Direct Cross-Primming by Th Lymphocytes Generates Memory Cytotoxic T Cell Responses

Richard Kennedy, Anita H. Undale, William C. Kieper, Matthew S. Block, Larry R. Pease and Esteban Celis

http://www.jimmunol.org/content/174/7/3967

**References**
This article cites 61 articles, 33 of which you can access for free at:
http://www.jimmunol.org/content/174/7/3967.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Direct Cross-Priming by Th Lymphocytes Generates Memory Cytotoxic T Cell Responses

Richard Kennedy, Anita H. Undale, William C. Kieper, Matthew S. Block, Larry R. Pease, and Esteban Celis

Under optimal Ag stimulation, CTL become functional effector and memory T cells. Professional APCs (pAPC) are considered essential for the activation of CTL, due to their unique capacity to provide costimulation and present exogenous Ags through MHC class I molecules. In this study, we report a novel means by which Th lymphocytes acquire and present MHC class I determinants to naive CTL. Although previous studies have looked at T cell Ag presentation to activated T cells, this study presents the first example of Ag presentation by Th cells to naive CTL. We report that activated Th cells can function as effective pAPC for CTL. Our results show that: 1) In addition to acquisition of cell surface molecules, including MHC class I/peptide complexes, from pAPC, Th cells can acquire and present MHC class I-binding peptides through TCR-MHC class II interactions with pAPC; 2) the acquired Ag can be functionally presented to CTL; and 3) Ag presentation by Th cells induces naive CTL to proliferate and preferentially differentiate into cells that phenotypically and functionally resemble central memory T cells. These findings suggest a novel role of Th cells as pAPC for the development of memory immune responses. The Journal of Immunology, 2005, 174: 3967–3977.

The generation of CD8+ T cell memory is crucial for the establishment of long-term immunity against reinfection and perhaps even for the prevention of tumor recurrences. Strong evidence points to the importance of CD4+ Th lymphocytes for the production of memory CD8+ CTL (1–3). Th cells activate professional APCs (pAPC) through CD40/CD40L to present Ag more efficiently to naive CD8+ T cells, generating effector and memory CTL responses (4–6). It has been proposed that a direct interaction between CTL and Th cells through CD40/CD40L is necessary for memory CD8+ T cell development (3). However, in other model systems, it was reported that the generation of CTL memory did not require CD40 expression by the CD8+ T cells (7, 8). Notwithstanding, both CD4+ and CD8+ T cells express a large number of receptor/ligand pairs with costimulatory properties, and direct Th cell-CTL interactions could still play a role in the development of CD8+ T cell responses. Hypothetically, direct interactions between Th cells and CTL would be most efficient if it occurred in an Ag-specific manner, for example with Th cells directly presenting MHC class I (MHC-I)-restricted epitopes to naive CTL. The likelihood of Th cells functioning as pAPC is not that far-fetched because activated Th cells possess many of the cell surface molecules characteristic of pAPC, including MHC-I, CD80, CD86, 4-1BBL, CD30L, and CD70 (9, 10). Moreover, the direct delivery of immune-potentiating lymphokines such as IL-2 to the CTL by activated Th cells via an immunological synapse would certainly be an efficient way for facilitating CTL clonal expansion. However, in contrast to the distinctive ability of conventional pAPC such as dendritic cells (DC) to acquire exogenous Ags (through phagocytosis, macropinocytosis, or receptor-mediated endocytosis) and to efficiently process and cross-present these Ags to CTL, T lymphocytes appear to lack the proficiency to capture exogenous materials for Ag processing and cross-presentation. Consequently, one would assume that Th cells might not be able to generate the necessary MHC-I/peptide complexes from exogenous sources to stimulate naive CD8+ T cells. The results presented in this study indicate that Th cells not only have the capacity to acquire peptide epitopes associated with either the MHC-I or the MHC-II of the pAPC, but, in addition, the Th cells are able to generate endogenous MHC-I/peptide complexes by processing exogenously acquired material. Moreover, our results demonstrate that Th cells can function as APC and efficiently stimulate the proliferation of naive CTL and induce their differentiation into cells that resemble and behave as typical memory CD8+ T cells.

Materials and Methods

Mice

TCR transgenic OT-I (11), DO11.10 (12), and OT-II (13) mice were bred and maintained at the Mayo Clinic animal housing facilities. C57BL/6 mice were purchased from The Jackson Laboratory. MHC-I knockout mice (K−/−, D−/−), the MHC-I mutant H-2Kbnull, H-2Kbnull mice (14), and the DO11.10 × C57BL/6 hybrid (DO11.B6F) mice were produced in our facilities. Mice were used for experiments between 6 and 12 wk of age and in accordance with institutional guidelines.

Cell lines

EL-4, EG7, LB27.4, and MC57G cells were purchased from the American Type Culture Collection and maintained as recommended by the vendor. T1-2−, a human cell line transfected with the mouse I-Aβ MHC-II, was kindly provided by A. Rudensky (University of Washington, Seattle, WA). MF2.2D9 T cell hybridoma (referred to simply as MF2) was a gift from K. Rock (University of Massachusetts Medical Center, Worcester, MA) and maintained in IMDM supplemented with 10% FCS, 2-ME, and gentamicin. The MF2 T cell hybridoma recognizes the OVA265–280 peptide presented by I-Aβ (15), which is adjacent to the well-known immunodominant CTL...
epitope OVA257–264. The OT-II T cell hybridoma was generated in our laboratory by fusing a T cell clone from OT-II TCR transgenic mice with the BW5147 TCR-negative thymoma fusion partner and selecting hybridomas by drug selection. Ag specificity was confirmed by the capacity of the hybridomas to produce IL-2 by stimulation with the OVA233–339 peptide (data not shown).

Peptides
All peptides were purchased from A&A Labs, and stock solutions of 20 mg/ml were made in DMSO + 0.1% trifluoroacetic acid. All peptides used were >98% pure based on analytical HPLC and mass spectrometry. The following peptides were used in these studies: SIINFEKL (OVA257–264), ISOAVHAHAINEAGR (OVA323–339), SIINFEKLISOAVHAHAINE (OVA237–246/OVA323–339), and SIINFEKLTWSSNVMEER KIVK (OVA257–264/OVA365–380).

