RESEARCH ARTICLE

IMMUNOTHERAPY

CMV-specific T cells generated from naïve T cells recognize atypical epitopes and may be protective in vivo

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Adoptive transfer of cytomegalovirus (CMV)–specific T cells derived from adult seropositive donors can effectively restore antiviral immunity after transplantation. However, CMV-seronegative donors lack CMV-specific memory T cells, which restricts the availability of virus-specific T cells for immunoprophylaxis. We demonstrate the feasibility of deriving CMV-specific T cells from naïve cells for T cell therapy. Naïve T cells primed to recognize CMV were restricted to different, atypical epitopes than T cells derived from CMV-seropositive individuals; however, these two cell populations had similar avidities. CMV-seropositive individuals also had T cells recognizing these atypical epitopes, but these cells had a lower avidity than those derived from the seronegative subjects, which suggests that high-avidity T cells to these epitopes may be lost over time. Indeed, recipients of cord blood (CB) grafts who did not develop CMV were found by clonotypic analysis to have T cells recognizing atypical CMVpp65 epitopes. Therefore, we examined unmanipulated CB units and found that T cells with T cell receptors restricted by atypical epitopes were the most common, which may explain why these T cells expanded. When infused to recipients, naïve donor–derived virus-specific T cells that recognized atypical epitopes were associated with prolonged periods of CMV-free survival and complete remission. These data suggest that naïve-derived T cells from seronegative patients may be an additional source of cells for CMV immunoprophylaxis.

INTRODUCTION

Adoptive immunotherapy is emerging as an attractive alternative to chemotherapy for both viral infections (1–5) and relapse (6–8) developing after hematopoietic stem cell transplantation (HSCT). Most, if not all, antigen-specific T cells adoptively transferred to humans are derived from memory T cell populations (9); hence, virus-specific T cells—although largely effective—have not been available for patients undergoing HSCT from virus-naïve donor sources including cytomegalovirus (CMV)–seronegative or umbilical cord blood (CB) donors (10, 11). Although preclinical data have been reported for human antigen-specific T cells generated from naïve T cells, with the exception of our previous study from CB (12), they are mostly restricted to Epstein-Barr virus (EBV)–specific T cells or mitogen-stimulated T cells bearing exogenous T cell receptors (TCRs) (13, 14), and therefore, the naïve T cell response to CMV, including epitope usage, avidity, and polyclonality, has not been addressed.

CMV-specific T cells were first described as those able to recognize the immunodominant antigen immediate early 1 (IE1) (15), but later reports emphasized the importance of T cells that target a tegument phosphoprotein of 65 kD (pp65) (16, 17). The original epitope identification studies focused on NLVPMVATV (hereafter NLV), a human leukocyte antigen (HLA)–A2–restricted epitope within pp65 (16) that we define as a "typical" epitope because of its common detection in HLA-A2–positive donors. Use of more advanced techniques, such as overlapping peptide pools, lentiviral vectors containing a chimeric CMV-pp65/IE1 protein, and bioinformatics, has allowed the identification of other less common ("atypical") epitopes targeted by CMV-specific T cells (18–20), and in murine models, subdominant epitopes have been shown to be protective (21). It should be stressed that all of these epitopes—both typical and atypical—have been identified in memory T cells. Whether the memory T cell repertoire mirrors that of naïve T cells in vivo remains to be determined.

HIV-seronegative women who are resistant to infection recognize epitopes that differ from those recognized by HIV-seropositive women who are not protected from HIV despite a CD8+ HIV-specific T cell response (22), suggesting a disparity between the immunodominant, persisting epitopes and the initial, protective epitopes, presumably generated from naïve T cells.

We have developed a protocol enabling the activation and expansion of multivirus (CMV, EBV, and adenovirus)–specific T cells from

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CB, a source of naïve T cells (12, 23). In previous studies of T cell responses to CMVpp65 from seropositive (CMVpos) donors, most HLA-A2 donors recognized the typical NLV epitope (24). By contrast, the HLA-A2–restricted pp65-specific T cell lines generated from CB did not recognize NLV but only atypical epitopes of CMVpp65 (12). We hypothesized that naïve T cells from CMV-seronegative (CMVneg) adult donors would also recognize atypical epitopes of CMVpp65. If so, it might be possible to increase the availability of CMV-specific T cells for patients at the greatest risk of CMV disease: CMVpos recipients receiving grafts from CMVneg donors. We therefore used CMVneg donors as a way to compare the epitope specificity of T cells expanded from the naïve T cells of CMVneg donors and CB to CMVpos donors.
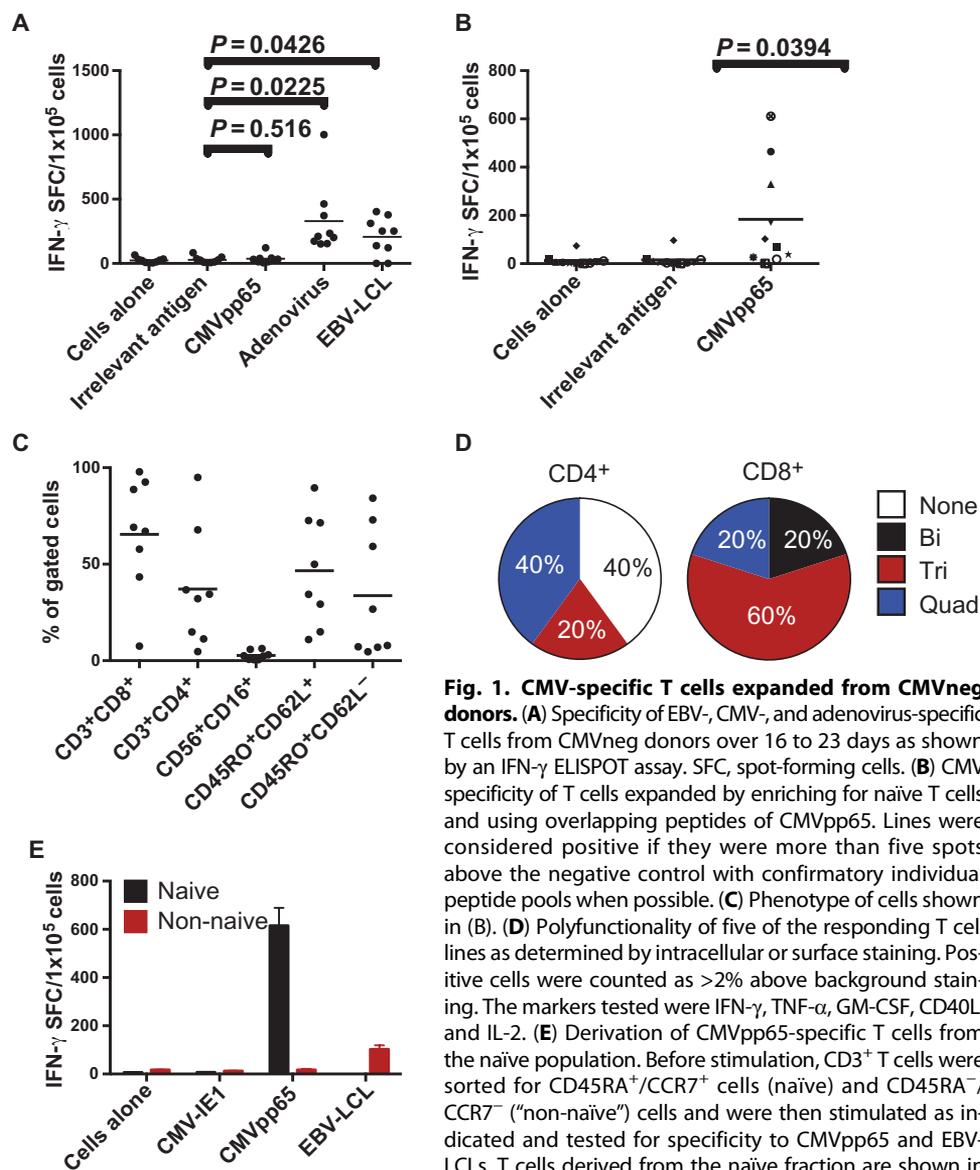
Here, we demonstrate not only the feasibility of generating pp65-specific T cells from CMVneg donors but also the ability of T cells of naïve origin to recognize atypical epitopes of pp65, supporting our working hypothesis. We further show that virus-specific T cells derived from a naïve T cell population may be protective in vivo despite their atypical epitope repertoire.

