In Vitro Human Memory CD8 T Cell Expansion in Response to Cytomegalovirus Requires CD4+ T Cell Help

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Requirements for human memory CD8+ T cell expansion are incompletely understood. We found that human cytomegalovirus (HCMV) induced expansion of memory CD8+ T cells in vitro without requiring intracellular viral peptide synthesis. Peptide–major histocompatibility complex class I tetramer binding confirmed expansion of cells with HCMV-peptide specificity. Expansion of memory CD8+ T cells was completely dependent on the presence and function of CD4+ T cells, whose “help” also could be induced by exposure to irrelevant antigen. Recombinant interleukin (IL)–2 or IL-15 could substitute for help provided by CD4+ T cells, whereas CD8+ T cell expansion was blocked by anti–IL-2 but not anti–IL-15 antibody. Human memory CD8+ T cells expand dramatically in vitro in response to cross-presentation of HCMV antigens, and, in contrast to observations made in murine systems, this proliferation was critically dependent on CD4+ T cells that provide essential IL-2. Thus, in humans, cross-presentation and expansion of memory CD8+ T cells may be compromised in disease states that result in deficits in CD4+ T cell numbers or function, such as may be seen in human immunodeficiency virus type 1 infection.

CD8+ cytotoxic T lymphocytes (CTLs) comprise an important host defense for controlling viral infections in humans. Chronic viral infections, such as those caused by human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), and HIV-1, are characterized by the expansion of virus-specific CD8+ T cells [1–5]. The sustained presence of virus-specific CD8+ T cells is essential to keep virus replication in check, and loss of these cells is associated with renewed virus growth [6–8]. Murine studies have shown that, although CD4+ T cells may not be necessary for the induction of CD8+ T cell responses in acute viral infection [9] or first antigen (Ag) exposure [10–13], memory CD8+ T cells generated in the absence of CD4+ T cell help may be less capable of adequate subsequent expansion and that secondary expansion of memory CD8+ T cells is otherwise not dependent on CD4+ T cell help [10].

Exogenous protein Ags are processed by Ag-presenting cells and are presented via major histocompatibility complex class I (MHC-I) molecules to CD4+ T cells. Cross-presentation to CD8+ T cells can occur, however, when exogenous protein Ags gain access to the MHC-I pathway and are processed and presented via MHC-I molecules. Two routes of cross-presentation have been proposed. The first involves transfer of exogenous Ags from vacuolar compartments to the cytosol of Ag-presenting cells for processing and presentation in the conventional MHC-I pathway [14]. The second route involves vacuolar processing and presentation of Ags [15–18] with peptides binding to MHC-I molecules either within vacuolar compartments or at the cell surface after recycling and regurgitation of peptides. These pathways have been well established in vitro, but direct evidence that this occurs in vivo during human viral infections is less clear.

HCMV is a β-herpesvirus that establishes chronic infection after what is generally considered to be a clinically benign infection. Approximately 50% of healthy
blood donors have serum IgG against HCMV. In HCMV-infected individuals, low-level persistent infection is thought to be controlled by virus-specific CD8+ T cells, and relatively large numbers of HCMV-specific CD8+ T cells persist during latent subclinical infection [19–24]. Clinically apparent reactivation of HCMV rarely occurs, except in immunocompromised patients.

In the present study, we used dye-dilution techniques and flow cytometry to study in vitro memory CD8+ T cell expansion in response to HCMV Ags. CD8+ T cells from otherwise healthy HCMV-seropositive individuals proliferated after exposure to HCMV Ags, even in the absence of new viral protein synthesis. Using HCMV peptide–HLA class I tetrameric complexes [25], in combination with other cell surface markers (CD8 and CD25), we visualized Ag-specific CD8+ T cell expansion. We found that CD8+ T cell expansion was entirely dependent on the presence of CD4+ T cells and was blocked by a monoclonal antibody (MAb) to IL-2. Exogenous IL-2 and/or IL-15 could replace the requirement for CD4+ T cells, but only MAb against IL-2 prevented Ag-driven CD8+ T cell proliferation in vitro. This system can be used to explore cooperation among CD4+ T cells, CD8+ T cells, and Ag-presenting cells that are permissive of memory CD8+ T cell expansion and cross-presentation in chronic viral infection.