Cell cultures
Single cell suspensions from mouse spleen and lymph nodes were washed once in RBC lysis buffer (0.15 M NH4Cl, 0.1 mM Na2EDTA, 10 mM KHCO3, pH 7.3), washed once in medium, and then resuspended in IMDM containing 5% FCS (HyClone), 5 × 10–3 M 2-ME, and 10 μg/ml gentamicin (all culture medium and supplements purchased from Invitrogen Life Technologies). DC were obtained from bone marrow-derived macrophages cultured in RPMI 1640 medium containing 10% FCS, 1-galutamine, penicillin/streptomycin, 10 ng/ml IL-4, 10 ng/ml IL-12, and 5 ng/ml GM-CSF for 1 wk. For those experiments using peptides, the DC were matured/activated with 10 μg/ml LPS overnight. For generating previously activated Th cells, lymph nodes and spleen cells from OT-II, DO11.10, or DO11.B6F, TCR transgenic mice were cultured in 24-well tissue culture plates at 4 × 104 cells/well with 1 μM OVA233–339 and 50 U/ml human rIL-2 for 3 days. CD4+ cells were purified by negative selection using magnetic beads (Miltenyi Biotec) and placed back in culture medium containing 50 IU/ml IL-2 until use. Naive, CD8+ T cells from the lymph nodes of OT-I TCR transgenic mice were purified by a two-step negative selection process using a magnetic bead isolation kit (Miltenyi Biotec). Lymph node cells were incubated with a mixture of Abs to CD4, B220, CD49d, CD11b, and Ter-119 and passed through an isolation column to remove non-CD8+ cells. These partially purified cells were then incubated with anti-CD44 Abs to remove the previously activated cells. The remaining CD8–CD44+ OT-I cells were untouched by this process. B cell cultures were generated by stimulating lymph node cells in RPMI 1640 medium containing 10% FCS, 10 ng/ml IL-4, 10 μg/ml LPS, and 5 μg/ml anti-CD40 mAb (clone 3/23; BD Pharmingen) for 3 days. Afterward, B cell cultures were given fresh medium containing 10 ng/ml IL-4, as needed. As necessary, live CD4+ T cells and B cells were isolated from their respective cultures using a dead cell removal kit (Miltenyi Biotec) before use in Ag acquisition assays.

Ag acquisition assays
The presence of Kb/OVA257–264 or Kk/mOVA237–264 complexes on Th cells was confirmed by flow cytometry by gating on CD4+ T cells (with FCTC anti-CD4 mAb) using PE 25-D1.16 mAb, which is specific for Kk/ OVA257–264 complexes (16). The indicated peptides were either pulsed onto the APC or added to the cultures without (dumb protocol), as indicated for each particular experiment. APC were coincubated with various Th cell lines for the indicated time periods, after which the cells were washed with flow buffer (PBS + 1% FCS and 0.1% NaN3) and stained with the indicated Abs for 30 min at room temperature. For viability analysis, cultures were washed with annexin-binding buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl2, pH 7.4) and stained flowcytochrome-labeled annexin V (BD Pharmingen) and propidium iodide (Sigma-Aldrich). Mitomycin C (Calbiochem)-treated Th cells or APC were used as positive controls for annexin V and propidium iodide staining. Flow cytometry analysis was performed on FACSscan or FACSCalibur flow cytometers (BD Biosciences).

CTL cross-priming of OVA protein by Th cell hybridomas
For these experiments, CD8+ T cells from OT-I mouse splenocytes and lymph node cells were purified by depletion of MHC-II-positive cells, followed by CD8-positive selection. The degree of purity was >98%. MF2 and OT-II hybridomas were treated with murine IFN-γ (10 ng/ml) for 24 h to up-regulate H-2K expression. DC were cultured with MF2 or OT-II hybridomas (±IFN-γ-treated) at 1:1 ratio (5 × 104 cells/well) in a 96-well plate in the presence of different concentrations of OVA protein. Purified OT-I T cells (1 ×104/well) were added to the cultures, and supernatants were collected 24 h later. IFN-γ production by the OT-I T cells was measured by using ELISA kit (BD Pharmingen), according to manufacturer’s instructions. As the MF2 and OT-II hybridomas do not produce IFN-γ after Ag activation (data not shown), all of the IFN-γ was derived from the OT-I T cells.

CFSE proliferation assays
Purified naive OT-I cells were stained with 3 μM CFSE (Molecular Probes) for 10 min at 37°C in serum-free medium, and then washed extensively to remove unbound CFSE. For in vitro priming studies, the CFSE-stained OT-I were mixed with peptide-pulsed or unpulsed APC at a ratio of 3:1 (OT-I:APC). At the indicated time points, the cells were washed with flow buffer, stained with the indicated Ab, and analyzed by flow cytometry, as described above.

Intracellular cytokine staining
For these experiments, we selected the immunodominant OVA peptide epitope OVA257–264, which binds to the mouse MHC-I molecule Kk, generating Kk/OVA257–264 complexes that can be recognized by Ag-specific CTL or by the mAb D5-25.16 (16). Previously activated and highly purified Th cells specific for the OVA233–339 epitope were obtained from three different TCR transgenic mouse strains: OT-II (H-2k), DO11.10 (H-2d), and DO11.B6F1 (H-2b). Mice expressing pAPC (LB27.4) were pulsed with OVA257–264 and washed extensively to remove unbound peptide before they were coincubated with the various Th cell lines. Flow cytometry analysis was performed with mAb D5-25.16 demonstrated the presence of Kk/OVA257–264 complexes on the surface of all three Th cell lines (Fig. 1a). As the DO11.10 Th cells do not express endogenous Kk molecules, these cells must have acquired intact Kk/OVA257–264 complexes from the pAPC. The OT-II and the DO11.B6F1 Th cells could also have acquired Kk/OVA257–264 from the pAPC, but it is also possible that OVA257–264 originally on the pAPC’s Kk molecules was transferred onto the Th cells’ own Kk molecules, which would explain the higher levels of Kk/OVA257–264 complexes observed on OT-II and DO11.B6F1 T cells.

In vivo CTL priming
T cells responses to Ag stimulation were measured by intracutaneous flow cytometry using the CytoFix/Cytoperm kit (BD Pharmingen), according to manufacturer’s instructions. OT-I cells were cultured for the indicated time points with an equal number of EL-4 or EG.7 target cells in the presence of monensin (GolgiStop). Surface staining with anti-CD8 (53.6-7) was performed for 20 min at 22°C. Cells were then fixed and permeabilized, and intracellular cytokine staining was performed with anti-IFN-γ (clone XMG1.2) for 30 min at 4°C. Cells were then washed, fixed with 0.1% formaldehyde, and analyzed by flow cytometry, as described above.

Cytotoxicity assays
At the indicated time points after activation, the CTL were centrifuged over a Ficoll gradient to remove dead cells and then cultured for 4 h with 3Cr-labeled targets at the various E:T ratios, as previously described (17). 3Cr released into the supernatant by lysed cells was measured on a TopCount NXT scintillation counter (PerkinElmer). Percentage of specific lysis was calculated, as described (17). All experimental determinations were performed in triplicate.

