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B Lymphoblastoid Cell Lines as Efficient APC to Elicit CD8\(^+\) T Cell Responses Against a Cytomegalovirus Antigen\(^1\)

Qi Sun,* Robert L. Burton,* Li-Jun Dai,* William J. Britt,† and Kenneth G. Lucas\(^2\)*

Potent and readily accessible APC are critical for development of immunotherapy protocols to treat viral disease and cancer. We have shown that B lymphoblastoid cell lines (BLCL) that stably express CMV phosphoprotein 65 (BLCLpp65), as a result of retroviral transduction, can be used to generate ex vivo CTL cultures that possess cytotoxicity against CMV and EBV. In this report, we demonstrate that the EBV-specific cytotoxicity in the BLCLpp65-primed culture had a spectrum of EBV-Ag recognition similar to that of the BLCL-primed counterpart, suggesting that retroviral transduction and expression of the CMV Ag would not compromise the Ag-presenting capacity of BLCL. In addition, BLCLpp65 appeared to present multiple natural pp65 epitopes, because pp65-specific CTL, which recognized different CMV clinical isolates, were generated in BLCLpp65-primed cultures from individuals with various HLA backgrounds. Consistent with a polyclonal expansion of virus-specific CTL, T cell lines established from the BLCLpp65-primed CTL cultures expressed different TCR-V\(_\beta\). Although most of the virus-specific T cell isolates were CD8\(^+\), EBV-specific CD4\(^+\) T cell lines were also established from BLCLpp65-primed cultures. Western blot analysis revealed that the CD8\(^+\) lines, but not the CD4\(^+\) line, expressed granzyme B, consistent with features of classic CTL. Thus, our results suggested that BLCL stably expressing a foreign Ag might be used as a practical APC to elicit CD8\(^+\) T cell responses. *The Journal of Immunology, 2000, 165: 4105–4111.

Epstein-Barr virus and CMV infections are often self-limiting in immunocompetent individuals (1, 2). However, they can be serious and potentially fatal in immunocompromised patients, such as recipients of allogeneic, T cell-depleted stem cell transplants (3, 4). The increased susceptibility to CMV and EBV disease has been attributed to defective cellular immunity. In T cell-depleted stem cell transplant patients, immunodeficiency is a result of manipulation of the graft to lower T cell dose as well as pharmacological immunosuppression aimed at preventing graft-versus-host disease and graft rejection (5–7). Clinical observation and animal models suggest that CD8\(^+\) T cells are particularly important for controlling CMV and EBV reactivation in this group of patients. Over 65% of patients at day 40 posttransplant are deficient in CD8\(^+\) T cell responses to CMV and at risk for CMV disease (7, 8). A correlation also exists between the peak incidence of EBV disease and levels of CD8\(^+\) EBV-specific CTL in stem cell transplant patients, who are most susceptible to developing this complication during the first few months posttransplant (5). Furthermore, adoptive transfer of virus-specific CD8\(^+\), rather than CD4\(^+\), T cells most effectively conveys protection against CMV reactivation in animal models (9). Due to the side effects of pharmacologic agents, such as myelosuppression and nephrotoxicity, as well as problems with CMV drug resistance, immunotherapy has been actively explored to restore host virus-specific cellular immunity (6, 10, 11).

Adoptive immunotherapy involves the infusion of ex vivo expanded, virus-specific CTL into susceptible patients (12). This strategy has been proven to be efficacious in treating and preventing EBV and CMV complications (11, 13–16). EBV-specific CTL can be prepared from EBV-seropositive PBMC by cocultivation with autologous B lymphoblastoid cell lines (BLCL) (13, 11), which can be readily established from B cells by ex vivo infection with a laboratory strain of EBV (17). CMV-specific T cells have also been generated with CMV-infected autologous skin fibroblasts (SF) as stimulator cells (14–16). Although infusion of in vitro-expanded BLCL-stimulated CTL has been shown to provide long-term protection against EBV (11, 13), the protection against CMV from infecting CD8\(^+\) clones is more transient, perhaps as a result of failed reconstitution of CD4\(^+\) T cell functions (18).