**MATERIALS AND METHODS**

**Cell preparation and culture conditions.** Blood from 7 healthy HCMV-seropositive blood donors was drawn into sodium heparin-containing Vacutainer tubes (Becton Dickinson). Peripheral blood mononuclear cells (PBMCs) were purified by Ficoll-Hypaque (Pharmacia) density sedimentation. PBMCs were either left unstained or immediately stained with 10 µmol of 5-(and–6) carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes) for 10 min in PBS containing 0.1% bovine serum albumin (BSA) at 37°C. Cells were washed 3 times in RPMI 1640 medium (BioWhittaker) with 10% heat-inactivated serum albumin (BSA) at 37°C. This system can be used to explore cooperation among CD4+ T cells, CD8+ T cells, and Ag-presenting cells that are permissive of memory CD8+ T cell expansion and cross-presentation in chronic viral infection.

**Magnetic bead separations.** CD8+ T cells were positively selected by use of magnetic beads (Dynal). In brief, PBMCs (5 × 10⁶ cells) were incubated with 144 µL of prewashed mouse anti-human CD8 microbeads at 4°C for 30 min with constant rotation/mixing. Bead-bound cells were isolated by magnetic separation, were washed 4 times with RPMI 1640 medium with 1% FBS, and were resuspended in 100 µL of RPMI 1640 medium with 1% FBS. Beads were detached by use of DETACHaBEAD, according to the manufacturer’s instructions (Dynal), and were incubated for 1 h at room temperature with gentle mixing. In some experiments, CD14+ cells were removed by binding to mouse anti-human CD14 Microbeads (Miltenyi Biotec), and CD4+ T cells were removed by binding to mouse anti-human CD4 Microbeads (Miltenyi Biotec). Bead-bound cells were cultured in a selection column attached to a magnet, whereas non-bead-bound cells were passed though the column.

**In vitro proliferation of CFSE-labeled cells.** To assess the responses to Ag stimulation, CFSE-labeled PBMCs were cultured with HCMV lysate (1:40 dilution; BioWhittaker); in some experiments, HCMV preparations were either exposed for 100 min to UV light in a tissue culture hood or boiled for 10 min to inactivate virus. An uninfected control lysate (HCMV control lysate; BioWhittaker) was used at the same dilution as the HCMV lysate (1:40 dilution). A murine anti–CD3 MAb (333 ng/mL; BD Pharmingen), tetanus toxoid (4 lfu/mL; Wyeth Ayerst), and HCMV pp65905–903 peptide NIVPMVATV (NILV9; Invitrogen) were used in some experiments to induce cellular proliferation. HCMV pp65905–903 peptide was dissolved in PBS (pH 7.2) and was used at a concentration of 10 µg/mL, in addition to other Ags, or supplemented with 3.6 IU/mL IL-2 (Chiron) or 10 ng/mL IL-15 (Immunex). Antibody-blocking experiments were performed by use of 1 µg/mL concentrations of MAb directed against human (hu) IL-2 (clone 5334.21; R&D Systems) and against huIL-15 (clone 34505.11; R&D Systems) or an IgG1 isotype control (clone 11711.11; R&D Systems).

In some experiments, HCMV was purified from viral lysate. In brief, 1 mL of HCMV lysate was clarified by centrifugation for 20 min. Supernatant was overlayed onto a 60% sucrose (wt/wt) cushion. Virus was subjected to ultracentrifugation at 28,000 g for 1 h in a Beckman SW41Ti rotor at 4°C without braking. The virus band was removed, washed, resuspended in 1 mL of PBS, passed through a 0.45-µm filter (Millipore), and used at 1:40 dilution.