Results
Th cells produce surface MHC-I/peptide complexes from exogenous Ag
To address the hypothesis that Th cells may function as pAPC for CTL, we first examined the capacity of activated CD4+ Th cells to generate specific surface MHC-I/peptide complexes from an exogenous Ag acquired while interacting with conventional pAPC. For these experiments, we selected the immunodominant OVA peptide epitope OVA257–264, which binds to the mouse MHC-I molecule Kk, generating Kk/OVA257–264 complexes that can be recognized by Ag-specific CTL or by the mAb D5-25.16 (16). Previously activated and highly purified Th cells specific for the OVA233–339 epitope were obtained from three different TCR transgenic mouse strains: OT-II (H-2k), DO11.10 (H-2d), and DO11.B6F1 (H-2b). Mice expressing pAPC (LB27.4) were pulsed with OVA257–264 and washed extensively to remove unbound peptide before they were coincubated with the various Th cell lines. Flow cytometry analysis using mAb D5-25.16 demonstrated the presence of Kk/OVA257–264 complexes on the surface of all three Th cell lines (Fig. 1a). As the DO11.10 Th cells do not express endogenous Kk molecules, these cells must have acquired intact Kk/OVA257–264 complexes from the pAPC. The OT-II and the DO11.B6F1 Th cells could also have acquired Kk/OVA257–264 from the pAPC, but it is also possible that OVA257–264 originally on the pAPC’s Kk molecules was transferred onto the Th cells’ own Kk molecules, which would explain the higher levels of Kk/OVA257–264 complexes observed on OT-II and DO11.B6F1 T cells.
relative to the DO11.10 cells. Because the pAPC were not pulsed with the Th cell epitope OVA323-339, the transfer of K\(^b\)/OVA257-264 from the pAPC to the previously activated Th cells occurred in an Ag-independent fashion. To control for the possibility that Th cells were nonspecifically acquiring cellular debris from dead APC, we repeated this and the following experiments using CFSE-labeled APC, and staining with annexin V and propidium iodide, demonstrating a relative absence of apoptotic and necrotic Th cells and APC (data not shown). Additionally, the CD4\(^+\) T cells did not stain with CFSE, indicating that large fragments of APC were not simply taken up by the Th cells (data not presented).

To test the possibility that OVA257-264 could indeed be transferred from the MHC-I of the pAPC to the endogenous K\(^b\) molecules of the Th cells, we used pAPC expressing two different MHC-I alleles: K\(^b\) and the mutant Kbm3 (14), both capable of binding OVA257-264 and reacting with mAb 25-D1.16. K\(^b\)/OVA257-264 complexes can be distinguished from Kbm3/OVA257-264 by using mAb B8-24-3 (18), which blocks the binding of 25-D1.16 to K\(^b\)/OVA257-264, but not to Kbm3/OVA257-264 (Fig. 1e). Therefore, if Th cells directly acquire Kbm3/OVA257-264 complexes from the pAPC, the Th cells will stain with 25-D1.16, regardless of the presence of B8-24-3. In contrast, if the OVA257-264 peptide is transferred onto the K\(^b\) molecules of the Th cells, then 25-D1.16 staining will be blocked by B8-24-3. Thus, this system allows precisely differentiating between the MHC-I/peptide complexes directly acquired from the Kbm3 pAPC (Kbm3/OVA257-264), and the endogenous MHC-I molecules of the K\(^b\) Th cells (K\(^b\)/OVA257-264). Kbm3 expressing pAPC (B cell blasts) were pulsed either with OVA257-264 or a mixture of the CTL epitope OVA257-264 and Th epitope OVA323-339, and were coincubated with previously activated OT-II Th cells. In these experiments, two populations of Th cells expressing MHC-I/OVA257-264 complexes could be distinguished based on their forward and side scatter: resting/small and blasted/large. On resting Th cells, most of the complexes were K\(^b\)/OVA257-264 because the majority of 25-D1.16 staining was blocked by B8-24-3 (Fig. 1b). Interestingly, the formation of endogenous K\(^b\)/OVA257-264 complexes on the resting Th cells was greatly increased through cognate recognition of the Th OVA323-339 epitope by the TCR. Conversely, on the Th blasts, most of the complexes were Kbm3/OVA257-264 (25-D1.16 staining was not blocked by B8-24-3), so they had to be derived directly from the pAPC. Again, the acquisition of Kbm3/OVA257-264 by blasted Th cells from the pAPC did not require cognate Ag presentation to the Th cells. However, cognate recognition of OVA323-339 by OT-II Th blasts resulted in the production of some endogenous K\(^b\)/OVA257-264 complexes. As the T cell blasts were presumably more activated, this raised the question of how T cell activation status alters the ability of the Th cells to acquire MHC-I-binding peptides. The small cells in these experiments had received prior antigenic stimulation and so were not truly naive, but presumably had transitioned into a resting state. We therefore repeated these experiments with freshly isolated, naive Th cells with similar results to those shown for the resting cells. Namely, naive Th cells required the presence of their cognate MHC-II Ag to acquire the MHC-I-binding peptide, and the acquired peptide was mostly found associated with the endogenous MHC-I of the Th cell and not with the acquired MHC-I molecule (data not shown). In the subsequent experiments described below, we also used naive Th cells (Figs. 1, c and d, and 2),

**FIGURE 1.** Production of endogenous peptide/MHC-I complexes by Th cells. a, Activated Th acquire of MHC-I-restricted epitopes from pAPC in an Ag-independent fashion. Previously activated (7 days with 1 \(\mu\)M OVA232-339) purified (>98%) CD4\(^+\) Th from TCR transgenic mice were cocultured at a 1:1 ratio with OVA257-264-pulsed LB27.4 APC for 4 h. Cell cultures were then stained with anti-CD4 FITC and 25-D1.16 PE mAbs and analyzed by flow cytometry. Histograms show the 25-D1.16 PE staining of CD4\(^+\) T cells incubated with either unpulsed LB27.4 (dotted lines) or OVA257-264-pulsed LB27.4 (bold lines). b, Kbm3 B cell blasts were pulsed for 2 h with 10 \(\mu\)M indicated peptides and cocultured with CD4\(^+\) purified OT-II cells for 9 h. Cells were then stained with anti-CD4 FITC and 25-D1.16 PE mAbs after pretreatment with 10 \(\mu\)g/ml either the blocking B8-24-3 mAb (filled histogram) or isotype control mAb (solid line). Flow cytometry analysis was done on both the resting and the blasting cell populations by setting up appropriate gates in the side vs forward scatter plots. Values of \(p\) show significant differences observed by the addition of the OVA232-339 peptide in the absence of B8-24-3 mAb (c, K\(^b\)/OVA257-264 solid line, or isotype control mAb (solid histogram) or isotype control mAb (solid histogram). The dotted histogram represents the staining pattern of CD4\(^+\) T cells in the absence of peptide. d, Purified (>98%) OT-II Th cells were incubated with 10 \(\mu\)M indicated peptides and cocultured with CD4\(^+\) purified OT-II cells for 9 h. Cells were then stained with anti-CD4 FITC and 25-D1.16 PE mAbs after pretreatment with 10 \(\mu\)g/ml either B8-24-3 mAb (filled histogram) or isotype control mAb (solid histogram). The dotted histogram represents the staining pattern of CD4\(^+\) T cells in the absence of peptide. e, Purified (>98%) OT-II Th cells were incubated with 10 \(\mu\)M indicated peptides in the presence/absence of an equal number of T1-A\(^b\) APC for 9 h and analyzed by flow cytometry. Shaded area, OVA232-339; bold line, OVA257-264 solid line, OVA257-264/OVA232-339; and dotted line, OVA257-264/OVA265-280. e, Lymph node cells from K\(^b\) and Kbm3 mice were isolated and unpulsed (dotted line) or pulsed with 5 \(\mu\)g/ml OVA257-264 for 2 h at 37°C. Before staining with 25-D1.16, cells were pretreated for 20 min with mouse IgG (nonspecific Ab, bold line) or B8-24-3 (anti-H-2K\(^b\), solid line).

