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*J Immunol* 2000; 164:1216-1222; doi: 10.4049/jimmunol.164.3.1216

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Naive CD8$^+$ T Cells Do Not Require Costimulation for Proliferation and Differentiation into Cytotoxic Effector Cells$^1$

Bo Wang,* Robert Maile,* Roberta Greenwood,* Edward J. Collins,*† and Jeffrey A. Frelinger$^{2*}$

Most current models of T cell activation postulate a requirement for two distinct signals. One signal is delivered through the TCR by engagement with peptide/MHC complexes, and the second is delivered by interaction between costimulatory molecules such as CD28 and its ligands CD80 and CD86. Soluble peptide/MHC tetramers provide an opportunity to test whether naive CD8$^+$ T cells can be activated via the signal generated through the TCR-cytoplasm in the absence of any potential costimulatory molecules. Using T cells from two different TCR transgenic mice in vitro, we find that TCR engagement by peptide/MHC tetramers is sufficient for the activation of naive CD8$^+$ T cells. Furthermore, these T cells proliferate, produce cytokines, and differentiate into cytolytic effectors. Under the conditions where anti-CD28 is able to enhance proliferation of normal B6 CD4$^+$, CD8$^+$, and TCR transgenic CD8$^+$ T cells with anti-CD3, we see no effect of anti-CD28 on proliferation induced by tetramers. The results of this experiment argue that given a strong signal delivered through the TCR by an authentic ligand, no costimulation is required. *The Journal of Immunology, 2000, 164: 1216–1222.*

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aive T lymphocytes need to be activated and subsequently differentiate into effector cells to perform their immune functions. The current model of T cell activation postulates that full activation of T lymphocytes requires two signals (1, 2). The first signal is postulated to be elicited by the engagement of TCR by the peptide/MHC complex. The second, costimulatory signal is provided by the interaction between accessory molecules, such as CD28, on the surface of T cells (3, 4) and their ligands, such as CD80 and CD86, on APCs (5–8). The role of costimulation has been studied extensively in vivo and in vitro in the activation of CD4$^+$ T cells (9–11). Most studies have shown that a costimulatory signal is required for the activation of CD4$^+$ cells. For example, in the absence of costimulation, engagement of the TCR on CD4$^+$ T cells by Abs to CD3 (12), Abs to TCR (13), or Ags presented by MHC class II on APCs (14–16) generally leads to functional inactivation. Blocking the interaction between CD28 and CD80 or CD86 molecules in vivo severely inhibits the primary Ab responses to T cell-dependent Ags and impairs Ab class switching (17–20). These data are consistent with a failure to activate CD4$^+$ Th cells in the absence of costimulation. There are many reports that very efficient signals via the CD3/TCR complex (for example, by anti-CD3ε alone) are able to overcome the lack of signal 2.

The data for CD8$^+$ T cells regarding the requirement of costimulation for activation are limited and contradictory. In vivo studies of tumor rejection have demonstrated that nonimmunogenic tumor cells could induce protection against a subsequent challenge if the immunizing tumor cells were transfected with CD80 (21–25). Treatment of mice with CTLA-4/Ig fusion protein designed to interrupt the interaction of CD28 with its ligands prolonged the survival of xenogeneic islet transplants (26) and cardiac allografts and was found to induce donor-specific tolerance (27, 28).

Other experiments suggest that costimulation is not required for CD8 activation. Some CD80/CD86-expressing tumor cells do not induce anti-tumor immune responses upon immunization (29–32). While CD28-deficient mice infected with vesicular stomatitis virus have impaired anti-virus Ab production, their primary CTL response against lymphocytic choriomeningitis virus (LCMV)$^3$ is intact (17). CD28-deficient mice can still reject allografts (33). The disruption of the CD28 gene in NOD mice does not prevent T cell-mediated destruction of pancreatic β-cells (34). Therefore, the role of a costimulatory signal in the activation of naive CD8$^+$ cells remains inconclusive.

MHC class I tetramers are molecules comprised of four identical class I heavy chains, each bound to β2-microglobulin, and a single peptide. The class I heavy chains are biotinylated and added to avidin, which forms a stable, but noncovalent, complex. These complexes bind to T cells bearing TCRs specific for the peptide/MHC complex in the tetramers (35). We used these class I tetramers to determine whether a costimulatory signal is required for the activation of naive CD8$^+$ T cells. Naive CD8$^+$ T cells isolated from two different TCR transgenic (tg) mice, P14 (specific for LCMV gp33–41) and HY-TCR, both restricted by H2D$^b$, were stimulated with their cognate peptide/MHC tetramers. Our results demonstrate that naive CD8$^+$ T cells could be activated by the engagement of TCR with only the correct peptide/MHC complexes. Following stimulation, these CD8$^+$ T cells proliferated and differentiated into functional effector cells. The gp33-specific CTL activity of CD8$^+$ cells activated in vitro with gp33/D$^b$ tetramers
was comparable to that of spleen cells isolated from P14 TCR transgenic mice primed in vivo by LCMV infection. The activation of CD8+ T cells by tetramers required engagement of the CD8 coreceptor. Addition of anti-CD8 mAb in the cultures inhibited the activation of CD8+ T cells induced by tetramers.