RESULTS

CMVpp65-specific T cells can be expanded from CMVneg donors

Using dendritic cells (DCs) and EBV-positive lymphoblastoid cell lines (EBV-LCLs) expressing CMVpp65 from an adenoviral vector (Ad5f35pp65) as sources of antigen and antigen-presenting cells (APCs), we previously showed that we could expand CMV-, EBV-, and adenovirus-specific T cells from the naïve T cells of CB (12, 23). To test whether CMV-specific T cells could be expanded from CMVneg donors, we used the same method of T cell expansion. The resultant T cells recognized EBV and adenovirus, but CMVpp65-specific T cells were below the limits of detection (Fig. 1A). To limit the expansion of memory T cells specific for adenovirus and EBV, we first selected for CD45RA$^+$ cells from the peripheral blood mononuclear cells (PBMCs), and as a source of antigen for the first stimulation, we used DCs pulsed with an overlapping peptide library (PepMix) spanning the entire amino acid sequence of CMVpp65. For the second and subsequent stimulations, we used PepMix-pulsed EBV-LCLs. With this revised method together with the cytokines interleukin-7 (IL-7), IL-12, and IL-15 as before, we were able to expand CMVpp65-specific T cells in a reproducible manner from 8 of 10 CMVneg adult donors as detected by

interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT) assay (Fig. 1B). Most (mean, 65%; range, 8 to 98%) of these T cells were CD8$^+$ and comprised both central and effector memory cells with only a minimal number of natural killer cells (CD3$^-$ CD16$^+$ CD56$^+$) (mean, 3%; range, 1 to 6%) (Fig. 1C). Because recent studies suggest that polyfunctional antigen-specific T cells have superior in vivo function (25, 26), we evaluated the polyfunctionality of five donor T cell lines by staining for IFN-γ, GM-CSF (granulocyte-macrophage colony-stimulating factor), IL-2, TNF-α (tumor necrosis factor–α), and CD40L (CD40 ligand) and found that most of the CD8$^+$ T cells released at least three of the five cytokines or activation markers tested (Fig. 1D and fig. S2). To demonstrate that the T cells expanded from CMVneg donors were derived from the naïve population, we sorted naïve donor PBMCs for CD45RA$^+$CCR7$^+$ or CD45RA$^-$CCR7$^-$ by flow cytometry,

Fig. 1. CMV-specific T cells expanded from CMVneg donors. (A) Specificity of EBV-, CMV-, and adenovirus-specific T cells from CMVneg donors over 16 to 23 days as shown by an IFN-γ ELISPOT assay. SFC, spot-forming cells. (B) CMV specificity of T cells expanded by enriching for naïve T cells and using overlapping peptides of CMVpp65. Lines were considered positive if they were more than five spots above the negative control with confirmatory individual peptide pools when possible. (C) Phenotype of cells shown in (B). (D) Polyfunctionality of five of the responding T cell lines as determined by intracellular or surface staining. Positive cells were counted as >2% above background staining. The markers tested were IFN-γ, TNF-α, GM-CSF, CD40L, and IL-2. (E) Derivation of CMVpp65-specific T cells from the naïve population. Before stimulation, CD3$^+$ T cells were sorted for CD45RA$^+$/CCR7$^+$ cells (naïve) and CD45RA$^-$/CCR7$^-$ ("non-naïve") cells and were then stimulated as indicated and tested for specificity to CMVpp65 and EBV-LCLs. T cells derived from the naïve fraction are shown in black and the non-naïve fraction in red. Error bars represent the SD from the mean. *$P < 0.005$ versus non-naïve by two-tailed t test. In (A) to (C), each symbol represents a T cell line taken from 10 CMVneg donors, and the black bars indicate the mean.

followed by expansion with DC, EBV-LCL, and CMV PepMixes. As shown in Fig. 1E, the CMVpp65-specific T cells were indeed derived from the naïve population and not the memory (non-naïve) population.

CMVpp65-specific T cells from the naïve T cell population recognize atypical epitopes

We previously showed (12) that CMVpp65-specific T cells from CB did not recognize the expected typical peptides such as the HLA-A2–restricted peptide NLV. To determine whether this result was unique to NLV, we compared the peptide repertoires of pp65-specific T cells from HLA-A2 adult CMVneg and CMVpos donors using CMV peptide pools. In this approach, the pp65 protein is dissected into 20-mer peptides overlapping by 15 amino acids, which are then divided into 22 pools such that each peptide is uniquely present in 2 pools, allowing us to map the specificities of the T cell line. With this method, T cells from three of three CMVpos donors recognized pools 2 and 21 (Fig. 2A), corresponding to the well-characterized epitope NLV. By contrast, the pp65-specific T cells from HLA-A2 CMVneg donors failed to respond to the 9-mer NLV; instead, many of these cells recognized alternative peptides such as those found in pools 7/8 and 13, corresponding to the 20-mer DTPVLPHETRLLQTGIHVRV (Fig. 2B). To fine-map this epitope, we tested the reactive T cells for their recognition of all 9-mers contained within the 20-mer DTPVLPHETRLLQTGIHVRV, showing that the 9-mer recognized by the HLA-A2+ pp65-specific T cells was LQTGIHVRV (hereafter LQT; fig. S1A). Besides LQT, pp65-specific T cells from HLA-A2+ naïve donors also recognized the atypical

epitope MLNIPSINV (MLN), whereas those from an HLA-B35+ donor recognized the novel HLA-B35–restricted epitope DANDIYRIF (DAN) as determined by fine mapping of the 20-mer DANDIYRIFAELEGVVWQPAA (fig. S1B). Epitope recognition by all of the T cell lines derived from CMVneg donors is shown in table S1.

T cells recognizing atypical epitopes are detectable in CMVpos donors

Given that the atypical epitopes we identified have either not been identified previously or not been commonly reported, we considered that T cells specific for atypical epitopes are not naturally expanded in response to CMV infection. To examine this possibility, we expanded T cells from seropositive donors by stimulation with the entire pp65 antigen using our standard T cell expansion protocol (1, 24). As shown in Fig. 3 (A and B), the response of T cells from five CMVpos HLA-A2 donors was largely directed against NLV, but a small proportion of the population recognized MLN or LQT, suggesting that such circulating T cells are infrequent and likely outcompeted during the ex vivo expansion step by more frequent clones that recognize NLV. To determine whether the T cells recognizing the atypical epitopes from HLA-A2+ CMVpos donors could be amplified, we used DCs pulsed with only the atypical peptide, resulting in the expansion of MLN- and LQT-specific T cells without the requirement for a naïve T cell isolation step (Fig. 3, C and D). Knowing that we could force the expansion of atypical epitope–specific T cells from CMVpos donors, we next asked whether we could force the expansion of NLV-specific T cells from HLA-A2+ CMV naïve sources. As shown in Fig. 3E, we were unable to generate such cells from any of the eight CB and CMVneg donor lines.

Because T cells specific for the atypical epitopes MLN and LQT could be expanded ex vivo, we considered whether the avidities of these TCRs for their respective peptide/major histocompatibility complexes might be lower than those recognizing the previously identified typical epitopes. To explore this idea, we used limiting dilutions of peptides in IFN-γ release assays. For NLV-specific T cells from four CMVpos donors, the mean peptide concentration needed to induce a half-maximum IFN-γ response (EC$_{50}$) was 0.23 ng/ml (range, 0.03 to 0.4 ng/ml) (Fig. 3F and Table 1), as previously reported (27). By contrast, CMVpos donor–derived T cells that recognized the atypical epitope MLN showed a substantially weaker avidity: mean of 4.8 ng/ml (range, 0.4 to 11 ng/ml) (Fig. 3F and Table 1). The mean values for T cells derived from CMVneg donors or CB specific for MLN were 0.45 ng/ml (range, 0.3 to 0.6 ng/ml) and 0.52 ng/ml (range, 0.2 to 0.8 ng/ml), respectively (Fig. 3F and Table 1), indicating a 17-fold stronger avidity than seen with CMVpos donor–derived T cells (P = 0.019679; paired t test on paired samples of log-transformed data) and only slightly weaker than observed for the typical epitope NLV, suggesting that T cells recognizing atypical epitopes may indeed be protective but do not persist.