**Cell surface and tetramer staining for flow cytometry.** Cells were stained with 1 µg of CD4-allophycocyanin ( APC; Becton-Dickenson), 10 µL of CD8-peridinin chlorophyll protein (PerCP; Becton-Dickenson), and 5 µL of CD25-phycocyanin (PE; Immunotech) or 5 µL of IgG2a-PE isotype control (BD Pharmingen) for 10 min at room temperature. Cells were washed with PBS containing 1% BSA and 0.1% sodium azide (Sigma) and were fixed in PBS containing 1% paraformaldehyde (Electron Microscopy Science) before analysis.

To assess the frequency of tetramer-reactive CD8+ T cells, unlabeled PBMCs were stained immediately after Ficoll separation (or after cultivation) with 10 µL of CD8-PerCP, 5 µL of CD4-APC, and 10 µL of MHC-I HLA-A*0201 HCMV pp65905–903 (NILV9)-PE tetramer (Beckman Coulter Immunomics) or HLA-A*0201 EBV BMLF1 (GLCTLVAML)-PE tetramer (Beckman Coulter Immunomics) for 30 min at room temperature. Cells were washed with 4-mL PBS/BSA/azide solution, fixed with PBS/
duced proliferation of CD8+ T cells, there was no expansion of
PE [25], to examine the Ag specificity of the expanding cell
population had completed ≥7 rounds (the limit of resolution) of
cell division and infrequently expressed CD25. The delay in
CD8+ T cell proliferation suggested that CD4+ T cell activation
might be a prerequisite for CD8+ T cell expansion.

To determine whether the CD8+ T cell expansion represented
a T cell receptor (TCR)–driven expansion or a nonspecific “by-
stander” cytokine-driven proliferation of CD8+ T cells, we used
pseud:MH C-I tetramers composed of 4 HLA-A*0201 MHC
cellular responses, CFSE-labeled PBMCs were incubated with me-
ning or with tetanus toxoid. CD4+ and CD8+ T cells (figures 1A and
Pe tetramer showed that the addition of tetanus toxoid supported
CD8+ T cells (figure 2). To determine whether an unrelated Ag
could induce “help” to expand HCMV-peptide specific CD8+ T
cell responses, CFSE-labeled PBMCs were incubated with me-
dium or with tetanus toxoid Ag, and HCMV pp65 NLV9 peptide
was added to some wells. Staining with HCMV pp65<sup>405–903</sup>
NLV9–

CD8+ T cells proliferate in response to HCMV and
tetanus toxoid Ag.  PBMCs were cultured with HCMV or
with tetanus toxoid, CD4+ and CD8+ T cells (figures 1A and
1B, respectively) transiently expressed CD25 and then prolif-
erated in response to both HCMV and tetanus toxoid Ags.
Typically, CD4+ T cells expressed CD25 by day 4, and prolif-
eration was detected by this time (figure 1A). Evidence of Ag-
specific CD8+ T cell activation was delayed (figure 1B), but, by
day 8, both CD4+ and CD8+ T cells responding to Ag stimu-
lization had completed ≥7 rounds (the limit of resolution) of
cell division and infrequently expressed CD25. The delay in
CD8+ T cell proliferation suggested that CD4+ T cell activation
might be a prerequisite for CD8+ T cell expansion.

To determine whether the CD8+ T cell expansion represented
a T cell receptor (TCR)–driven expansion or a nonspecific “by-
stander” cytokine-driven proliferation of CD8+ T cells, we used
teptide:MHC-I tetramers composed of 4 HLA-A*0201 MHC
molecules, each bound to a specific 9-mer peptide derived from
the HCMV pp65 peptide (NLVPMAVT) and conjugated with

CD8+ T cell proliferative responses require CD4+ T cells.
Because CD4+ T cells are important in maintaining Ag-specific
CD8+ T cell responses [9, 26–28] and in light of the relative
delay in CD8+ T cell responses, we examined the role of CD4+
T cells in CD8+ T cell proliferative responses. We depleted CD4+
T cells by use of magnetic beads and MAB to CD4. After de-
pletion, <1.0% of the remaining T cells expressed CD4 (data
not shown). Monocytes were readily detectable in these prep-
arrations after depletion, as determined by CD4<sub>intermediate</sub>
and CD4<sub>bright</sub> staining (data not shown). Before CD4+ T cell de-
pletion, both CD4+ and CD8+ T cells proliferated in response
to HCMV (figure 3A and 3B, respectively). Depletion of CD4+
T cells from the preparation resulted in a failure of CD8+ T
cell proliferation in response to both HCMV and tetanus toxoid
(figure 3C).