**Th cell acquisition of MHC-I-binding peptides through Ag presentation by MHC-II**

B lymphocytes can be very efficient pAPC for T cells by selectively capturing Ag via their Ag receptor (19). Likewise, it is possible that Th cells could carry out a similar function when the peptides that they recognize in the context of MHC-II contain an adjoining CTL epitope. This situation becomes possible because there is no tight constraint on the length that peptides must have to be recognized by the T cell. This becomes important when they recognize a small peptide (15–20 aa) harboring both a T and a CTL epitope. In fact, as shown in Table I, many well-known CTL...
epitopes lie within or are adjacent to Th epitopes (20–37). Following this rationale, a peptide containing the OVA257–264 CTL epitope linked to OVA323–339 (OVA257–264-OVA323–339) and a second peptide containing the same CTL epitope, but linked onto a different I-A^b-restricted Th epitope from OVA, OVA265–280 (OVA257–264-OVA265–280) (20), were tested for their capacity to produce K^b/OVA257–264 complexes on OT-II Th cells. Equal amounts of these linked peptides contain similar amounts of the class I epitope, SIINFEKL (i.e., OVA257–264-OVA265–280) contains 94% of the amount of SIINFEKL found in the same mass quantity of OVA257–264-OVA323–339. These experiments were done using three to five mice per group.

### Table 1. Adjoining or nested CTL/Th epitopes from various Ag sources

<table>
<thead>
<tr>
<th>Ag</th>
<th>CTL Epitope</th>
<th>Th Epitope</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Peptide position MHC-I</td>
<td>Peptide position MHC-II</td>
<td></td>
</tr>
<tr>
<td><strong>Foreign Ags</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OVA</td>
<td>257–264 K^b</td>
<td>265–280 I-A^b</td>
<td>20</td>
</tr>
<tr>
<td>Hen egg lysozyme</td>
<td>91–99 K^d</td>
<td>91–105 I-A^d</td>
<td>21</td>
</tr>
<tr>
<td>Hen egg lysozyme</td>
<td>116–124 K^d</td>
<td>106–116 I-E^d</td>
<td>21</td>
</tr>
<tr>
<td><strong>Autoantigens</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAD</td>
<td>206–214 K^d</td>
<td>206–220 I-A^d</td>
<td>21</td>
</tr>
<tr>
<td>GAD</td>
<td>505–513 K^d</td>
<td>509–527 I-A^d</td>
<td>21</td>
</tr>
<tr>
<td>GAD</td>
<td>546–554 K^d</td>
<td>524–543 I-A^d</td>
<td>21</td>
</tr>
<tr>
<td>Insulin</td>
<td>15–23 K^a</td>
<td>9–23 I-A^a</td>
<td>22, 23</td>
</tr>
<tr>
<td><strong>Viral Ags</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rubella capsid</td>
<td>264–272 HLA-A3, A11</td>
<td>263–275 HLA-DR</td>
<td>24</td>
</tr>
<tr>
<td>Influenza B NP</td>
<td>335–349 HLA-B37</td>
<td>335–349 HLA-DQw5</td>
<td>25</td>
</tr>
<tr>
<td>HBVenv</td>
<td>10–17 HLA-A1</td>
<td>10–19 HLA-DQ5</td>
<td>26</td>
</tr>
<tr>
<td>HIV-1 gp160</td>
<td>318–327 D^a</td>
<td>315–327 I-A^d</td>
<td>28</td>
</tr>
<tr>
<td>HIV-1 RT</td>
<td>38–52 H-2^b MHC I</td>
<td>36–52 H-2^b MHC II</td>
<td>29</td>
</tr>
<tr>
<td><strong>Tumor Ags</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ras oncogene</td>
<td>4–12 K^d</td>
<td>4–16 I-A^d</td>
<td>30, 31</td>
</tr>
<tr>
<td>P1A</td>
<td>35–43 L^d</td>
<td>33–44 I-A^d</td>
<td>32, 33</td>
</tr>
<tr>
<td>p53</td>
<td>234–242 K^d</td>
<td>234–242 H-2^d MHC II</td>
<td>34</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>80–88 HLA-Cw6</td>
<td>87–98, 80–109 HLA-DR7</td>
<td>35, 36</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>92–100 HLA-Cw3</td>
<td>87–98, 80–109 HLA-DR7</td>
<td>35, 36</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>84–102 HLA-B51</td>
<td>87–98, 80–109 HLA-DR7</td>
<td>35, 36</td>
</tr>
<tr>
<td>NY-ESO-1</td>
<td>157–165 HLA-A2</td>
<td>157–170 HLA-DR4</td>
<td>35, 36</td>
</tr>
<tr>
<td>Melanoma gp100</td>
<td>177–186 HLA-A2</td>
<td>175–189 HLA-DR53, DQ6</td>
<td>37</td>
</tr>
</tbody>
</table>

^The first column indicates the Ag in which the epitopes were discovered. The second and fourth columns indicate the amino acid position of the peptides from each Ag. The third and fifth columns correspond to the MHC-I or MHC-II allele binding to the peptide. The sixth column lists the references for each nested epitope. GAD, glutamic acid decarboxylase; NP, nucleoprotein; HBVenv, hepatitis B virus envelope; RT, reverse transcriptase.