Live CMV does not stimulate T cells specific for typical epitopes from CB or CMVneg donors

A possible cause of the expansion of T cells recognizing atypical epitopes in this study is that the epitopes presented to naïve T cells under in vitro priming conditions (including overlapping peptide libraries or the Ad5f35pp65 vector–transduced DCs and LCLs) differ from those prevalent during in vivo priming by live CMV. Thus, to test whether CMV-infected APCs would restore the typical pattern of epitope
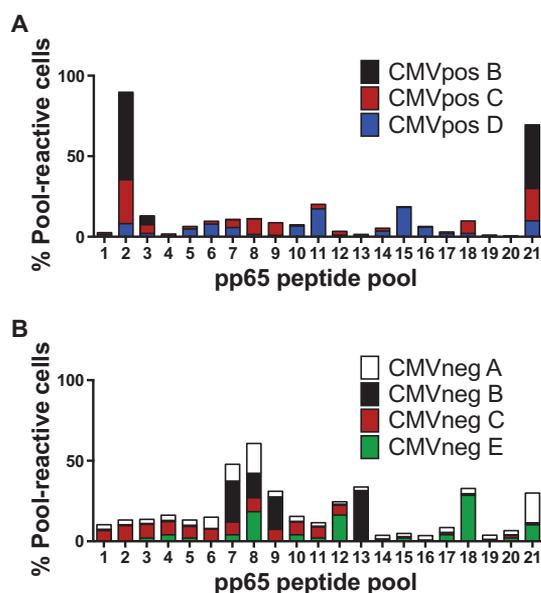

Fig. 2. Comparison of recognition of typical and atypical epitopes of CMVpp65 by CMVpos versus seronegative donors. (**A**) Epitope specificity for the three CMVpos HLA-A2+ donors based on overlapping peptide pools of CMVpp65. The pp65 protein was divided into 20 amino acids overlapping by 11 amino acids. The peptides were then distributed into a total of 22 pools, with each peptide present in 2 pools. Pools 2 and 21 contain the 20-mer peptide 98, harboring the typical HLA-A2–restricted epitope NLV. The percentages of cells recognizing each pool from each of the three donors are shown. This was calculated by totaling the total number of spots across all pools and dividing by spots per pool. (**B**) Peptide pool recognition by four CMVneg HLA-A2+ donors.
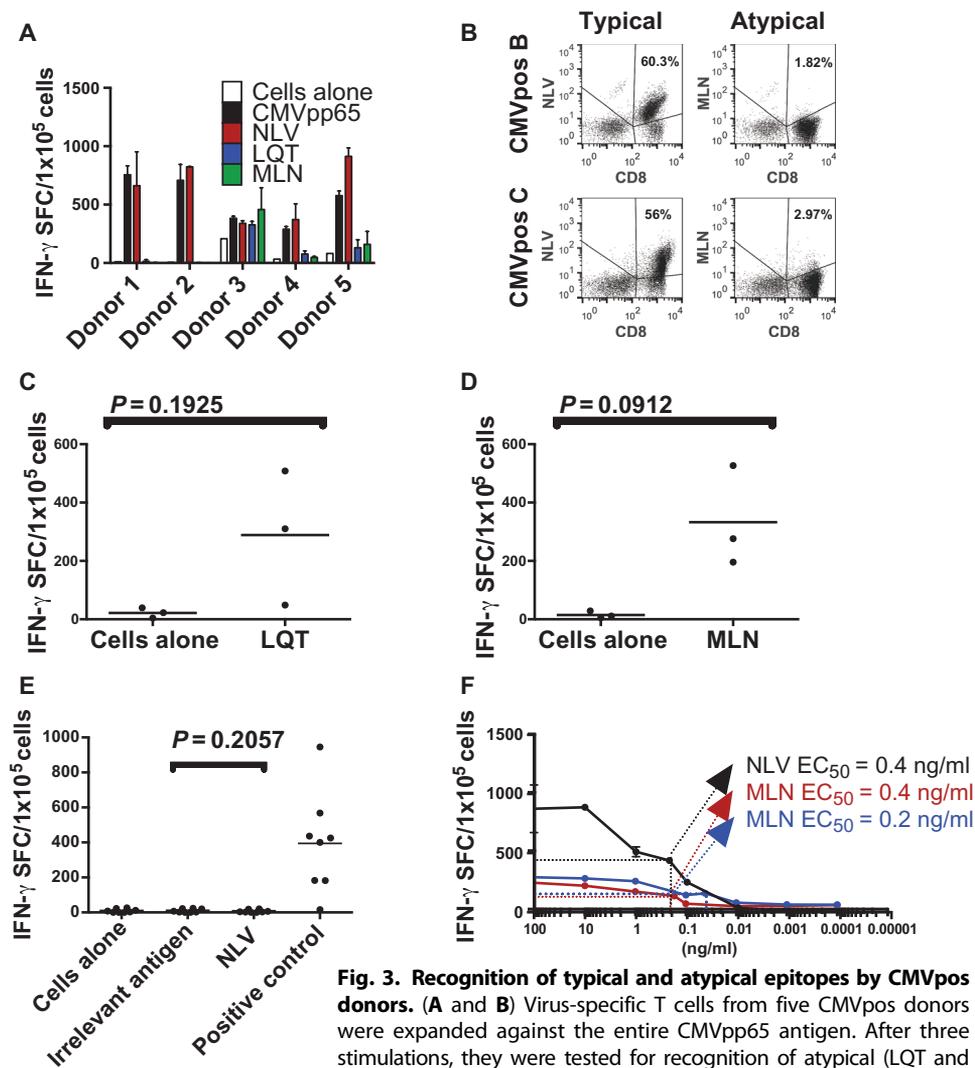
Fig. 3. Recognition of typical and atypical epitopes by CMVpos donors. (**A** and **B**) Virus-specific T cells from five CMVpos donors were expanded against the entire CMVpp65 antigen. After three stimulations, they were tested for recognition of atypical (LQT and MLN) and typical (NLV) epitopes as determined by the IFN-γ ELISPOT assay (A) and pentamer analysis (B). (**C** and **D**) T cells from CMVpos donors were stimulated three times with DCs pulsed with the indicated peptide. The number of cells that secrete IFN-γ in response to stimulation with LQT (C) or MLN (D) peptide is shown on about day 21 of culture. Gray bars indicate mean n values. (**E**) Failure to generate NLV-specific T cells from CMVneg donors (gray diamonds, $n = 3$) or CB (black diamonds, $n = 5$). T cells were stimulated with DCs pulsed with the peptide NLV in the absence of other peptides. After three stimulations, the resulting cells were tested for their ability to recognize NLV. Gray bars indicate mean values. (**F**) T cells from CMVpos donors were stimulated as above with the peptide NLV or MLN and tested by limiting dilution for the mean one-half effective concentration (EC_{50}). Error bars indicate the SD from the mean of triplicate wells; a representative result is shown (see Table 1 for the avidity of other lines). Values shown in (E) and (F) are the numbers of spots above background.

recognition, we infected HLA-A2$^+$ allogeneic fibroblasts with CMV AD169 and used them together with DCs to stimulate the T cells. As shown in Fig. 4A, this modification resulted in pp65-specific T cells from CB as well as CMVneg and CMVpos donors. The T cells from CMVpos donors recognized typical epitopes of pp65 (Fig. 4B), whereas stimulation with CMV-infected fibroblasts did not restore typical epitope recognition by T cells from either CMVneg donors (Fig. 4C) or CB (Fig. 4D). Instead, these CMVpp65-specific T cells continued to recognize atypical epitopes (Fig. 4E).