Irrelevant Ag can provide help to support HCMV peptide-
specific CD8+ T cell proliferation.  We found that stimulation
with tetanus toxoid Ag provided adequate “help” to support
CD8+ T cell proliferation in response to tetanus toxoid, yet did
not induce a “bystander” expansion of HCMV–NLV9–binding
CD8+ T cells (figure 2). To determine whether an unrelated Ag
could induce “help” to expand HCMV-peptide specific CD8+ T
cell responses, CFSE-labeled PBMCs were incubated with me-
dium or with tetanus toxoid Ag, and HCMV pp65 NLV9 peptide
was added to some wells. Staining with HCMV pp65<sup>405–903</sup>
NLV9–

CD8+ T cell proliferation in response to HCMV is blocked by
anti–IL-2 but not anti–IL-15 antibody, yet “help” can be pro-
vided by either IL-2 or IL-15.  To identify potential mecha-
nisms of CD4+ T cell “help” for HCMV-induced CD8+ T cell
proliferation, we tested the effects of anti–cytokine MAb in this
system. Mouse anti–huIL-2 and mouse anti–huIL-15 were
added to PBMC cultures stimulated with HCMV. Expression
of CD25 and cell proliferation by CFSE dye dilution were ex-
amined after 6 days in culture. MAB to IL-2 reduced prolif-
eration to near background levels, but MAB to IL-15 had no
effect (figure 5A). These results suggested that IL-2 is necessary
to support HCMV-induced CD8+ T cell proliferation, whereas
IL-15 was not. To ascertain whether helper cytokines could
substitute entirely for CD4+ T cells in supporting CD8+ T cell
proliferation in response to HCMV, IL-2 and IL-15 were added
to cell cultures depleted of CD4+ T cells (containing <1% con-
taminating CD4 T cells). Cells characteristic of monocytes, as
determined by CD4<sub>intermediate</sub> or CD4<sub>bright</sub> staining, were not
depleted by this treatment (data not shown). Addition of IL-2
(3.6 IU/mL) or IL-15 (10 ng/mL) increased background pro-

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Figure 1. CD4^{bright} and CD8^{bright} T cells proliferate in response to human cytomegalovirus (HCMV) and to tetanus toxoid antigen (Ag). Peripheral blood mononuclear cells (PBMCs) were stained at t = 0 with carboxyfluorescein diacetate succinimidyl ester (CFSE) and were incubated for 4, 6, or 8 days with medium, anti-CD3, HCMV, HCMV control lysate, or tetanus toxoid. PBMCs were gated either for CD4^{bright} (A) or CD8^{bright} (B) T cells. The X-axis represents CFSE fluorescence intensity, and the Y-axis reflects CD25 intensity. Nos. in the quadrants represent the percentage of gated cells in each quadrant. Data are representative of experiments with PBMCs from 7 HCMV-seropositive donors.
HCMV-induced CD8⁺ T cell proliferation does not require infectious virus. The HCMV Ag preparation contained infectious virus capable of high-level replication in mink lung fibroblasts (data not shown). Because Ags presented by MHC-I to CD8⁺ T cells generally are synthesized intracellularly, we wanted to determine whether infectious virus in the Ag preparation was required to induce activation and proliferation of the CD8⁺ T cells. UV irradiation for 100 min or boiling for 10 min resulted in complete inactivation of HCMV infectivity (data not shown), yet live, UV-inactivated, or heat-inactivated virus preparations induced similar proliferative responses of both CD4⁺ and CD8⁺ T cells (figure 6A). Thus, neither synthesis of viral proteins nor intact virus structure was required for the proliferation and expansion of CD8⁺ T cells in response to HCMV. This indicates that cross-presentation of viral Ags (alternate MHC-I processing and presentation) might underlie these findings. To assure that contaminating viral peptides within the cell lysate were not responsible for these results, intact HCMV was purified over a 60% sucrose cushion, washed, and resuspended in PBS at the original starting volume. CD8⁺ T cell proliferative responses were enhanced by this purification (figure 6B), which indicates that CD8⁺ T cell proliferation was driven by purified virus and not by contaminating peptide.