We have reported a novel system to generate ex vivo CTL cultures that are cytotoxic to both EBV and CMV (19). This strategy takes advantage of BLCLpp65, which are retrovirus-transduced BLCL expressing CMV phosphoprotein 65 (pp65) (20) to present both EBV and CMV Ags. CTL cultures primed with BLCLpp65 have EBV- and CMV-specific cytotoxicity, which is associated with CD8\(^+\), MHC class I-restricted cellular components (19). Although this system promises a feasible alternative treatment for posttransplant complications with EBV and CMV, several questions need to be addressed before its clinical application. The most important ones include whether the transduced CMV gene would affect the presentation of EBV Ags, and whether the pp65-primed CTL would recognize wild-type CMV.

We show in this report that: 1) pp65 expression in BLCL did not affect the spectrum or effectiveness of EBV Ag presentation, 2) BLCLpp65-primed CTL recognized multiple clinical CMV isolates, and 3) BLCLpp65 stimulated the expansion of multiple CD8\(^+\) virus-specific CTL clones. Our results indicated that, in

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addition to providing an immediate application in treatment of posttransplant CMV and EBV complications, BLCL could potentially be a versatile APC for T cell immunotherapy.

**Materials and Methods**

**Donors**

Four healthy CMV-seropositive and two seronegative donors provided PBMC, SF, and serum for this study under protocols approved by the Institutional Review Board of the University of Alabama at Birmingham (UAB). CMV serostatus was determined by the UAB Core Immunology Laboratory with an IgG enzyme-linked immunosorbent assay (Abbott Laboratories, Chicago, IL). MHC class I and II typing was performed by the UAB Histocompatibility Laboratory.

**Expression vectors and viruses**

MSCVpp65, a recombinant retrovirus encoding CMV pp65, has been described elsewhere (19). In brief, CaCl2-purified construct DNA was electroporated into psi-cir cells, an ectopic packaging cell line (21). Culture medium from the transfected psi-cir cells was used to infect the amphotropic producer cell line GP-envAM12 (22). The producer cells were grown in DMEM (Mediatech, Herndon, VA) with 10% FBS (HyClone, Logan, UT) and were selected with G418 (Geneticin; Life Technologies, Rockville, MD) at 100 μg/ml for 7 days. The virus-containing media was collected after the producer cell line was grown to confluence, and this supernatant was used to infect either human SF or BLCL. Retrovirus titer ranged from 5 × 10^5 to 10^6/ml.

Recombinant vaccinia viruses encoding EBV peptides were gifts from Michael Kurilla (University of Virginia, Charlottesville, VA). Their preparation has been described previously (23). Briefly, virus stocks were prepared in human AD169 cells (American Type Culture Collection, ATCC, Manassas, VA). After infection at a multiplicity of infection (MOI) of 0.1, the cells were cultured for 48 h and then harvested and underwent three cycles of freeze and thaw. Virus suspension was cleared of debris by centrifugation, and stored in aliquots at −20°C. Vaccinia virus titers ranged from 1 × 10^7 to 10^8/ml as determined by plaque assays in BSC-40 cells.

Human CMV AD169 strain was obtained from ATCC and propagated in human SF. The initial infection was at an MOI of 0.1. Five days after cytopathological effects appeared in more than 90% cells, the virus-containing medium was collected, passed through a 0.45-μm filter, and frozen in liquid nitrogen as aliquots. CMV titer was between 1 × 10^6 and 10^7/ml by plaque assays in human SF. Clinical CMV isolates were collected from patients undergoing bone marrow transplantation or neonates at Children's Hospital (Birmingham, AL). Preparation of clinical isolates has been described (24), and all the isolates were passed no more than 10 times. Titers were 1 × 10^6 PFU/ml for isolates DD and PO, and 1 × 10^7 PFU/ml for CO. The pp65-defective mutant CMV RV (25) is a gift from B. Kopp (Institut National de la Santé et de la Recherche Médicale, Marne-La-Vallée, France) and was propagated in human SF. The initial infection was at an MOI of 0.1. Five days after cytopathological effects appeared in more than 90% cells, the virus-containing medium was collected, passed through a 0.45-μm filter, and frozen in liquid nitrogen as aliquots. CMV titer was between 1 × 10^6 and 10^7/ml.