T cells recognizing atypical epitopes are polyclonal and exhibit TCR diversity

The marked disparity in the T cell avidity for atypical epitopes between naïve and CMVpos donors (Fig. 3) suggested a difference in clonality. We therefore sorted T cells from CMVpos donors that were specific for NLV and MLN, as well as those specific for MLN from CB and CMVneg donors on the basis of pentamer positivity, extracted RNA from the sorted cells, and sequenced the TCRs. As shown in table S2, CMVpos donor–derived T cells that recognized NLV were oligoclonal, whereas T cells specific for MLN, regardless of donor origin, were polyclonal ($P = 0.02$, Fisher's exact test), suggesting a possible difference when priming naïve T cells.

Because CB-derived T cells represent an immature population of T cells that have not undergone clonal expansion in the presence of antigen and hence are representative of the starting naïve T cell population in vivo, we also considered that the TCR repertoire in CB and the frequency of atypical epitope recognition might be increased as compared to TCRs identified in mature T cells that recognize NLV. To pursue this hypothesis, we isolated HLA-A2$^+$ cells from five unmanipulated HLA-A2$^+$ CB units, extracted the DNA from these units, and then compared the TCR sequences with those identified in table S2 that represent T cells able to recognize MLN, LQT, or NLV. Five of the five CB units had detectable typical and/or atypical TCRs in the unmanipulated CB unit (table S3); however, of the 22 TCRs specific for NLV, only 1 was found in any of the CB units sequenced, whereas 5 different TCRs specific for MLN or LQT were identified in the HLA-A2$^+$ CB units. These results, which underscore the very low frequency of TCRs for the NLV epitope, help to explain the difficulty of generating T cells specific for canonical CMVpp65 epitopes from naïve sources.

Virus-specific T cells derived from a naïve source are safe and may be protective in vivo

To determine whether T cells recognizing atypical epitopes are protective, we first showed that T cells specific for either MLN or NLV could prevent the dissemination of CMV from human fibroblasts (table S4). We next evaluated MLN- and LQT-specific T cells in PBMC samples obtained at 3, 6, and 12 months after CB transplantation (CBT) from 22 HLA-A02$^+$ patients. As shown in Table 2, in seven of these patients, TCRs recognizing either MLN or LQT epitopes

Table 1. Avidity of CMVpos, CMVneg, and CB T cells recognizing typical and atypical epitopes.

T cell source	NLV [mean EC_{50} (ng/ml)]	MLN [mean EC_{50} (ng/ml)]
CMVpos A	0.4	3.0
CMVpos E	0.08	Not determined
CMVpos B	0.03	0.4
CMVpos C	0.4	11
Mean	0.23*	4.8*
CMVneg H		0.3
CMVneg C		0.6
Mean		0.45
CB line 1		0.7
CB line 2		0.2
CB line 3		0.8
CB line 4		0.5
CB line 5		0.4
Mean		0.52

*$P = 0.019679$ of paired t test of log-transformed values from CMVpos A, CMVpos B, and CMVpos C.

were detected. Five patients demonstrated TCRs recognizing the NLV epitope, whereas three patients recognized both typical and atypical epitopes. One transplant recipient whose T cells recognized MLN did not develop CMV reactivation despite the absence of detectable T cells recognizing typical epitopes, which are generally considered protective in this setting (28).

In more rigorous testing, we transferred multivirus-specific T cells that had been generated from the 20% fraction of CB units into a different set of patients who had been transplanted with the 80% fraction. The infused T cell lines had a predominantly effector memory phenotype; ELISPOT and/or pentamer staining of T cells from HLA-A2+ donor P3275 confirmed specificity for the atypical CMVpp65 epitope LQT but not the typical epitope NLV (Fig. 5, A to C). Three consecutive patients (aged 1 to 5 years) received CB-derived multivirus-specific T cells between 63 and 85 days after transplantation at doses ranging from $5 \times 10^6/m^2$ to $1 \times 10^7/m^2$. Patients received CB grafts from five of six or six of six donors for Fanconi anemia, severe combined immunodeficiency (SCID), or acute lymphoblastic leukemia (ALL) after receiving myeloablative (n = 1) or submyeloablative (n = 2) conditioning regimens (Table 3). After T cell infusion, there were no episodes of infusion-related toxicity or graft-versus-host disease (GVHD) (Table 3). Two of these patients, including one whose multivirus-specific T cell line comprised T cells specific for the atypical CMVpp65 epitope LQT (Fig. 5, B and C), remained free of CMV as well as EBV and adenovirus at more than 2 years after CBT following infusion of CB-derived T cells. The third patient was on foscarnet at the time of T cell infusion and was transiently positive for CMV in the blood shortly after infusion #1. Foscarnet was discontinued 4 weeks after T cell infusion, but the CMV reactivated soon after and he was treated with a second dose of CTL and antiviral pharmacotherapy was reinitiated (foscarnet followed by ganciclovir). As shown in Fig. 5D, the rise in CMV-specific T cells in the peripheral blood began around the time of the first T cell

infusion and was observed at the time when the viral load reduced from 1900 to zero copies/ml. Despite clearance of the CMV viremia within 16 days of T cell therapy, antiviral pharmacotherapy was continued for an additional 4 weeks, followed by a subsequent reactivation after it was stopped. After administering a third dose of CTL, the virus cleared without requiring additional pharmacotherapy. Three months after the first T cell infusion, this patient was also antigen-positive for adenovirus in his stool associated with diarrhea, which resolved spontaneously without additional antiviral therapy. Patient PBMCs were also analyzed for pp65 specificity by peptide pool analysis and for the detection of T cells recognizing atypical CMV epitopes when possible. However, the CMV response was barely above the limits of detection of the ELISPOT assay in recipients who did not have CMV reactivation. Unfortunately, patient P2891, who did reactivate CMV, received a CB-derived T cell line recognizing unknown CMV epitopes (fig. S3). However, TCR sequencing was performed on the infused T cell line and the patient PBMCs obtained before infusion and at 6 months after T cell infusion. Unique TCRs that were detected in the infused T cell product but not in patient PBMCs obtained before infusion were detected in patient PBMCs collected 6 months after infusion as shown in fig. S4.

DISCUSSION

The objective of this study was to test the ability of T cells derived from naïve populations to recognize atypical epitopes of CMVpp65. After expanding CMV-specific T cells from two naïve sources (CB and CMVneg donors), we established their suitability for clinical use in a series of experiments designed to elucidate the epitope-specific activity, clonality, and TCR diversity of these novel cells. The safety and efficacy of these naïve-derived T cells was assessed in three consecutive patients who had undergone CBT and were at high risk for CMV infection. One caveat with this approach is that the efficacy of the naïve-derived T cells cannot be irrefutably determined with such a small sample size. However, the study was designed as a phase 1 pilot study to evaluate the safety of the CB-derived T cells and was not designed to test efficacy. An additional limitation of our approach is the relatively limited number of unmanipulated HLA-A2+ CB samples that could be used to detect atypical and typical epitopes. Because of the high cost of TCR sequencing, we were limited to only five CB units, and hence, the results were too small to determine the significance.