DISCUSSION

In the present study, we provide clear evidence that human memory CD8⁺ T cells proliferate in vitro after exposure to HCMV and that these responses are HCMV peptide specific. CD8⁺ T cell proliferation also can be seen in response to the soluble Ag tetanus toxoid and killed HCMV, which suggests that Ag cross-presentation drives this response. In this system, CD8⁺ T cell proliferation is dependent on CD4⁺ cells. Although both IL-2 and IL-15 can substitute for CD4⁺ cells, CD4⁺ T cell help for CD8⁺ T cell proliferative responses was blocked by MAbs to IL-2 but not by MAbs to IL-15, which indicates that IL-2 plays a central role in this process.

Murine models of viral infection support an important role for CD4⁺ T cell help in CD8⁺ T cell responses. For example,
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Figure 3. CD4+ T cells are required for CD8+ T cell proliferation. Peripheral blood mononuclear cells (PBMCs) or CD4+ T cell–depleted PBMCs (<1.0% CD4+) were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and were cultured for 8 days with medium or tetanus toxoid. Frequency histograms show dye dilution (proliferation) of CD4+ T cells in PBMC cultures (A), CD8+ T cells in PBMC cultures (B), or CD8+ T cells in PBMCs depleted of CD4+ T cells before cultivation (C). Data are representative of 4 separate experiments. FITC, fluorescein isothiocyanate; HCMV, human cytomegalovirus.

in acute lymphocytic choriomeningitis virus (LCMV) infection, CD4+ T cells were not necessary for the generation of virus-specific CD8+ T cells [9, 29, 30]; however, CD4+ T cells were essential for the expansion and maintenance of virus-specific CD8+ T cell responses during chronic infection [9, 26–28] that led to the resolution of infection [9]. More recently, several reports have suggested that the functional capacity of murine memory CD8+ T cells may depend on the nature and timing of the help that they receive [10–13]. In these studies, memory CD8 T cell proliferation and cytokine expression were impaired in mice that had first seen Ag in the absence of CD4+ T cell help. Moreover, the secondary expansion of memory CD8+ T cells in response to antigenic challenge did not require CD4+ T cell help at the time of secondary challenge [10], but, rather, the ability of these memory cells to expand after rechallenge was somehow “programmed” into them through mechanisms dependent on the presence of CD4+ T cells during primary exposure.

In the present study, we examined the secondary memory CD8+ T cell expansion in response to whole HCMV. In all healthy HCMV-seropositive persons studied, memory CD8+ T cell expansion in response to cross-presented HCMV antigens was readily demonstrable. However, in contrast to observations in murine systems, we found that this expansion was critically dependent on the presence of CD4+ T cells. Likewise, MHC-I–restricted CD8+ T cell proliferation responses to inactivated hepatitis B virus (HBV) Ags also were diminished by CD4+ T cell depletion [31], and, more recently, “effector” CD8 T cell expansion in response to HCMV was shown to be CD4+ T cell dependent [32]. We do not know whether CD4+ T cell help is required for cross-presentation in our system, but CD4+ T cells and/or IL-2 are required for memory CD8+ T cell expansion in response to whole HCMV (figure 3 and figure 5A), and CD8+ T cell proliferation in response to the NLV9 HCMV peptide is minimal in the presence or absence of CD4+ T cells (figure 4B and figure 5B).