---

**FIGURE 2.** DO11.B6 F1 mice were immunized with 80 μmol/mouse OVA257–264 epitope, OVA257–264-OVA233–339, or no peptide in CFA in the footpads. One day later, the draining lymph nodes were excised and the cells were stained with 25-D1.16 PE and anti-CD4 mAb, as described above. The 25-D1.16 staining (K^b/OVA257–264 complexes) of CD4^+ cells of all the mice in that group beside each histogram represent the average percentage of 25-D1.16-positive cells of all the mice in that group ± the SD within that group. Experiments were done using three to five mice per group.
complexes in a more biologically relevant situation. TCR transgenic mice (DO11.10.B6Fl) received footpad injections of either OVA257–264 or OVA257–264-OVA323–339, and 24 h later, cells from the draining lymph nodes were analyzed by flow cytometry for the surface expression of Kβ/OVA257–264. In those mice injected with OVA257–264-OVA323–339, a significant proportion (~35%) of the lymph node-draining Th cells expressed Kβ/OVA257–264, while fewer Th cells (<5%) of mice that received OVA257–264 displayed these MHC-I-peptide complexes (Fig. 2). In contrast, similar levels of Kβ/OVA257–264 complexes were observed on the CD4-negative lymph node cells (mostly B cells, macrophages, and DC), regardless of the peptide used for vaccination. These results not only reiterate the important role of Ag recognition by the TCR in the generation of MHC-I-peptide complexes on naive Th cells, but also demonstrate that the formation of these complexes on Th cells can also take place in vivo.

Peptide Ags acquired by Th cells can be functionally presented to CTL

Next, we determined whether the CTL peptide epitope acquired from larger, MHC-II-binding peptides could be functionally active. Previously activated Th cells (OT-II) were incubated with Kβ-negative APC (T1-Aβ) loaded with various concentrations of either OVA257–264-OVA323–339 or OVA257–264-OVA265–280. Subsequently, the Th cells were washed extensively, and purified preactivated OT-I CTL, which specifically recognize the Kβ/OVA257–264 complex (11), were added for 12 h after which the CTL response was assessed by intracellular cytokine staining. For both linked peptides, the percentage of IFN-γ-producing OT-I cells as directly proportional to the concentration of peptide loaded onto the APC (Fig. 3a). However, the activation of the CTL in the presence of Th cells and the Th-stimulatory OVA257–264-OVA323–339 peptide was slightly more effective than with the OVA257–264-OVA265–280 peptide. In the absence of Th cells, neither peptide was able to activate the OT-I CTL (data not shown), indicating that the peptides were not simply being cleaved and presented to the CTL by their own MHC-I. Thus, these results suggest that Th cells are capable of acquiring Ag from the pAPC’s MHC-II molecules and effectively present MHC-I/peptide complexes to preactivated CTL. Moreover, the observation that cognate recognition of Ag by the Th cells increased the capacity of these cells to serve as APC suggests that the transfer of Ag from pAPC to Th cells may take place via TCR/MHC-II interactions.

Th cell acquisition of MHC-I-binding peptides from protein-loaded APC

The biological relevance of the last experiments could be questionable because the results were obtained using synthetic peptides that artificially link together Th and CTL epitopes. Ideally, one would like to assess the capacity of Th cells to present Ag to CTL in a situation in which the Th and CTL epitopes lie naturally contiguous to each other, as it frequently occurs in nature (Table I). In addition, it would be important to demonstrate that the process of Ag capture by Th cells from pAPC does not only occur with synthetic peptides, but also takes place with a natural Ag (i.e., protein). Because the OT-I and OT-II T cell epitopes from OVA are not contiguous, it was not possible to carry out these experiments using the respective TCR transgenic T cells. However, thanks to the existence of a T cell hybridoma (MF2) specific for the MHC-II-restricted Th cell epitope OVA265–280, which lies adjacent to the OVA257–264 CTL epitope, we were able to study the capacity of Th cells to serve as APC in a more biologically relevant situation. For these experiments, we compared the ability of MF2 T cells with OT-II hybridoma cells (recognizing the distant MHC-II epitope) to serve as APC to naive OT-I CTL. Because both of these T cell hybridomas express low levels of surface MHC-I, the cells were pretreated with IFN-γ, which increases their expression of Kβ (data not shown). Cell mixtures containing DC, T cell hybridomas (treated or not with IFN-γ), and purified naive OT-I CTL were cultured with various concentrations of OVA protein. After a 24-h incubation period, the activation of the OT-I CTL was measured by IFN-γ production (the T cell hybridomas MF2 and OT-II do not produce this cytokine upon Ag activation; data not shown). It should be noted that in this assay, direct cross-priming by DC required high concentrations of protein (>300 μg/ml; data not shown). At the protein concentrations used in this study (≤100 μg/ml), the DC were unable to directly cross-prime the CTL (Fig. 3b). Thus, under these experimental conditions, the presence of the MF2 or the OT-II T cell hybridomas was necessary for the activation of the OT-I CTL (Fig. 3b). Moreover, the MF2 cells were ~3 times more effective than the OT-II cells in enhancing the response of OT-I cells to Ag. In the absence of DC, neither of the T cell hybridomas was able to activate the OT-I cells at any of the concentrations of protein tested (data not shown). The Ag-induced activation of OT-I was found to be significantly lower if the MF2 cells were not previously treated with IFN-γ (Fig. 3c), which was required to increase MHC-I surface expression. Furthermore, the addition of anti-MHC-II mAbs to the cultures blocked the activation of the OT-I CTL (Fig. 3c), reinforcing the requirement for cognate Ag

FIGURE 3. Functional presentation of acquired Ag and acquisition of class I peptide from protein-loaded APC. a. Purified (~98%) OT-II Th cells were incubated with 10 μM indicated peptides in the presence/absence of an equal number of T1-Aβ APC for 6 h, washed three times, and placed in a fresh tissue culture plate. Purified (>98%), preactivated OT-I cells were added to each well for 8 h in the presence of Brefeldin A. Cells were then stained with anti-CD8 and anti-IFN-γ using the BD Pharmingen Cytofix/Cytoperm kit. Results represent the percentage of OT-I cells positive for intracellular IFN-γ. b and c. C57BL/6 DC were loaded with the indicated concentrations of OVA protein in the presence/absence of T cell hybridomas. Freshly isolated, CD8+ OT-I cells were then added for 24 h. The IFN-γ released by the OT-I cells into culture supernatants was then analyzed by ELISA. d. As in b and c, but using Kβ/−Dβ−/− DC.
recognition by the Th cells. Lastly, addition of IL-2 to the cultures was not able to substitute for the requirement of Th cells to promote OT-I CTL activation (Fig. 3c), suggesting that the role of the Th cells is not simply due to an enhancement of CTL response to Ag presented by the DC by their production of IL-2.