Here, we show that CMV-specific T cells can be generated from the naïve T cell populations of CMVneg donors in a good manufacturing practice–compliant manner. The presence of memory T cells specific for other viral antigens, such as adenovirus, limited the expansion and detection of CMVpp65-specific T cells from the naïve population in adult CMVneg donors, but removing the adenoviral antigens and isolating the naïve T cells allowed the specific expansion of pp65-specific T cells. When tested for epitope specificity, the naïve T cells from CMVneg donors, like those from CB, recognized only atypical epitopes of pp65, whereas T cells from each of three HLA-A2+ CMVpos donors recognized the typical HLA-A2–restricted epitope NLV. Of the atypical epitopes identified in this study, at least two (DAN and LQT) have not previously been reported, whereas MLN (29), although reported previously, is nonetheless atypical. Moreover, the T cells recognizing atypical CMV epitopes appear to protect against CMV in vivo as demonstrated by (i) the recovery of T cells specific for
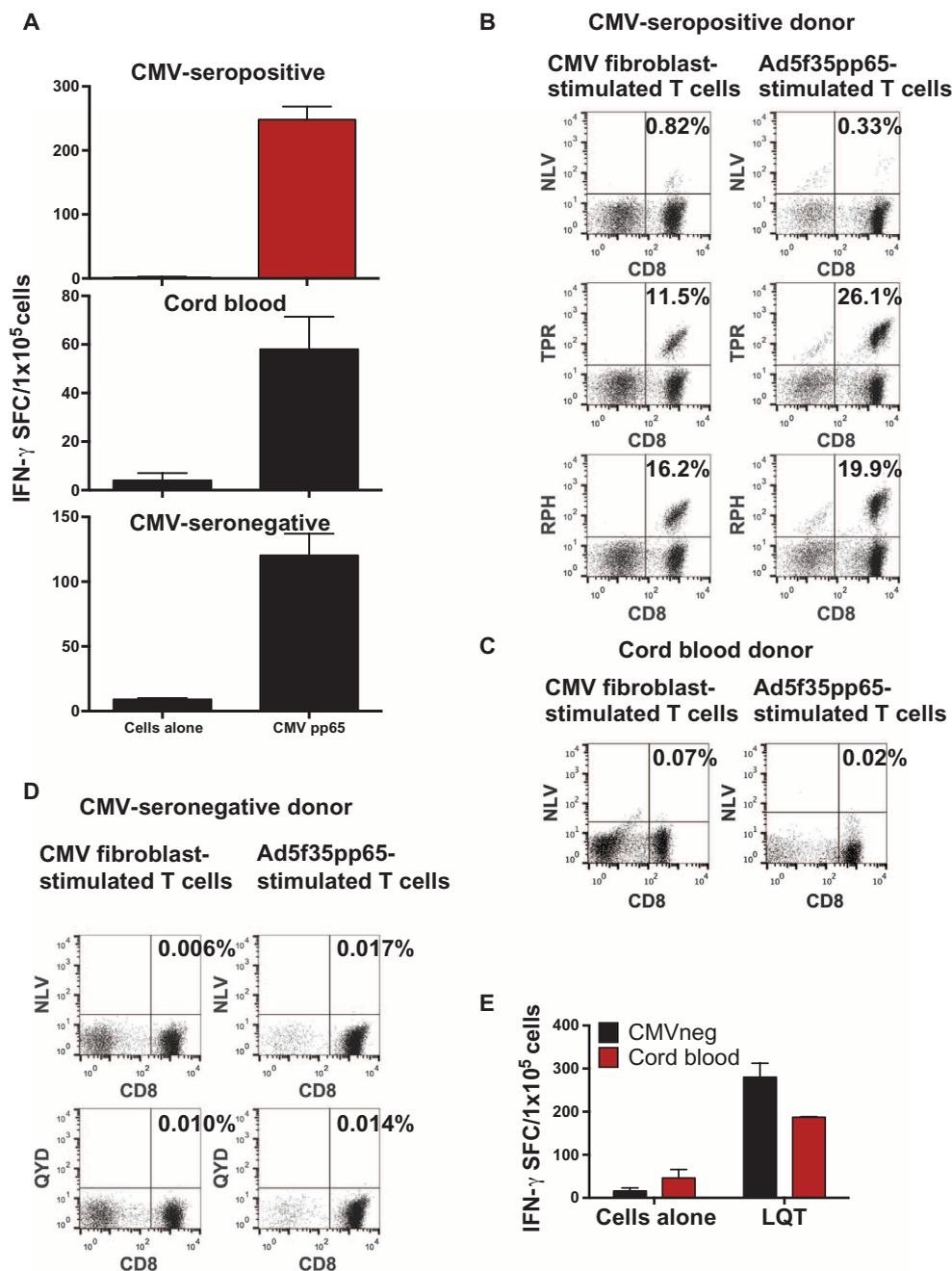
Fig. 4. Epitope recognition by T cells stimulated with live CMV. (A) Comparison of response to CMVpp65 by T cells from HLA-A2$^+$ CMVneg donors, CMVpos donors, and CB. The T cells were stimulated with CMV-infected HLA-A2$^+$ fibroblasts that were cocultured with DCs and then tested with an IFN-γ ELISPOT assay. Mean ± SD values from triplicate wells are shown. **(B)** Typical epitope (HLA-A2–restricted NLV, B7-restricted TPR and RPH, or A24-restricted QYD) recognition of cells from a CMVpos donor line stimulated with CMV-infected fibroblasts or an adenoviral vector expressing CMVpp65 as described. **(C)** Typical epitope recognition by CB T cells stimulated with CMV-infected fibroblasts or the adenoviral pp65 vector. **(D)** Typical epitope recognition by CMVneg T cells stimulated with CMV-infected fibroblasts or the adenoviral pp65 vector. **(E)** Atypical epitope recognition by CB-derived T cells generated with CMV-infected fibroblasts. Pool 13 contained the atypical epitope LQT.

undergoing CBT. T cells recognizing atypical CMV epitopes are detectable in vivo in CBT recipients in a similar fraction of patients and at similar frequencies as T cells recognizing typical epitopes and persist up to a year after transplant.

Aside from our previous work with CB T cells, the only other report describing the reactivation of CMV-specific T cells from naïve populations in adult donors was described by Jedema *et al.* (*30*), who showed that primary CMV-specific responses can be elicited from the naïve donor T cell repertoire, although the extensive manipulation required makes translation of this strategy to the clinic problematic. Further, here, CD45RO cells were depleted, and nondepleted T cells were stimulated with single peptides (including NLV), followed by an enrichment of activated cells after the second stimulation using CD137 immunomagnetic selection. In some cases, cells were further enriched after subsequent stimulations using immunomagnetic tetramer sorting. Given the extensive manipulation needed to expand these cells, the Jedema study corroborates our finding that NLV-specific T cells are at a low frequency before exposure to CMV, and in our system, the naïve T cells were generated in competition with all pp65 peptides and could be generated in the presence of naturally infected APCs. It remains to be tested whether T cells targeting atypical epitopes would require such extensive manipulations in the system tested above.

A pivotal question at the outset of our study was whether T cells specific for atypical epitopes circulate in the peripheral blood of CMVpos donors. Even after T cells from CMVpos donors were expanded with the entire CMVpp65 antigen, the frequency of such T cells remained low. Yet, by using only a single atypical peptide such as LQT, we were able to expand atypical epitope–specific T cells from both the naïve and memory T cell populations. This result stands in marked contrast to our unsuccessful attempts to expand NLV-specific T cells from HLA-A2$^+$ CB or CMVneg donors (*31*).

What could account for the different epitope recognition patterns of naïve versus memory T cells? (i) Fibroblasts or epithelial cells (not DCs) are typically the first cells infected by CMV (*32*) and therefore would be expected to present different antigens than do DCs. (ii) CMV expresses at least 15 proteins that modulate the

atypical CMVpp65 epitopes in patients who do not have CMV reactivation and (ii) the successful transfer of naïve CB or CMVneg donor–derived CMV-specific T cells to prevent or treat infections in patients

Table 2. Identification of TCR sequences specific for typical and atypical epitopes in CB transplant recipients. CMVneg, CMV-seronegative recipient; CMVpos, CMV-seropositive recipient; CDR3, complementarity-determining region 3. # and * represent the time point at which the samples were taken.