In vivo CD4+ T cell depletion as a result of HIV-1 infection is characterized by dramatic CD8+ T cell dysfunction, such as impaired proliferation, cytokine production, cytolytic activity, and increased anergy and apoptosis [33–39]. Moreover, in HIV-
Figure 4. Irrelevant antigen (Ag) can induce help to support human cytomegalovirus (HCMV) peptide-specific CD8\(^+\) T cell proliferation. Peripheral blood mononuclear cells (PBMCs) were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and were cultured for 8 days with medium, HCMV, or tetanus toxoid Ag in the presence or absence of HCMV pp65 NLV9 peptide. A, Day 0 indicates HCMV pp65 tetramer\(^+\) staining of CD8\(^{bright}\) T cells before Ag stimulation. B, Dot plots show CFSE dye dilution on day 8 of CD8\(^+\) T cells stained with either CD25–phycoerythrin (PE) (left panels) or HCMV pp65 tetramer-PE (right panels). Data are representative of experiments with PBMCs from 2 HCMV-seropositive HLA-A*0201 donors. FITC, fluorescein isothiocyanate; PerCP, peridinin chlorophyll protein.

1 infection, CD8\(^+\) T cell dysfunction probably related to CD4\(^+\) T cell dysfunction also may predict clinical outcome. Diminished CD4\(^+\) T cell responses to HCMV were associated with an inability to sustain high levels of HCMV-specific CD8\(^+\) T cells and an increased risk of HCMV-associated end organ disease [40]. Recently, Migueles et al. [41] showed that the ability of CD8\(^+\) T cells to proliferate in response to HIV-1 Ags distinguished HIV-1–infected long-term nonprogressors from persons with progressive HIV-1 disease. Thus, depletion of CD4\(^+\) T cells in vivo may be associated with and underlie a failure of memory CD8\(^+\) T cell expansion in response to secondary antigenic challenge.

In the present study, the CD4\(^+\) T cell help required for CD8\(^+\) T cell expansion in response to HCMV was largely mediated...
Figure 5. CD8⁺ T cell proliferation is mediated by interleukin (IL)-2, yet both IL-2 and IL-15 can provide help for CD4⁺ T cell–independent CD8⁺ T cell expansion in vitro. A, Peripheral blood mononuclear cells (PBMCs) were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and were cultured for 6 days with human cytomegalovirus (HCMV), with or without anticytokine monoclonal antibodies. Proliferation by CFSE dye dilution (X-axis) and CD25 expression (Y-axis) was determined by flow cytometry. B, CD4-depleted PBMCs were stained with CFSE (<1.0% CD4⁺) and were cultured with NLV9 peptide, with or without IL-2 and/or IL-15 for 6 days. Nos. in each quadrant represent the percentages of CD8⁺bright-gated cells in that quadrant. Data are representative of experiments with PBMCs from 2 HCMV-seropositive HLA-A*0201 donors.
Figure 6. Neither peptide synthesis nor contaminating peptides drives CD8+ T cell proliferation, because UV-irradiated, boiled, and sucrose density-purified human cytomegalovirus (HCMV) each induces CD8+ T cell proliferation. A, Peripheral blood mononuclear cells (PBMCs) were stained with carboxyfluorescein diacetate succinimidyl ester (CFSE) and were cultured for 8 days with medium, anti-CD3 antibody, “live” HCMV, or HCMV that was inactivated by UV light (HCMV-UV) or by boiling (HCMV-B). After 8 days, PBMCs were stained with CD4–allophycocyanin (APC), CD8–peridinin chlorophyll protein (PerCP), and CD25–phycoerythrin (PE) and were gated on CD4+bright or CD8+bright T cells, as indicated. Data are representative of experiments with 3 HCMV-seropositive persons.