The results presented in Fig. 3, b and c, suggest, but do not prove that the Th hybridomas can acquire Ag from the DC via MHC/peptide complexes and present it to the OT-I CTL. To assess whether the Th hybridoma cells are able to present Ag directly to the OT-I CTL under these conditions, these experiments were repeated using DC from MHC-I-deficient (K−/−D−/−) mice. With this system, we assured that the DC would be unable to directly present Ag to the OT-I cells. The results clearly show that in the presence of MF2 cells, the OT-I cells became activated, while in this case the OT-II hybridoma failed to show any Ag-presenting activity (Fig. 3d). As previously noted, treatment of the MF2 with IFN-γ to increase their surface MHC-I expression resulted in a significant increase in the capacity of these cells to serve as APC (Fig. 3d).

Th lymphocytes function as APC to stimulate primary CTL proliferative responses

To date, the results indicate that Th cells have the capacity to acquire exogenous Ags from pAPC (mainly through MHC-II interactions) and produce MHC-I/peptide complexes. Moreover, the data suggest that CTL can become activated (produce IFN-γ) as a consequence of recognizing Ag presented by Th cells. It has been previously noted that T lymphocytes are able to present Ag to other T lymphocytes with sometimes contradicting end results such as eliciting activation, the generation of anergy, or inducing T cell fratricide (38–42). However, in most of these examples, activated T cells were used to present Ag to other, previously activated T cells, and, to our knowledge, no one has examined whether activated Th cells can present Ag to naive CTL and determined the consequences of this interaction. Some of the results presented in Fig. 3 indicate that Ag presentation by Th cells can activate naive CTL to produce lymphokines, but this does not imply that such activation can lead to proliferation and clonal expansion, which are necessary for attaining protective immunity. Therefore, we compared purified, activated Th cells with conventional pAPC (DC) and a poor APC (MC57G fibrosarcoma) for their capacity to stimulate the proliferation of purified naive OT-I CTL. Notably, both the DC and the Th cells were equally effective in inducing the Ag-mediated cell division of CTL, as measured by the serial decrease of CFSE staining, while the MC57G cells were considerably less effective APC (Fig. 4a). In separate experiments, titrated numbers of irradiated, OVA257–264-pulsed APC (both DC and Th cells) were mixed with naive OT-I cells, and 3 days later, proliferation of the OT-I cells was evaluated by [3H]thymidine incorporation into DNA. DC induced ~2-fold greater levels of thymidine incorporation compared with Th cells. However, similar numbers of both APC types (~5 × 105) were required for optimal proliferative responses (Fig. 4b). Cell division (Fig. 4a) and DNA synthesis (Fig. 4b) do not necessarily imply that the cells will remain viable (i.e., expand) and will maintain function after activation. Thus, the total numbers of live CD8+ T cells were enumerated at various time points to evaluate the capacity of the various APC to actually mediate CTL clonal expansion. As shown in Fig. 4c, both the DC and the Th cells stimulated a robust, Ag-induced expansion of the naive CD8+ T cells (~50-fold in 7 days). In multiple experiments, Th cells consistently triggered a higher level of CTL expansion than DC at the early time points (day 3), but by day 7 the cultures stimulated with DC had greater numbers of viable cells. Under the same conditions, although some CTL stimulated by the MC57G cells underwent two or three divisions (Fig. 4c), there was no substantial CTL expansion (Fig. 4c). It should be noted that simply dumping OVA257–264 peptide (~1 μM) to purified naive OT-I CTL resulted in limited proliferation, similar to that observed with the MC57G APC (data not shown). In these experiments, there was a small number of contaminating cells in the purified T cell preparations (~2% CD8-negative cells). However, the contaminating cells did not express MHC-II or CD11c, so it is unlikely that they were conventional pAPC (data not shown). Nevertheless, to rule out this possibility, cultures containing unpulsed Th cells and naive OT-I cells were spiked with 1, 2, or 5% contaminating, peptide-pulsed pAPC. Under these experimental conditions, no significant loss of CFSE or CTL expansion was seen (Fig. 5), indicating that the proliferative response was a direct result of Ag presentation by the Th cells.

Phenotypic characterization of Th-primed CTL

The presence and levels of various activation markers of naive CTL primed in vitro with either Th cells or pAPC were examined by flow cytometry. As shown in Fig. 6, 3 days after stimulating
OT-I cells with either DC or Th cells, all the dividing CTL expressed high levels of CD25, CD44, and CD69, which are considered classic T cell activation markers (43, 44). However, the level of expression of these markers, as determined by the mean fluorescence intensity of y-axis (FL2-Height), was consistently higher on the DC-primed CTL as compared with the Th-primed CTL. Another notable difference between the two CTL populations was the expression of CD66L, a marker expressed on naive T cells that is usually lost upon activation (45). Although the majority (89%) of the DC-primed CTL lost expression of CD66L, a large proportion (72%) of the Th-primed CTL continued to express this cell surface marker, even after multiple rounds of cell division. We also noted that the CD66L+ CTL from the Th-primed cultures coexpressed high levels of the Ly-6C surface marker (data not shown).

**Functional characterization of Th-primed CTL**

The CD44+CD66L+Ly-6C+ surface phenotype observed in the Th-primed CTL is characteristic of central memory CD8+ T lymphocytes (46–49). Therefore, we examined whether Th-primed CTL exhibited any of the functional traits attributed to typical memory cells. These traits include: 1) the production of IFN-γ in response to either Ag or proinflammatory lymphokines such as IL-12 and IL-18 (50); 2) the enhanced survival and proliferation in response to IL-15 and IL-7 (51, 52); 3) the capacity to generate Ag-specific killer cells upon Ag restimulation; 4) the capability to expand upon Ag re-encounter; and 5) the ability to persist for a long period of time after their original antigenic stimulation.

Th cell-primed CTL were as efficient as pAPC-primed CTL in their capacity to produce IFN-γ when restimulated with Ag (Fig. 7a), indicating that these cells can produce effector cytokine cells upon Ag rechallenge. CTL that were primed by Th cells were compared with naive CD8+ T cells for their ability to produce IFN-γ soon after (8 h) treatment with proinflammatory cytokines IL-2, IL-12, and IL-18. A significant proportion (21%) of Th-primed CTL produced IFN-γ in response to the proinflammatory lymphokine mixture (but not to IL-2 alone) in the absence of TCR stimulation, while as expected, naive CD8+ T cells failed to respond under either of these conditions (Fig. 7b). CTL that were primed with Th cells expanded better in the presence of IL-2, IL-7, and IL-15 than CTL primed with DC, while both CTL populations

![FIGURE 5](http://www.jimmunol.org/)

**FIGURE 5.** CFSE proliferation profile of CTL upon stimulation with various APC. A total of 3.5 × 10⁶ CFSE-labeled OT-I was incubated for 3 days with peptide-pulsed (1 μg/ml for 2 h) or unpulsed 5.0 × 10⁵ APC (Th cells or T cell-depleted splenocytes). To some wells, the indicated percentage of peptide-pulsed, T cell-depleted splenocytes was added to cultures containing OT-I cells and sufficient unpulsed Th cells to keep the total number of APC in each well constant. HTL, Th cells.