		CB transplant recipients (% TCRs)								
		CMVneg		CMVpos						
		1	2	1	2	3	4	5	6	7
CMV reactivation?		No	Yes	No	No	No	No	Yes	No	Yes
Peptide	CDR3									
NLV	CASSLDRVTGELFF	0.00578								
	CASSPGTGREQFF			0.00536*						
	CASSLTNEQFF	0.00486			0.01544					
	CASSLTSEQFF					0.04603*			0.00448	
	CASSLAERSYEQYF									
	CASSSVNEQFF					0.33467#				
	CASSLAPGATNEKLFF	0.00026								
MLN	CASSFGVNTEAFF						0.0053	0.00614		
	CASSLGLNYEQYF		0.00027							
	CSARDRDRGYEQYF								0.00055	
	CASRAVSTDTQYF									
	CATSPTANTEAFF									
	CASSPSGYNEQFF			0.00921#					0.00382	
	CASSLDLGASTDTQYF	0.00263								
	CASSFRGDTEAFF									0.0015
LQT	CASSPPGGSGNTIYF		0.00127						0.00216	
Time points observed		12 mo	12 mo	3mo* 12 mo#	12 mo	3 + 12 mo* 3 + 6 + 12 mo#	6 mo	12 mo	12 mo	12 mo
Time points not observed		3 mo	Only time pt	6 mo	3 or 6 mo	3 mo	3 mo	3 mo	3 or 6 mo	6 mo

immune response to the virus and the peptides presented by the APC (33, 34), so that in the absence of these immunomodulatory factors, naïve T cells might recognize different epitopes of CMVpp65. (iii) T cells recognizing atypical epitopes are more abundant at the time of primary infection but are replaced by T cells recognizing typical epitopes. To pursue possibilities (i) and (ii), we generated CMV-specific T cells using live CMV-infected fibroblasts cocultured with DCs as APCs. This modification did not lead to the preferential activation of typical epitopes by naïve T cells, and these APCs were also able to activate T cells specific for atypical epitopes, indicating that these epitopes are naturally processed and presented during infection. To address the third possibility, we searched for known NLV, MLN, and LQT TCRs in five HLA-A2 CB units. Here, we showed that TCRs recognizing atypical epitopes are about 60-fold more common than NLV-specific clones in unmanipulated CB units. This finding suggests that the initial naïve T cell response to CMV is a stochastic response where T cells of similar avidities but different precursor frequencies compete. Soon after, these T cells are overwhelmed by the typical epitope responses, like those from NLV. Alternatively, high-avidity T cells initially responding to atypical epitopes may undergo exhaustion, allowing for the expansion and detection of typical epitopes.

In other disease models, notably those driven by SIV (simian immunodeficiency virus), EBV, and HIV, naïve T cells have been reported to recognize different epitopes (and even antigens) than do memory T cells (22, 35, 36). The HLA-A*02–restricted HIV peptide SLYNTVATL is found in chronically infected individuals but not in those with primary infection (22). In an analysis of EBV infection, T cells recognizing lytic epitopes were always present and dominant during primary infection but decreased over time, whereas T cells recognizing the latent peptides were at a low frequency during the primary infection but then increased at later times (37, 38). Indeed, clonotypic analysis of these cells showed that the original T cells were replaced by different T cells over time, leading us to believe that this is possible during CMV infection as well. Finally, our results add clinical perspective to a recent report (39) showing that in an SIV model, rhesus CMV (RhCMV) vectors expressing the simian immunodeficiency viral gene elicit SIV-specific CD8$^+$ T cells that do not recognize canonical epitopes and instead target unusual, diverse, and highly promiscuous epitopes but only when the RhCMV gene Rh189 (US11) is present, suggesting that CMV may be responsible for the atypical epitope recognition. Although we were unable to detect typical epitopes in vitro when using APCs infected with CMV, this study corroborates our previous report
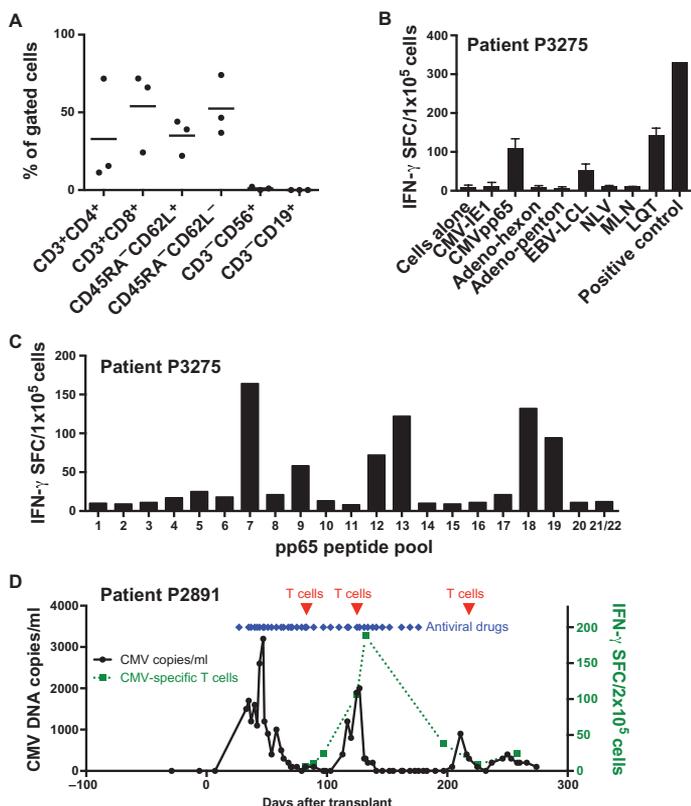
Fig. 5. CB-derived T cells for clinical use. (**A**) Phenotype of three CB-derived T cell lines manufactured from the 20% fraction of a clinical CB unit. (**B**) Reactivity of the CB line infused into patient P3275 to viral antigens and epitopes of pp65 including the typical epitope NLV and the atypical epitope LQT. (**C**) The specificity of the pp65 response of the CB line infused into patient P3275 was evaluated using overlapping pp65 peptide pools. (**D**) CMV load of P2891 (left) in relation to the number of reactive cells from the peripheral blood (right). The timing of T cell infusions is indicated by arrows.

Table 3. Characteristics of CB transplant recipients. CSA, cyclosporin A; MMF, mycophenolate mofetil; Pred, prednisone; Bu, busulfan; Cy, Cytoxan; Flu, fludarabine; TBI, total body irradiation.

	P2891	P3010	P3275
Sex	M	M	M
Age (years)	5	2	1
Disease	Fanconi anemia	ALL	SCID
Donor HLA	A*23:01,30:01; B*58:01,57:01; C*07:18,07:01; DRB1*15:03,15:02; DQB1*08:02,06:01	A*02:AGA,33:01; B*07:ANVB,14:02; C*05:01,08:02; DRB1*03:01,15:01; DQB1*02:01,06:02	A*01:BMMP,02:01; B*08:01:01,39:05; C*07:WTR,07:WCP; DRB1: 03:01,04:07:01; DQB1: 02:01:01,03:02:01
HLA match	5 of 6	5 of 6	6 of 6
Conditioning regimen	Flu/Cy/TBI	Flu/Cy/TBI	Bu/Cy/Flu
GVHD prophylaxis	CSA and MMF	CSA	Pred and MMF
Immune suppression at time of T cell infusion	CSA	CSA	Pred and MMF
Recipient CMV serostatus	Positive	Positive	Not determined but PCR-negative
Viral infections	CMV, adenovirus	None	None
Cell dose (cells/m²)	5 × 10⁶	5 × 10⁶	1 × 10⁷
Concurrent antiviral drugs	Foscarnet → ganciclovir	None	None
Viral outcome	CMV reactivation and adenovirus infection resolved	No viral reactivations	No viral reactivations
Cell dose (cells/m²)	5 × 10⁶	5 × 10⁶	1 × 10⁷
Duration of cell culture (days)	57	50	55

of atypical epitope specificity in CB and suggests that the T cell response to CMV is more complicated than initially believed. Testing the evolution of CMVpp65 epitope recognition in patients undergoing primary infection with CMV might help understand the mechanism for the eventual dominance of the response to typical epitopes.

Much recent emphasis has been placed on identifying the optimal T cell population for use in adoptive immunotherapy. Studies reported over the last few years have been largely restricted to comparisons of different memory subsets (*40*), with only scant attention paid to T cells from naïve sources. On the basis of the data presented here, we argue that despite the unusual epitope repertoire, it is possible to use naïve-derived T cells to expand CMV-specific T cells for at-risk patients and also begs the question whether T cells recognizing epitopes identified for other viral and tumor antigens undergo the same selection process. Therefore, the optimal phenotype (*14, 41*) for adoptive transfer and also the ideal epitope targets remain to be determined (*22*). This study, however, establishes the proof of concept for using antigen-specific T cells from naïve donors. However, the lengthy procedure for T cell generation and the use of the B95-8 EBV virus required for to manufacture EBV-LCL make this therapy difficult to translate beyond small center studies. Therefore, for this approach to become broadly

applicable, there is a need to eliminate the use of autologous EBV-LCL, perhaps by using artificial APCs, and to reduce the culture time from naïve donors. Another approach would be to isolate T cells secreting IFN-γ shortly after they are stimulated with viral peptides, as used with T cells derived from CMVpos donors (*4*), or to use multimers targeting atypical epitopes to select out the virus-specific T cell fraction that could be transferred to the recipient (*42, 43*). Whether enough cells could be isolated using either of these techniques remains unanswered.