**Protein analysis**

Immunoblotting used 0.2% SDS-polyacrylamide gel, and transferred to Hybond-P membranes (Amersham, Arlington Heights, IL). The blots were blocked with 5% nonfat milk in PBS-T (0.1 M phosphate buffer containing 200 mM NaCl and 0.05% Tween 20), and then probed with the mAb against granzyme B (2C5; Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:100 dilution for 60 min at room temperature. An HRP-labeled sheep anti-mouse IgG Ab (Amersham) was used as the secondary Ab at a dilution of 1:2000. Enhanced chemiluminescence (ECL) detection was performed with ECL detection agents (Amersham) and recorded on Hyperfilm-ECL film (Amersham).

**Ex vivo expansion of CTL**

Ex vivo CTL cultures were established as described (19). Briefly, PBMC were cocultivated with autologous BLCLpp65 in 24-well-plates (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ) in RPMI 1640 supplemented with 10% FBS and 50 μM 2-ME (Sigma). BLCL were exposed to 100 Gy of γ irradiation before use as stimulator cells. The CTL cultures were primed weekly following a regimen of decreasing responder-stimulator ratios from 40:1 at day 0, 20:1 at day 7, and 5:1 on day 14 over a period of 3 wk. IL-2 (Collaborative Biomedical Products, Bedford, MA) was added 10 days after the initial stimulation to a final concentration of 2.5 IU/ml, and medium was then changed every 3 days by replacing one-half of the supernatant with fresh medium.

**Chromium release assays**

Chromium release assays were performed as previously described (5) with an E:T ratio of 12:5:1 (11, 19). Target cells were labeled with 51Cr (New England Nuclear, Boston, MA) overnight (100 μCi/10^6 cells) and labeled cells were either trypsinized for SF or by centrifugation for BLCL. Cells were then washed in PBS and dispensed in triplicate into 96-well V-bottom plates (ICN, Costa Mesa, CA) at × 10^3 cells/well. Spontaneous release and total release for each target were used to calculate percent specific release as follows: % specific release = (experimental cpm − spontaneous cpm)/(total cpm − spontaneous cpm).

**RT-PCR for TCR-Vβ**

Whole RNA was isolated from 3–5 × 10^6 T cells using 1 ml Tri Reagent (Molecular Research Center, Cincinnati, OH). First-strand cDNA was synthesized with the SuperScript premplification system (Life Technologies). The primers used for Vβ amplification were a common 3′ primer and 1 of the 24 5′ primers according to Genevee (26). A pair of TCR Cα primers was included in each reaction as an internal control. The reaction cycles were: 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min, with a total 30 cycles, followed by 72°C for 5 min. The PCR products were separated by agarose gel electrophoresis, stained with ethidium bromide, and evaluated visually.

**Flow cytometric determination of cell surface and intracellular markers**

Flow cytometry was performed on a FACScan (Becton Dickinson). Surface markers of EBV/CMV CTL were determined as described previously (19) by staining with directly conjugated mAbs specific for CD3, CD4, CD8, and CD16/56 (Becton Dickinson). Multiple-color staining of immunophenotypic markers, both surface and intracellular, was performed according to Kuzushima (27) with modifications. In brief, PBMC or cultured T cells were incubated with equal numbers of stimulators, including BLCLpp65, SF, or SFpp65, in RPMI 1640 with 10% FCS and 10 μg/ml brefeldin A (Sigma) at 37°C for 5 h. After incubation with 1 μM EDTA in PBS at 37°C for 15 min, cells were then fixed in 4% paraformaldehyde in PBS at 37°C for 5 min. After incubation in FACS permeabilization buffer (Becton Dickinson) for 10 min, cells were aliquoted and stained with the following labeled Abs (Becton Dickinson): CD69-FITC, γ-IFN-PE and CD8-peridinin chlorophyll protein for IFN staining, or CD3-APC, CD4-phycoerythrin, and CD56-FITC and perforin-PE for perforin detection. Control Abs were the respective isotypes.