![FIGURE 6](http://www.jimmunol.org/)

**FIGURE 6.** Phenotypic characteristics of CTL primed by Th cells. CFSE-labeled purified naive OT-I cells were primed with peptide-pulsed DC or Th cells for 3 days and double stained for CD8 and the indicated cell surface marker. Dot plots of CD8+ T cells are shown indicating the percentage of the cells in the top left quadrants, which represents the OT-I cells that divided and express the corresponding marker. Quadrant gates were previously set using naive OT-I cells. HTL, Th cells.

![FIGURE 7](http://www.jimmunol.org/)

**FIGURE 7.** Functional characteristics of CTL primed by Th cells. a. OT-I cells were primed with the indicated APC (pulsed with OVA257–264) and maintained in culture for 3 wk in the presence of IL-2, IL-7, and IL-15. The OT-I cells were then stimulated with either EL-4 (negative control) or EG.7 (transfected with OVA and expressing Kb/OVA257–264 complexes) cells for 24 h and then stained for CD8 and intracellular IFN-γ. Numbers represent the percentage of CD8+ cells producing IFN-γ. b. Naive OT-I cells and CD66L+ Th-primed OT-I cells were stimulated for 6 h with 50 IU/ml IL-2 alone (thin lines) or in combination with 10 ng/ml IL-12 and 10 ng/ml IL-18 (bold lines). The histogram gates (M1) represent the CD8+ cells positively staining for intracellular IFN-γ. The percentages of cells within the M1 gates are shown for each condition (dark bars; IL-2, IL-12, IL-18, red numbers, IL-2; IL-12, IL-18, gray numbers, IL-2). c and d. 3.5 × 10⁶ OT-I CTL primed on day 0 with peptide-pulsed DC (●) or activated Th cells (□) were maintained in culture for 1 wk with the indicated cytokines added on days 3 and 5 at the following concentrations: IL-2 (50 IU/ml), IL-7 (10 ng/ml), and IL-15 (5 ng/ml). Live CD8+ T cells for each culture done in duplicate were counted at the indicated time points. HTL, Th cells.
responded equally to IL-2 alone (Fig. 7, c and d). When the recently primed CTL were tested in a 4-h cytotoxicity assay, the DC-primed CTL showed a much higher level of cytotoxicity than the Th-primed CTL (Fig. 8a). These Th-primed CTL were then restimulated with peptide-pulsed splenocytes. As would be expected of memory cells, the restimulated CTL exhibited enhanced killing activity compared with prerestimulation, lost CD62L expression, and expanded efficiently (~6-fold in 7 days; Fig. 8, b–d).

The capacity of Th to prime CTL responses in vivo was also examined. CFSE-labeled, purified naive OT-I cells were adoptively transferred into C57BL/6 mice, and 1 day later, the mice were vaccinated with DC or Th cells that were pulsed with OVA 257–264. Under these in vivo conditions, peptide-pulsed DC and Th cells produced equivalent CTL proliferation, while no significant effect was observed in the absence of Ag (Fig. 9a). To eliminate the possibility that host pAPC could be presenting OVA257–264 peptide released by the Th cells, these experiments were repeated using Th cells expressing the mutant Kbms8 allele, which binds OVA257–264, but cannot stimulate OT-I cells (53). OT-I CTL proliferated extensively in response to OVA257–264-pulsed Kb-Th cells, but not to unpulsed Th cells or to OVA257–264-pulsed Kbms8 Th cells (Fig. 9b). In these experiments, the recipient mice had to be sublethally irradiated to eliminate the host’s response against the Kbms8 APC, so the transferred OT-I cells underwent a few (~3) rounds of homeostatic proliferation in the control samples. Nevertheless, Ag presentation by Kb Th cells induced significantly greater level of cell division above this background level. In contrast, the OT-I proliferation induced by the Kbms8 Th cells was indistinguishable from that observed in the absence of vaccine (Fig. 9b). Being aware that typical memory CD8+ T cells should last for a considerable period of time, we examined the capacity of Th-primed CTL to persist long after vaccination. Adoptively transferred OT-I cells that were stimulated in vivo either with peptide-pulsed Th, peptide-pulsed pAPC, or peptide/IFA were observed at
similar cell numbers 10 wk after vaccination and continued to express memory phenotypic markers CD62L, CD44, and Ly-6C (Fig. 9c). Overall, these observations demonstrate that Th-primed CTL have the capacity to persist in vivo for an extended period of time, behaving as typical central memory cells.