In summary, we show that CMV-specific T cells can be generated from CB and blood from CMVneg adult donors and that these naïve-derived cells recognize atypical epitopes of pp65 because their precursor frequency is higher than that of T cells recognizing typical epitopes. When transferred to CB transplant recipients, these cells are safe

and were efficacious in one patient experiencing viral reactivation. Our findings have important implications for the transplantation field, in which increasing emphasis is being placed on the use of third-party "off-the-shelf" virus-specific T cell therapies for all patients undergoing HSCT or solid organ transplantation, irrespective of their HLA type (44–47). The use of a T cell product that can recognize unusual and diverse epitopes may be useful for avoiding immune escape when the T cells are not completely HLA-matched between donor and recipient.

MATERIALS AND METHODS

Study design
The objective of this study was to test the ability of T cells from naïve populations to recognize atypical epitopes of CMVpp65 and in immunocompromised recipients of transplants from virus-naïve donors. After expanding CMV-specific T cells from two naïve sources (CB and CMVneg donors), we established their suitability for clinical use in a series of experiments designed to elucidate the epitope-specific activity, clonality, and TCR diversity of these novel cells. The safety and efficacy of such T cells was assessed in three consecutive patients who had undergone CBT and were at high risk for CMV infection.

Patients
The clinical trial (#151992 at Baylor College of Medicine) was open to candidates for CBT from five of six or six of six HLA antigen–matched or HLA antigen–mismatched CB donors. Patients with a life expectancy <6 weeks or severe kidney or liver disease were excluded, and GVHD (grade 3 or 4) was a contraindication for the infusion of T cells. The investigation was reviewed by the U.S. Food and Drug Administration (FDA), the Recombinant DNA Advisory Committee, and the Institutional Review Boards (IRBs) of Baylor College of Medicine and the National Marrow Donor Program. All participants or their guardians gave informed consent upon enrollment. The characteristics of the three patients who were studied are reported in Table 3.

Generation of multivirus-specific T cell cultures
Virus-specific T cells were generated from fresh or frozen CB units obtained from the MD Anderson Cancer Center Cord Blood Bank or from mothers who consented to the protocol approved by the Baylor College of Medicine IRB. For patients enrolled on clinical trial 151992, the 80% fraction of the clinical CB unit was infused in the patient as specified by the protocol. The 20% fraction of the unit was used to manufacture virus-specific T cells according to the investigational new drug application with the FDA and as detailed below. Subjects enrolled signed informed consent on the protocols approved by the FDA and the IRB. In addition, CMV-specific T cells were derived from healthy human donors who had consented to a protocol approved by the IRB of Baylor College of Medicine. The donors were HLA-typed by the HLA laboratory of Houston Methodist Hospital and tested for CMV seropositivity by the Gulf Coast Regional Blood Center.

Generation of DCs from PBMC or CB mononuclear cells
CB was thawed and then purified by Ficoll (Lymphoprep, Nycomed) gradient separation. PBMCs from healthy CMVneg donors were isolated using Ficoll. Both CB and PBMCs were washed twice and resuspended in CellGenix medium and plated at 1×10^7 cells per well in DC medium (CellGenix medium plus 2 mM L-glutamine) (GlutaMAX, Invitrogen) in a six-well plate (Costar) for 1 hour at 37°C in a humidified CO_2 incubator. Nonadherent cells were removed by rinsing with 1× phosphate-buffered saline (Sigma) and frozen. Cells adherent after 1 hour were cultured in DC medium with GM-CSF (800 U/ml) (sargramostim, Leukine; Immunex) and IL-4 (500 U/ml) (R&D Systems) for 5 days. On day 5, the cells were harvested for maturation.

Maturation of DCs and transduction with Ad5f35pp65 vector
CB-derived DCs as well as adult CMVpos and CMVneg DCs were transduced and matured using a cytokine cocktail consisting of GM-CSF, IL-4, IL-1β, TNF-α, IL-6 (R&D Systems), and PGE_2 (prostaglandin E_2) (Sigma) ("cytokine cocktail") for 1 day. On day 6, the DCs were harvested and used to stimulate T cells. CB-derived DCs were also transduced at a multiplicity of infection of 1000 virus particles on day 5, whereas PBMC-derived DCs were pulsed with 200 ng of pp65 PepMix per 1×10^6 DCs for 1 hour and then washed on day 6.

Generation and pulsing of EBV-transformed B cell lines from PBMCs
As the source of APCs for the second and third stimulations, 5×10^6 PBMCs were infected with concentrated supernatants from a B95-8 working cell bank as previously described (1) and subsequently used to establish EBV-LCLs. The day of the second and third T cell stimulation, LCLs were pulsed for 1 hour with 200 ng of pp65 PepMix (JPT Peptide Technologies) per ~5×10^6 LCLs at 37°C. The cells were irradiated at 40 Gy, washed, resuspended at 5×10^5 cells/ml of complete medium [RPMI (HyClone) plus human serum and GlutaMAX], and then used as stimulators at a ratio of one stimulator to four effectors in a 24-well plate or five stimulators to one effector in a G-Rex10 gas-permeable culture device as published (23).

Generation of CMVpp65-specific cultures derived from CMVneg donors
PBMCs were isolated using Ficoll as described above. Adherent cells were used for DC generation, and T cell–containing nonadherent cells were frozen for use on day 7. After thawing, nonadherent cells were labeled for immunomagnetic selection of $CD45RA^+$ cells, washed, and then selected by magnetic cell sorter (MACS). The $CD45RA^+$ cells were then resuspended in 45% RPMI (HyClone) and 45% Click's (Irvine Scientific) with 10% human serum plus GlutaMAX (T cell medium). Cells were resuspended at 2×10^6/ml and cocultured with autologous, PepMix-pulsed DCs at a ratio of 20 PBMCs to 1 DC in the presence of the cytokines IL-7 and IL-12 (10 ng/ml) (R&D Systems) and IL-15 (5 ng/ml) (CellGenix). Cultures were restimulated on days 10 and 17 with irradiated (40 Gy), pp65 PepMix–pulsed autologous LCLs at a responder-to-stimulator ratio of 4:1 plus IL-15 (5 ng/ml) on day 10 and IL-2 (50 U/ml) (Proleukin) on days 17 and 20. To confirm the origin of the pp65-specific T cell populations, we sorted CD45RA/CCR7 double-positive and double-negative T cell populations by flow cytometry and stimulated them with pp65 PepMix–pulsed DCs, followed by pp65 PepMix–pulsed LCLs as described above.

Introducing CMV antigens with CMV AD169–infected fibroblasts
CMV AD169–infected irradiated (40 Gy) allogeneic $HLA-A2^+$ foreskin fibroblasts were passaged 3 days after the initiation of DC culture.

On day 5, CMV-infected fibroblasts were harvested, counted, and plated with DCs at a ratio of one fibroblast to four DCs in medium containing the cytokine cocktail. On day 6 or 7, the DCs were used to stimulate T cells.

ELISPOT assay

ELISPOT analysis was used to determine the frequency and function of T cells in the T cell lines secreting IFN-γ when stimulated with PepMixes for CMV-pp65, Ad-hexon, Ad-penton, and CMV-IE1 as previously described (24). Spot-forming cells were enumerated by ZellNet Consulting and compared with input cell numbers to obtain the frequency of virus-reactive T cells.