**Results**

**BLCL transduced with pp65 retained Ag-presenting characteristics**

To generate bispecific CTL for adoptive immunotherapy, we stimulated PBMC with autologous BLCLpp65 derived from transduction of BLCL with the recombinant retrovirus MSCVpp65 (19). Because retroviral transduction involves selective growth of cells expressing a drug selection marker, one concern was whether the transduced BLCL would retain its native Ag-presenting characteristics. Also pertinent to this question was that pp65 has been shown to be involved in down-regulating Ag presentation of CMV IE protein (28, 29). To ensure that the expression of pp65 would not affect Ag presentation by BLCL, BLCL and the derivative
BLCLpp65 were tested in parallel as stimulators for priming autologous PBMC. The resultant CTL cultures were then tested against a panel of autologous SF that were infected with recombinant vaccinia viruses encoding individual EBV peptides.

Fig. 1A shows that, when tested at day 21, BLCLpp65-, but not BLCL-, primed CTL cultures from donor ND0104 (HLA A02/29, B50/44, C06/16) killed the pp65-expressing autologous SF, but not the untransduced SF. The same culture also showed specific lysis of autologous BLCL. This is consistent with our previous observation that BLCLpp65-stimulated PBMC contain specific cytotoxicity against both pp65 and EBV. When tested against fibroblast targets infected with vaccinia viruses encoding individual EBV peptides, BLCLpp65-primed CTL showed 54%, 19%, and 16% specific lysis against targets expressing EBV-encoded nuclear Ag (EBNA3C), latent membrane protein (LMP)2A, and EBNA3B, respectively. No cytotoxicity was detected against EBNA2- and EBNA3A-expressing targets. Significantly, the BLCLpp65-primed CTL had a spectrum of EBV Ag recognition very similar to the BLCL-primed CTL, although the specific cytolytic activity was marginally but consistently higher in the latter. The somewhat lower EBV-specific cytolytic activity seen in the BLCLpp65-primed CTL may reflect a reduced E:T ratio, because pp65-specific CTL could account for a significant proportion in the BLCLpp65-primed culture. For instance, nearly 50% of the CD8$^+$ cells were pp65-specific in the BLCLpp65-primed CTL culture from donor ND1917 (see below, Fig. 3).

Similar results were obtained with CTL cultures from two additional donors. BLCLpp65 stimulation also activated pp65- and EBV-specific cytotoxicity in CTL cultures from donors ND1920 (HLA A02/11, B44/55, C05/03) (Fig. 1B), ND1917 (HLA A24/30, B13/37, C06/06) (Fig. 1C), and ND1802 (HLA A25/26, B38/51, Cw4) (data not shown). Although CTL from ND1920 recognized LMP2A and EBNA3C, the major Ag was LMP2A, rather than EBNA3C, as seen in ND0104 (Fig. 1B). In contrast, the BLCLpp65-primed CTL from donor ND1917 recognized EBNA3B and 3C as the major EBV Ags (Fig. 1C). In all the cases tested, the spectrum of EBV Ags recognized by BLCLpp65-primed CTL was similar to that by those stimulated with BLCL. This suggested that expression of pp65 did not compromise presentation of EBV Ags by BLCL. It is also noted that, whereas ND0104 and ND1920 shared the relatively common HLA alleles A2 and B44, donors ND1917 and ND1802 carried neither A02 nor B44. Thus, BLCLpp65 was capable of presenting pp65 epitopes in individuals with various HLA backgrounds.

**FIGURE 1.** BLCL and BLCLpp65-primed CTL had similar spectra in EBV Ag recognition. CTL cultures from donors ND0104 (A), ND1920 (B), and ND1917 (C) were primed with BLCL or BLCLpp65, followed by cytotoxicity assays against SF infected with recombinant vaccinia encoding individual EBV peptides (BHFR, LMP2A, EBNA2, EBNA3A, EBNA3B, or EBNA3C). SFvv65 and SFvv150 are SF infected with vaccinia encoding CMV pp65 and pp150. SF MSCVpp65 is SF transduced with pp65.