Discussion
From the results presented in this study, we propose that a novel role for Th cells is to function as APC to naive CD8+ T cells for the preferential generation of central memory CTL responses. Help for CTL by Th cells is considered to be Ag specific and was believed until recently to occur solely via conventional pAPC, because only these cells were deemed to be proficient at acquiring exogenous Ag and presenting the corresponding MHC-I/peptide complexes to CTL in a stimulatory manner. Cross-priming has been thought to be an exclusive feature of specialized pAPC (54, 55), and has given rise to some controversy with respect to its biological significance when Ag concentrations are not excessively high (56). We hypothesize that CTL cross-priming may also occur by Th under physiological conditions when Th cells are able to capture Ags while interacting with conventional pAPC. Our results indicate that Th cells are capable of acquiring CTL peptide epitopes from pAPC via at least two different means. First, we observed direct acquisition of intact specific MHC-I/peptide complexes by activated Th cell blasts while interacting with pAPC (Fig. 1a). Studies by Sprent and colleagues (42) have shown that activated T lymphocytes (in their case CD8+ T cells) were capable of absorbing and later internalizing MHC-I/peptide complexes from pAPC. Thus, it should not be all that surprising that the DO11.10 Th cells, which lack endogenous K\(^{b}\) molecules, displayed significant levels of surface K\(^{b}\)/OVA257–264 complexes after interacting with APC (Fig. 1a). However, it was unexpected that the levels of K\(^{b}\)/OVA257–264 complexes formed by Th cells expressing endogenous K\(^{b}\) molecules (OT-II and DO11.B6F1) were nearly 20-fold greater as compared with the K\(^{b}\)-negative Th cells. These results together with the subsequent experiments that used K\(^{b}\)m3 pAPC (Fig. 1b) indicate that Th cells somehow are capable of transferring the CTL peptide epitope from the passively absorbed MHC-I molecule to their own MHC-I. The main consequence of such transfer is that while the passively acquired MHC-I/peptide complexes are rapidly internalized and degraded, the endogenously formed MHC-I/peptide complexes would be more stable and persist for a sufficiently long time period to be presented to MHC-I-restricted T cells. The second source of CTL epitopes for Th, which perhaps bears more biological relevance for Ag cross-presentation, are MHC-II/peptide complexes when these contain adjoining or overlapping CTL epitopes. Interestingly, the Ag most commonly used in arguing in favor of cross-presentation is OVA, whose well-known immunodominant CTL epitope OVA257–264 contains the adjoining and not so well-known Th cell epitope OVA265–280 (20). In contrast, the immunodominant CTL epitopes present in the Ags used for arguing against physiological cross-priming, lymphocytic choriomeningitis virus-gp and lymphocytic choriomeningitis virus-nucleoprotein (56), are not known to have neighboring Th cell epitopes. Although transfer of MHC-II/peptide complexes from APC to activated Th cells can occur in an Ag-independent manner by simple absorption, as it occurs with MHC-I/peptide complexes, this transfer would be more effective if it occurred in an Ag-specific manner through the interaction of the TCR with MHC-II/peptide, and the data presented in Figs. 1, c and d, and 3, b–d, provide support for this hypothesis. To assess the biological significance of Th cells as APC in CTL cross-priming, we used a model system in which low amounts of protein Ag (10–100 \(\mu g/ml\)) were fed to pAPC and the capacity of MHC-II-restricted T cells to serve as APC to stimulate naive CTL was evaluated. Two types of Th cells were evaluated, one recognizing an epitope adjoining the CTL epitope (MF2 hybridoma) and the other one specific for a distant epitope (OT-II hybridoma). With wild-type APC (C57BL/6 DC), the presence of either one of the Th cells was required to activate the CTL, although the MF2 cells were clearly more potent than the OT-II cells (Fig. 3b). In the absence of Th cells, much higher concentrations of Ag (~1 mg/ml) were needed to observe the activation of the CTL (data not shown). The most obvious mechanisms for explaining the effects of the Th cells in Ag cross-priming of CTL are via CD40/CD40L interactions and/or through the production of IL-2 by the Th cells. Nevertheless, neither of the Th cell hybridomas used in this study expressed CD40L, even after IFN-\(\gamma\) treatment (data not shown). Furthermore, the addition of IL-2 to these cultures could not substitute for Th cells to achieve CTL activation (Fig. 3c). More definite proof that the Th cells can function as APC were the observations that IFN-\(\gamma\) treatment of the Th cells, which increases their expression of MHC-I (data not shown), enhanced the cross-priming effect (Fig. 3c), and that CTL activation took place in the presence of Th cells with pAPC lacking MHC-I molecules (Fig. 3d).

The mechanisms involved in Ag processing by Th cells will need to be addressed. As it occurs with passively absorbed materials, during cognitive Ag recognition the TCR-MHC/peptide complexes are also internalized by the T lymphocytes (42, 57). Most importantly, our results indicate that the CTL epitope portion of the MHC-II-binding peptide somehow ends up associated with the Th cells’ endogenous MHC-I molecules. It is clear that minimal CTL epitopes acquired from the APC’s MHC-I will not require any major processing before being transferred onto the endogenous MHC-I molecules on the Th cells. In contrast, it is evident that linked Th-CTL peptides bound to MHC-II will need additional processing and/or trimming by the Th cells to generate the corresponding MHC-I-binding epitopes. Although we currently do not know the precise mechanism and cellular compartment in which these MHC-I-binding peptides are generated in the Th cells, it is not difficult to speculate that this process could easily occur in the endosomal compartment. First, it is known that both the passively absorbed and TCR-acquired materials from APC end up in the T cell’s endosomes, where presumably they are destined for degradation by numerous proteases (42, 58). Thus, in these compartments, the peptides would dissociate from denatured MHC molecules and could undergo any necessary proteolytic processing to generate the minimal CTL epitopes. Because MHC-I molecules on T cells are known to frequently recycle through endocytic compartment (59), where, in many cases, the free exchange of binding peptides takes place (60), it is conceivable that CTL epitopes originally bound to the APC’s MHC could associate with the Th cells’ recycling MHC-I molecules, while en route back to the cell surface. Preliminary results showing that chloroquine treatment of Th cells inhibits the formation of CTL epitopes from Th-CTL-linked peptides presented by pAPC provide support to this possibility (A. Undale, unpublished results). Nevertheless, additional work will be required to elucidate the complex Ag-processing mechanisms of Th cells.

We have shown in this study that Th cells can present Ag to naive CD8+ T cells and induce activation and a robust clonal expansion (Fig. 4), in which the resulting CTL resemble both phenotypically and functionally typical central memory CTL (Figs. 5–8). It has been proposed that strong Ag presentation stimulates the development of effector CTL, while less efficient Ag presentation can lead to the generation of memory CTL (46). Thus, it
seems reasonable to conclude that due to the lower level of activation/costimulation signals provided by the Th cells to the naive CTL, as compared with a PAPC such as a DC, the Th-primed CTL would preferentially differentiate into memory cells. This supposition coincides with the results showing that Th-primed CTL express lower levels of activation markers (CD44, CD25, and CD69) as compared with the DC-primed CTL (Fig. 6). Although the present work has focused solely on the role of Th cells to serve as APC for CTL, it should be mentioned that under special conditions CD8 T cells could also have a similar function. For example, as previously mentioned, CD8 T cells have been shown to acquire MHC-I/peptide complexes from APC, and when these cells present Ag to other, previously activated, CD8 T cells, fratricidal effects are produced, which could play a role in the down-regulation of CTL responses (42, 58). However, we have observed a different outcome when activated CD8+ T cells present Ag to naive CD8+ T cells in vitro. Under these circumstances, the naive CD8 T cells become activated, proliferate, and differentiate into cells that, again, resemble memory CTL (data not presented).

Whether this phenomenon occurs in vivo and has a physiological role in immune responses remains to be determined. Finally, the present findings have practical implications for designing T cell epitope-based vaccines capable of generating strong and lasting CD8 T cell memory responses. One would predict that vaccines made from synthetic peptides containing linked CTL-Th epitopes should be more effective than the mixtures of the individual epitopes. Experimental evidence that confirms this prediction was elegantly presented nearly a decade ago (61).

Acknowledgments

We thank Drs. K. L. Rock, P. Marrack, H. Schreiber, and A. Rudensky for providing various cell lines used in these studies.

Disclosures

The authors have no financial conflict of interest.

References