Immunophenotyping, multimer analysis, and intracellular cytokine staining

T cell lines were analyzed with monoclonal antibodies to CD3, CD4, CD8, CD56/16, CD45RA, and CD62L (Becton Dickinson). To detect CMV-pp65– and CMV-IE1–specific T cells in the T cell lines, we used the soluble CMV-pp65 pentamers HLA-A*02–NLV, HLA-A*02–LQT, HLA-A*02–MLN, HLA-A*24–QYD, HLA-B*7–TPR, HLA-B*7–RPH, and HLA-A*01–YSE (prepared by ProImmune Inc.). Tetramers for HLA-A*02–LQT, HLA-A*02–MLN, and HLA-B*35–DAN were first created by the Protein Core Facility of Baylor College of Medicine and the Dan L. Duncan Cancer Center. Functionality of the T cells was assessed by the flow cytometric detection of intracellular accumulation of IFN-γ, GM-CSF, IL-2, TNF-α, and CD40L (all from BD Biosciences) as previously described (48, 49). Briefly, T cells were stimulated with a final concentration (1 μg/ml) of each PepMix; medium alone was used as the negative control. After incubation for 1 hour at 37°C, brefeldin A (10 μg/ml) (Sigma) was added for an addition 5 hours. Cells were then washed, fixed, and analyzed using a FACSAria or LSR II flow cytometer (BD Biosciences). When possible, at least 200,000 live events were acquired per tube. Data analysis was performed using FlowJo.

Peptide pools to evaluate the breadth of the virus-specific response

Panels of 20-mer peptides (overlapping by 15 amino acids and covering the entire amino acid sequence of CMVpp65 from the CMV AD169 strain) were synthesized (ProImmune). For CMV-pp65, 22 peptide pools comprising 2 to 12 20-mer peptides were prepared, so that each 20-mer peptide was represented in 2 pools. The 20-mer peptides overlap by 15 amino acids, such that the minimal epitope likely overlaps multiple sequential peptides. These CMV-pp65 peptide libraries were designed to identify HLA class I– and HLA class II–restricted epitopes.

Cytotoxicity assay

T cells were tested for specific cytotoxicity against autologous LCLs or phytohemagglutinin (PHA) blasts and autologous LCLs (or PHA blasts) pulsed with CMV-IE1, CMV-pp65, Ad-hexon, and Ad-penton PepMixes. ^{51}Cr-labeled target cells were mixed with effector cells at doubling dilutions to produce the specified effector/target (E:T) ratios. Target cells incubated in complete medium or 5% Triton X-100 (Sigma) were used to determine spontaneous and maximal ^{51}Cr release, respectively. After 4 hours, supernatants were collected, and radioactivity was measured on a γ counter. The mean percentage of specific lysis of triplicate wells was calculated as 100 × (experimental release – spontaneous release)/(maximal release – spontaneous release). The ability of effector cells to lyse CMV-infected targets was assessed by using HLA-A*0201$^+$ fibroblasts infected with the AD169 laboratory strain of CMV.

Viral dissemination assay

Human HLA-A02$^+$ fibroblasts were infected with CMV AD169 at various dilutions in the presence of IFN-γ (10 ng/ml). After ~4 hours, the virus was removed, and NLV-specific T cells, MLN-specific T cells, or no T cells were added to the culture together with IL-2. After 3 to 5 days, the supernatant or cell lysate was added to fresh human fibroblasts. After 8 days, the fibroblasts were scored for their cytopathic effect (table S3).

Evaluating TCR precursor frequency

HLA-A2$^+$ CB units were stained and selected for CD3$^+$ cells using MACS selection (Miltenyi Biotec). These cells were then sent to Adaptive Biotechnologies for DNA extraction and TCR sequencing.

Identifying atypical epitopes

The 20-mer peptide was identified as above using overlapping peptide pools. The 20-mer peptide was then divided into all possible 9-mer peptides, and these peptides were synthesized and tested for specificity with the T cell line by IFN-γ.

TCR sequencing

Frozen peripheral blood samples were obtained from recipients of double CB transplants who had been consented to a protocol approved by the IRB of Harvard Cancer Center/Dana-Farber Cancer Institute/Massachusetts General Hospital. PBMCs from 10 ml of whole blood were isolated using Ficoll as described above and incubated with fluorophore-conjugated monoclonal antibodies: anti-CD3 V450 (clone UCHT1, BD Biosciences), anti-CD4 APC-H7 (clone RPA-T4, BD Biosciences), anti-CD8 Pacific Orange (clone 3B5, Invitrogen), anti-CD25 PE (phycoerythrin)–Cy7 (clone M-A251, BD Biosciences), and anti-CD127 PE-Cy5 (clone eBioRDR5, eBioscience). Cell analysis was performed with the FACSCanto II system (BD Biosciences) and FACSDiva software (BD Biosciences). Genomic DNA was extracted from mixed PBMCs using the QIAamp DNA Blood Mini Kit as per the manufacturer's instructions (Qiagen). A median of 96 ng of genomic DNA (range, 0.8 to 400 ng) for mixed PBMCs was sent to Adaptive Biotechnologies for TCRβ sequencing on their immunoSEQ platform, based on multiplex PCR (polymerase chain reaction) amplification with 45 Vβ and 13 Jβ primers, followed by sequencing of TCRβ CDR3 regions on the Illumina Genome Analyzer Cluster Station. Analysis and compilation of resulting sequences was performed by Adaptive Biotechnologies. HLA typing was performed by the HLA Laboratory of Brigham and Women's Hospital (Boston, MA) or Massachusetts General Hospital (Boston, MA) or was sent to the American Red Cross and tested for CMV seropositivity by the Brigham and Women's Hospital Virology Laboratory, the Massachusetts General Hospital Virology Laboratory, or the Rhode Island Blood Center.

Recipient PBMC samples thawed and analyzed were originally obtained at 3, 6, and 12 months after transplantation from recipients of double CB grafts who received reduced-intensity conditioning consisting of fludarabine, melphalan, and anti–thymocyte globulin either on clinical trials or standard treatment protocols between the years 2005 and 2012 at Dana-Farber Cancer Institute or Massachusetts General

Hospital. GVHD prophylaxis was tacrolimus and rapamycin. Samples were selected for analysis if recipients demonstrated >80% donor T cell chimerism and were not experiencing disease relapse. There were no restrictions based on duration of survival, infections, or GVHD history. The earliest samples were analyzed from 3 months after transplant. Sample availability in the tissue bank was occasionally a limiting factor.

TCRβ sequencing results from 43 PBMC samples representing 22 HLA-A02$^+$ cord recipients were searched for TCRβ sequences corresponding to those described as recognizing atypical CMV epitopes described in this article or typical epitopes as compiled after an extensive literature search. Six recipients, reflecting 13 samples, received an umbilical cord unit that was ex vivo stimulated (50).Time points from which CMV-specific sequences were identified are indicated on Table 2.

Statistical analysis

Student's t test (two-tailed) was used to test for significance in selected sets of values, assuming equal variance. A P value <0.05 was considered significant. When applicable, values were log-transformed to account for variability between donors. Other statistical tests are stated in the text. Unless otherwise stated, all values are reported as means ± SD.

SUPPLEMENTARY MATERIALS

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Fig. S1. Identifying atypical epitopes from CMVpp65.
Fig. S2. Polyfunctionality of pp65-specific T cells derived from naïve T cells.
Fig. S3. Atypical epitope recognition in patient receiving CB-derived virus-specific T cells.
Fig. S4. Shared TCRs in the CB-derived virus-specific T cells and patient P2891 6 months after T cell infusion.
Table S1. Epitope recognition by CMVneg donors.
Table S2. Polyclonality of T cells from CMVneg and CMVpos donors that recognize typical and atypical epitopes.
Table S3. Precursor frequencies of TCRs recognizing NLV and MLN from CB.
Table S4. Inhibition of CMV dissemination by T cells recognizing typical and atypical epitopes.
Source data

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CMV-specific T cells generated from naïve T cells recognize atypical epitopes and may be protective in vivo

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Sourcing CMV immunotherapy

Immunotherapy—such as adoptive T cell therapy—is making headway in treating cancer, autoimmunity, and infectious disease. Indeed, adoptive transfer of cytomegalovirus (CMV)–specific T cells can restore immunity to the virus. However, these cells have been primarily derived from memory cells from CMV-seropositive individuals, which limits the donor pool. Now, Hanley *et al.* demonstrate that CMV-specific T cells can be derived from naïve T cells taken from CMV-seronegative people. These cells react to different epitopes than the memory cell–derived T cells but with similar avidity. What's more, these cells were associated with periods of CMV-free survival when transplanted into patients. These data support expanded trials of adoptive therapy of CMV-restricted T cells from seronegative donors.

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