**BLCLpp65-primed CTL recognized low passage clinical CMV isolates**

We have shown that CTL cultures stimulated with BLCLpp65 possess specific cytotoxicity against autologous targets infected with the laboratory strain CMV AD169 (19), suggesting that the pp65-specific cytotoxicity could be effective in a setting of natural CMV infection. However, because the pp65 gene cloned in the retroviral construct is derived from the Towne strain CMV (20), which has been propagated in culture for an extended period of time, it was necessary to evaluate the cytotoxicity of the BLCLpp65-primed CTL with clinical CMV isolates that have been in culture for a lesser period of time.

BLCLpp65-primed CTL from donor ND1917 were tested against SF targets infected with a panel of four clinical isolates (DD, PO, CO and DE), all of which were below the tenth in vitro passage. Control viruses included AD169, a laboratory strain, and RV, a pp65-knockout mutant (25). Although AD169, DD, and PO could be propagated to titers sufficient for an MOI of 3, CO, DE, and RV could only be produced at substantially low titers, and, as a result, targets were only available at an MOI of 0.3. Fig. 2 shows that BLCLpp65-primed CTL lysed the DD- and PO-infected targets with efficiencies of 32% and 25%, respectively, comparable to the 32% for those infected with AD169. Furthermore, at a suboptimal MOI of 0.3, BLCLpp65-primed CTL also lysed CO- or DE-infected fibroblasts, although the efficiency was lower (15%). In contrast, no cytotoxicity was observed for the noninfected targets and those infected with the pp65-mutant CMV strain RV. These results confirmed our expectation that T cells stimulated by
BLCLpp65 would recognize clinical strains of CMV. It was consistent with the observation that pp65 sequences are well conserved among CMV strains (20). A very recent study also showed that pp65 HLA-A02-restricted epitopes are conserved among CMV strains (30).

BLCLpp65-primed CTL exhibited high relative frequencies of EBV- and pp65-specific CD8$^+$ T cells

To quantitatively evaluate the expansion of virus-specific effectors, we used triple-color flow cytometry to measure the relative frequencies of CD8$^+$ cells specific to EBV and pp65. Concurrent expression of CD69 and IFN-γ in response to the stimulation by Ag-expressing cells was used as the marker for activated specific T cells (27, 31). Quantification was achieved by enumerating CD69/IFN-γ-expressing CD8$^+$ cells after induction with BLCL or SFpp65. As controls, T cells not induced or induced with SF were also tested in parallel for background staining.

Fig. 3 shows that, after a 5-h incubation with BLCL or SFpp65, only 0.11% and 0.08% CD8$^+$ cells in PBMC from ND1917 coexpressed CD69 and IFN-γ. When compared with the 0.06% background staining, as measured from the noninduced PBMC and those induced with SF, the frequency of activated T cells specific to either EBV or pp65 was negligible in PBMC. In contrast, the Day 33 BLCLpp65-primed CTL culture, which was reprimed 12 days prior, displayed 3.38% and 44.9% CD8$^+$ T cells with an activated phenotype in response to the 5-h induction with BLCLpp65 or SFpp65, respectively. The same CTL culture, when not induced or induced with SF, contained only 0.03% and 0.12% CD8$^+$ T cells positive for IFN-γ/CD69, respectively, consistent with the established observation that IFN-γ and CD69 expression is tightly regulated in response to specific engagement between T cell and target cells (31, 32). Similar results were obtained for the CTL culture from donor ND1802 (data not shown). These data indicated that EBV-specific, and, more prominently, the pp65-specific, CD8$^+$ T cells were preferentially expanded in the BLCLpp65-primed CTL culture.

