PL AAPCclass I or AAPCclass I-pp65 can generate as many as 5 × 10^6 to 1 × 10^7 epitope-specific T cells over 3 wk of culture. Such numbers can provide doses of CMVpp65-specific T cells well within the range of numbers used in ongoing trials of adoptive cell therapy for the prevention and or treatment of CMV infections (2, 3, 50).

The yields of tetramer+ CMV pp65 epitope-specific T cells generated using AAPCclass I-pp65 were consistently higher than those generated in response to PL CAMs or AAPCclass I-pp65. This may reflect the potential of living APCs like AAPCclass I-pp65 to provide more effective sensitization of T cells and superior yields of Ag-specific T cells by continuously processing and presenting immunogenic epitopes throughout in vitro culture. Indeed, a greater or equal number of peptide-specific T cells have also been generated using dendritic cells transduced to express an immunogenic CMVpp65 peptide presented by HLA-A*0201(51).

Although T cell sensitization with AAPCclass I-pp65 regularly induced higher yields of CMVpp65-specific T cells, the utility of AAPCclass I-pp65 would be limited if the epitopes of CMVpp65 presented by these mouse 3T3-derived AAPCs differ from those recognized by T cells sensitized with PL autologous CAMs or AAPCclass I-pp65. To be presented by AAPCclass I-pp65, the CMVpp65 protein must be processed by the murine ubiquitin proteosome pathway and then loaded on the expressed HLA H chain/β2-microglobulin complex by murine TAP proteins (52–55). Earlier studies suggested that the antigenic peptides presented would differ significantly due to differences between the murine and human TAP proteins (56). However, in mice transgenic for HLA-A*0201, A*1101, or B*0702, immunization with peptide epitopes of either viruses or human tumor Ags with high binding affinity for these HLA alleles, and known immunogenicity in humans, elicited murine T cell responses specific for the same peptides when presented by the transgenic human HLA alleles (57–62). These studies thus provided evidence that mouse cells could transport and present on the transgenic HLA allele the same epitopes as naturally presented.

### Table II. T cell responses to dominant/subdominant epitopes of CMVpp65 presented by autologous CAMs or AAPCs

<table>
<thead>
<tr>
<th>Donors</th>
<th>HLA</th>
<th>% CD8</th>
<th>IFN-γ</th>
<th>Cytotoxicity</th>
<th>Sensitization with AAPCs Transduced with CMVpp65</th>
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<tr>
<td>1</td>
<td>Dominant</td>
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<td>33</td>
<td>60</td>
<td>A*0201 43 70</td>
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<tr>
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<td>A*2402</td>
<td>30</td>
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<tr>
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<td>43</td>
<td>B*0702 24 66</td>
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<td>0</td>
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<td>B*0801 11 45</td>
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</table>

N/D. Not performed. HLA alleles in bold represent the alleles presenting subdominant epitopes.
on human APCs. Reports from our own group have also demonstrated that 3T3-based HLA-A*0201 AAPCs transduced to express either CMVpp65 or telomerase can elicit human T cell responses specific for epitopes normally presented by HLA-A*0201 on human cells (20, 42).

The present report confirms and significantly extends previous studies with 3T3-based AAPCs expressing HLA-A*0201 (12, 20, 42), providing evidence that AAPCs expressing several HLA class I alleles can process and present the same epitopes of CMVpp65 that would normally be presented by human APCs. Sensitization with AAPC\textsubscript{class I}-pp65 expressing HLA-A*0201, A*2402, and B*0702 elicited tetramer\textsuperscript{+} T cell responses against the same CMVpp65 epitopes that were presented by these HLA alleles on autologous CAMs. The TCRs represented in the tetramer\textsuperscript{+} T cell responses to the same CMVpp65 epitopes that were presented by these HLA alleles on autologous CAMs. Therefore, we were able to use this panel of AAPCs to consistently generate CMVpp65 IFN-\gamma CD8\textsuperscript{+} T cells that lysed human cell targets presenting subdominant epitopes of CMVpp65 presented by the restricting HLA alleles expressed by the AAPCs from each of the donors tested. This included donors whose T cells, when sensitized with PL autologous CAMs, failed to respond to the epitopes presented by the AAPCs.

Our findings suggest that this panel of AAPCs provides a standardizable, renewable, and immediately accessible “off the shelf” source of cellular reagents to generate CMVpp65 peptide-specific IFN-\gamma- and cytotoxic CD8\textsuperscript{+} T cells of desired HLA restriction in numbers required for adoptive cell therapy. These AAPCs may be particularly useful for generating donor T cells restricted by HLA alleles shared by donor and host for use in HLA-disparate transplant recipients whose risk of mortality due to CMV infection remains as high as 15% despite the use of antiviral drugs (73). They may also permit early selection and expansion of HLA-restricted long-lived central memory T cells specific for a given epitope which may confer more sustained T cell-mediated resistance after adoptive transfer (74). As a research tool, these cells may also permit rapid identification of natural and heteroclitic HLA-binding epitopes that elicit viricidal T cell responses and the cloning of T cells of desired epitope specificity and HLA restriction.

Acknowledgments
We thank Lorna Barnett and Anam Khan for technical assistance, and both Dr. Ekaterina Doubrovin and Dr. Gloria Koo for technical advice and critical review.

Disclosures
The authors have no financial conflict of interest.

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