B, HCMV lysate (1 mL) was purified over a 60% sucrose (wt/wt) cushion, washed, passed through a 0.45-μm filter, and resuspended in PBS (1 mL). Control (uninfected cell lysate), unpurified HCMV-infected cell lysate, or sucrose-purified HCMV were added at a 1:40 dilution to CFSE-stained PBMCs and were cultured for 8 days. Cells were surface stained, as in panel A, and were gated on CD8+ T cells. Data are representative of experiments with PBMCs from 3 HCMV-seropositive donors.

by IL-2, and the requirement for CD4+ T cells could be replaced either by IL-2 or IL-15. The importance of IL-2 in CD8+ T cell expansion is well recognized [32, 42, 43]. The results of the present study demonstrate that, when CD8+ T cell expansion is driven either via the alternative pathways of exogenous Ag presentation or when immunodominant peptide is provided exogenously, IL-2 retains a critical role. Although we could not define a role for IL-15 in CD8+ T cell expansion in our system, we found that IL-15, like IL-2, could substitute for CD4+ T cells for supporting CD8+ T cell expansion in response to HCMV Ags. Additional studies will be required to determine whether the phenotype or function of CD8+ T cells expanded in the presence of CD4+ T cells, IL-2, or IL-15 are distinguishable or whether these sources of help are truly interchangeable. In vivo studies with IL-15– or IL-15R–deficient mice showed normal generation of LCMV-specific memory CD8+ T cells, but, with time, mice deficient in either IL-15 or its receptor had a failure of proliferative renewal of memory CD8+ T cells, and a decline in memory CD8+ T cell numbers [44]. It remains to be determined whether IL-15 plays a similar role in human CD8+ T cell expansion in vivo. Nonetheless, our findings provide some rationale for further exploration of IL-15 and IL-2 as strategies to expand virus-specific CD8+ T cells in persons with CD4+ T cell deficiency.

Using intracellular expression of interferon (IFN)–γ as a readout and high concentrations of a HCMV infected cell lysate, Maeker et al. [45] recently reported variable degrees of cross-presentation of HCMV Ags to CD8+ T cells of healthy subjects that was inhibited by lactacystin but not by chloroquine, which suggests that this phenomenon was dependent on MHC-I (but not MHC-II) presentation and was also dependent on the presence of HCMV-specific antibodies in the system. We were unable to confirm a role for HCMV-specific antibodies in our system, because CD8+ T cell proliferation in response to whole cytomegalovirus was comparable in the presence of HCMV-seropositive and -seronegative serum (data not shown). Moreover, in their studies, as in some of our studies, live virus and other viral Ags were contained within a cell lysate; therefore, it was not clear how much of the observed effect was a result of either viral infection or direct binding of viral peptides contained within the cell lysate. We purified virus from the cell lysate by sucrose density sedimentation to confirm that direct peptide binding without processing and cross-presentation could not account for CD8+ T cell activation and proliferation.
observed in our system. Buseyne et al. [46] found that cross-presentation of HIV-1 Ags to MHC-I restricted CTL lines and that a T cell clone was dependent on the presence of viral envelope capable of membrane fusion. In contrast, we found that cross-presentation of HCMV peptides was not dependent on the integrity of the virion, because boiling of the HCMV preparation had no effect on cross-presentation to stimulate CD8^+ T cell proliferation.

In contrast to the reported CD4-independent IFN-γ production by CD8^+ T cells [45], we found that CD4^+ T cell help was essential to support CD8^+ T cell proliferation in response to HCMV Ags. The degree of cellular activation required to induce IFN-γ production may be significantly less than the requirements for cellular proliferation [47–49], which might explain the differential requirements of these cellular responses for CD4^+ T cell help.

In summary, memory CD8^+ T cells can expand dramatically in vitro in response to whole cytomegalovirus and in response to other exogenous Ags. Cross-presentation of HCMV peptides underlies this expansion that is critically dependent on CD4^+ T cell help. Help for cellular expansion is mediated by IL-2, yet both IL-2 and IL-15 can support CD8^+ T cell expansion in the absence of CD4^+ T cells. The importance of this alternate pathway of Ag presentation during HCMV infection in vivo, as well as in other settings, remains to be determined. This system will provide a valuable model to explore the relationships among antigen-presenting cells, CD4^+ T cells, and CD8^+ T cells in HCMV disease and HCMV disease complicated by CD4^+ T cell deficiency or dysfunction.

References

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