**Table I. Cytotoxicity and immunophenotypes of T cell lines from BLCLpp65-stimulated CTL.**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>EBV Reactivity</th>
<th>pp65 Reactivity</th>
<th>Phenotype</th>
<th>$V_β^+$ Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1920-1</td>
<td>–</td>
<td>+</td>
<td>CD8$^+$</td>
<td>9</td>
</tr>
<tr>
<td>ND1920-2</td>
<td>–</td>
<td>+</td>
<td>CD8$^+$</td>
<td>9, 16</td>
</tr>
<tr>
<td>ND1920-5</td>
<td>–</td>
<td>+</td>
<td>CD8$^+$</td>
<td>9</td>
</tr>
<tr>
<td>ND1920-6</td>
<td>–</td>
<td>+</td>
<td>CD8$^+$</td>
<td>9</td>
</tr>
<tr>
<td>ND1920-16</td>
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<td>+</td>
<td>CD8$^+$</td>
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<td>13, 24</td>
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<td>ND1920-14</td>
<td>+</td>
<td>+</td>
<td>CD8$^+$</td>
<td>8, 24</td>
</tr>
<tr>
<td>ND1920-17</td>
<td>+</td>
<td>+</td>
<td>CD8$^+$</td>
<td>6, 9</td>
</tr>
</tbody>
</table>

BLCLpp65 stimulated expansion of multiple clones of virus-specific CTL

To further characterize the composition of effector cells in the BLCLpp65-primed CTL cultures, T cells were isolated by limiting dilution. The established cell lines were examined for the expression pattern of TCR-$V_β$ by RT-PCR with 24 pairs of specific primers, allowing us to distinguish the clonality between cell lines (33). In parallel, specific cytotoxicity was evaluated for the lines by chromium release assay against BLCL and SFpp65. Table I summarizes cytotoxicity and TCR-$V_β$ expression of the isolates from donor ND1920. Among the seven ND1920 lines recognizing only pp65, four lines expressed only TCR-$V_β$, whereas $V_β^{13/24}$ and $V_β^{9/24}$ were detected in lines ND1920-28 and ND1920-16, respectively. Because $V_β^9$ was expressed in ND1920-14 and $V_β^{24}$
in ND1920-17, both of which recognized EBV and pp65, pp65-specific cytotoxicity appeared to be associated with the expression of \( V_{\mu}9 \), \( V_{\mu}24 \), and possibly \( V_{\mu}13 \). Conversely, expression of \( V_{\delta}6 \) and \( V_{\rho}8 \) was correlated with EBV-specific cytotoxicity in CTL cultures from donor ND1920. Thus, both pp65- and EBV-specific CTL may have been derived from at least two independent precursors. These results also suggested that EBV- and pp65-specific cytotoxicities in the BLCLpp65-primed T cell cultures were two independent components mediated by separate effector T cells.

**EBV-specific cytotoxicity of CD8\(^+\), but not CD4\(^+\), T cells was associated with the expression of perforin/granzyme B**

We have shown that both EBV- and pp65-specific cytotoxicities in the BLCLpp65-primed CTL are associated with the CD8\(^+\), but not the CD4\(^+\), cell fraction (19). In our further studies, we were able to isolate not only CD8\(^+\), but also CD4\(^+\) T cell lines from CTL cultures stimulated with BLCL as well as BLCLpp65 (Table II). To investigate the function of the CD4\(^+\) lines, they were tested for specific cytotoxicity in parallel with selected CD8\(^+\) lines as controls (Fig. 4). In a standard 4-h test, ND1802-39, the CD4\(^+\) line from BLCLpp65-stimulated culture, showed significant cytotoxicity against BLCL, but not PHA blasts, SF, or SFpp65. ND1802-41 and -34, the CD8\(^+\) controls, were cytotoxic to BLCL and SFpp65, respectively. Similarly to ND1802-39, the CD4\(^+\) line from BLCL-stimulated culture, ND0612-126, was also BLCL-cytolytic (data not shown, summarized in Table II). EBV-specific CD4\(^+\) CTL have been reported by others (34–37) and very recently were shown to be specific to EBNA1 in a dendritic cells-based system (38).

The T cell lines were then examined for the expression of effector molecules involved in granzyme/perforin-mediated cytotoxicity (39, 40). For the lines isolated from BLCLpp65-stimulated cultures, Western blot with the Ab 2C5, which is specific to granzyme B, detected a single band in the CD8\(^+\) cells, CD4\(^+\) and CD4\(^+\) T cells, respectively. Although most somatic cells express HLA class I and II complexes to CD8\(^+\) cells, CD4\(^+\) T cells are capable of carrying out defined functions. The process of T cell activation involves the interplay between T cells and APC, in which APC present antigenic peptides in the context of HLA class I, or II complexes to CD8\(^+\), or CD4\(^+\) T cells, respectively. Although most somatic cells express HLA class I molecules

### Table II. Expression of granzyme/perforin in T cell lines from BLCLpp65 or BLCL-stimulated culture

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Stimulator</th>
<th>EBV Reactivity</th>
<th>pp65 Reactivity</th>
<th>Phenotype</th>
<th>Granzyme/Perforin</th>
</tr>
</thead>
<tbody>
<tr>
<td>ND1802-34</td>
<td>BLCLpp65</td>
<td>–</td>
<td>+</td>
<td>CD8(^+)</td>
<td>+</td>
</tr>
<tr>
<td>ND1802-39</td>
<td>BLCLpp65</td>
<td>+</td>
<td>+</td>
<td>CD4(^+)</td>
<td>–</td>
</tr>
<tr>
<td>ND1802-41</td>
<td>BLCLpp65</td>
<td>+</td>
<td>NT(^a)</td>
<td>CD8(^+)</td>
<td>+</td>
</tr>
<tr>
<td>ND0612-23</td>
<td>BLCL</td>
<td>+</td>
<td>NT(^a)</td>
<td>CD8(^+)</td>
<td>–</td>
</tr>
<tr>
<td>ND0612-90</td>
<td>BLCL</td>
<td>–</td>
<td>NT(^a)</td>
<td>CD8(^+)</td>
<td>–</td>
</tr>
<tr>
<td>ND0612-126</td>
<td>BLCL</td>
<td>+</td>
<td>NT(^a)</td>
<td>CD4(^+)</td>
<td>–</td>
</tr>
</tbody>
</table>

\( ^a \) NT, Not tested.

**Discussion**

The goal of adoptive immunotherapy is to establish specific immunity by infusing ex vivo stimulated T cells (8). This concept is based on the knowledge that T cell-mediated immunity is the major specific immune mechanism providing protection against viral infections. While CD8\(^+\) T cells efficiently destroy virus-infected cells, CD4\(^+\) T cells serve to regulate and coordinate functions of T cells, B cells, and other immune cells. However, only activated T cells are capable of carrying out defined functions. The process of T cell activation involves the interplay between T cells and APC, in which APC present antigenic peptides in the context of HLA class I, or II complexes to CD8\(^+\), or CD4\(^+\) T cells, respectively.

![Figure 4. Cytotoxicity of the CD8\(^+\) and CD4\(^+\) T cell lines established from BLCLpp65-primed CTL culture.](http://www.jimmunol.org/)

T cell line ND1802-34 recognized only pp65, whereas ND1802-39 and ND1802-41 were cytotoxic only to EBV-infected cells. Note ND1802-39 was CD4\(^+\), whereas ND1802-41 and ND1802-34 were CD8\(^+\).
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and are subject to specific lysis by CD8+ T cells, only specialized cells have potent and complete Ag-presenting potentials. These professional APC express both HLA class I and II molecules, together with costimulatory molecules that mediate physical interaction and signal transduction between T cells and APC. Due to their importance in initiating T cell responses, professional APC have been the focus of active research and remain a major hurdle in many cases for the development of effective immunotherapy.

We explored BLCL as practical APC for adoptive immunotherapy against CMV and EBV reactivation that may occur concurrently in BMT patients posttransplant (42). B cells play unique roles in T cell-mediated immunity (43), actively presenting Ags captured either with surface Ig receptors or by pinocytosis. Consistent with this Ag-presenting function, B cells express HLA class I, class II, and costimulatory molecules, including B7, ICAM-1, and LFA-3. Immortalized by EBV, BLCL have been shown to retain their differentiation status and immunophenotypes after transformation (44). In fact, BLCL represent a successful example for APC used in immunotherapy protocols, generating EBV-specific CTL for treatment of EBV-induced lymphoproliferative disease in patients during the posttransplant period (13, 45). However, the potential of BLCL for presentation of non-EBV Ags has not been demonstrated, although attempts have been made to study its Ag presenting characteristics (46). It has been reported that BLCL have inferior capacity to present soluble Ags in comparison to monocytes (47, 48). In contrast, BLCL preincubated with Ag preparations do stimulate proliferation of established CTL clones, either CD4+ or CD8+ (49, 50).

In this study, we systemically characterized the T cells activated and expanded ex vivo in response to stimulation by BLCLpp65. We demonstrated clearly by FACS analysis of T cell activation markers that the relative frequency of pp65-specific CD8+ T cells was greatly increased by BLCLpp65 stimulation (Fig. 3). The expansion of virus-specific CD8+ CTL is particularly important for adoptive immunotherapy, because CD8+ CTL, rather than CD4+ T cells, directly contribute to protection against CMV and EBV reactivation in patients posttransplant. Very recently, Chen et al. (51) reported that BLCL expressing HCV core Ag activate CD4+, rather than CD8+, T cells. Although many differences exist between their system and ours, most noteworthy is that we used a recombinant retrovirus to introduce the transgene, instead of transfection. Transgene expression mediated by retroviral transduction is stable and long lasting, and can be achieved at relatively high levels, in contrast to the transient and more variable expression in transfection-based delivery systems. Another explanation for the preferential stimulation of CD4+ cells by the endogenously synthesized HCV core Ag may lie in the inherent nature of this protein itself. Indeed, although specific cytotoxicity is detected only in the CD8+ T cell fractions (19), we isolated CD4+, EBV-specific CTL lines from the same culture (Fig. 4), consistent with Chen and other’s observation that BLCL could prime CD4+ T cells (34–38). A significant feature of our system is its versatile applicability, which in part was due to the full-length expression of pp65 in the transduced BLCL. Expression of the entire Ag enhances the possibility that multiple epitopes of a peptide would be presented in the context of various HLA alleles. Thus, unlike pulsing APC with peptide epitopes (30, 52), there would be no need of prior detailed knowledge on the epitopes and their corresponding HLA restrictions. This benefit was demonstrated by our data indicating that BLCLpp65 elicited pp65-specific T cell responses from individuals with distinct HLA backgrounds, including those carrying (ND0104 and ND1920) and not carrying (ND1917 and ND1802) A02, the allele upon which previous studies have been focused (30, 52). The existence of pp65-specific T cell lines with distinct lineages in the BLCLpp65-primed CTL culture is also consistent with the possibility that multiple, different epitopes on pp65 were presented by BLCLpp65.

Another important advantage of the BLCL-based APC system is its accessibility. BLCL can be readily established from PBMC, conveniently transduced with retroviral vectors encoding target Ags, and quickly expanded to large numbers. In contrast, dendritic cells (DC), potent APC capable of initiating naive T cell responses, have extremely low yields with current cultivation methods in comparison to BLCL. Because ex vivo T cell activation usually requires multiple primings, the availability of DC can be a restraint to applying DC for T cell therapy. Thus, BLCL would be a more convenient source of APC for stimulating T cells from seropositive individuals. In consideration of the proposal that B cells are inherently adept in priming naive T cell responses (53), a protocol combining BLCL and DC may find applications in eliciting naive T cell responses. In this scenario, for example, DC could be used in the initial priming, followed by BLCL to further promote T cell expansion.

In summary, we propose that BLCL could be developed as a versatile APC in adoptive immunotherapy. Based on the results from our studies, a phase I/II clinical protocol has been developed and approved for the use of BLCLpp65 as APC in adoptive immunotherapy against post-BMT CMV and EBV complications (54).

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References


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