The results from the present study indicate that engagement of TCR with peptide-MHC complex alone provides a sufficient signal for naive CD8+ cells to differentiate into cytotoxic effector cells.

Materials and Methods

Animals

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) or Charles River (Raleigh, NC). Tg mice, (B6-D2-Tg(N(Tcr-LCMV)327Sdz), expressing the P14 TCR specific for gp33 peptide (aa 33–41 of the LCMV glycoprotein) restricted by H2Dd (36) were obtained from The Jackson Laboratory and subsequently backcrossed an additional four times to C57BL/6J mice. These mice are referred to as P14 in this manuscript. B6-Tg(N(Tcr-HY)) mice that carry a transgene specific for male Ag were obtained from the National Institute of Arthritis and Infectious Diseases via Taconic Laboratories (Germantown, NY). These mice are referred to as HY-TCR. After backcrossing to C57BL/6 four times, the TCR tg mice were further bred to C57BL/6-rag-1−/− recombinase-deficient mice purchased from The Jackson Laboratory (37) to produce mice that express only the transgenic TCR. In some experiments transgenic rag−/− mice were used, in others the backcross stock was used. No differences were seen between responses. All animals used in this study were maintained under recirculating pathogen-free conditions in the American Association of Laboratory Animal Care-accredited University of North Carolina Department of Laboratory Animal Medicine Facilities.

Peptides

LCMV gp33 peptide (KAVYNFATM), Y4A peptide (KAVANFATM), and HY peptide (KCRSNRQYL), and HY peptide (KCSRNQRQYL) were synthesized by the University of North Carolina microchemical facility, purified by HPLC, and tested for purity by mass spectroscopy.

Tetramer preparation

Recombinant protein was prepared as previously described by Garboczi et al. (38). The plasmid encoding the complete wild-type ΔD (provided by Dr. Stanley Nathenson, Albert Einstein College of Medicine, Bronx, NY) was modified to fuse a BirA recognition sequence at the C-terminus (35). Later, another modified ΔD clone was provided by Dr. Altman (Emory College of Medicine, Atlanta, GA) that encodes a more efficient biotinylation signal for BirA. Proteins were expressed in Escherichia coli strain BL21-pLysS and isolated with isopropyl β-D-thiogalactoside (IPTG). Purification of naive CD8+ T cells by tetramers required engagement of the CD8 coreceptor. Addition of anti-CD8 mAb in the cultures inhibited the activation of CD8+ T cells induced by tetramers.

Purification of naive CD8+ TCR transgenic T cells from spleen

Cell suspensions were prepared from the spleens of TCR transgenic mice and RBC lysed with ACK lysis buffer (0.15 M NH4 Cl, 1 mM KHCO3, and 0.1 mM Na2EDTA in water). Cells were incubated at 37°C for 1 h in flasks to eliminate adherent cells before purification. CD8+ T cells were purified using the MACS magnetic separation system according to the manufacturer’s instructions (Miltenyi Biotech, Auburn, CA). Cells were resuspended at a concentration of 107 cells/ml in PBS containing 0.5% FBS. CD4+ and MHC class II+ cells were depleted by incubating with 10 μl of anti-CD4 (clone GK1.5) and anti-MHC class II Ab-conjugated microbeads/106 cells (clone MS114.15.2) at 4°C for 15 min. After incubation, cells were washed and resuspended at 2 × 106 cells/ml in PBS with 0.5% FBS. L/Ly/5S−/− columns (Miltenyi Biotech) were used for the selection of CD8+ T cells. In our initial studies we found that CD8+ T cells selected positively with anti-CD8 mAb-conjugated beads did not proliferate in response to tetramer stimulation. Therefore, the CD8+ T cells used in all experiments were purified by depletion of other cells.

Proliferation assay

Purified CD8+ T cells (4 × 105) were stimulated with tetramers at different concentrations in 200 μl of RPMI 1640 medium plus 10% FBS, antibiotics, glutamine, and 50 μM 2-ME in 96-well flat-bottom plates. The plates were incubated for 48 h, and 1 μCi [3H]thymidine was added to each well for the final 10 h of culture. Cells were harvested using a multiple sample harvester (Otto Hille, Madison, WI), and incorporation of [3H]thymidine was measured by scintillation counting using a Beckman LS5000 counter (Palo Alto, CA). All data represent the average counts per minutes of duplicate determinations. All proliferation experiments were repeated at least three times.

Cytotoxicity assay

CTL activity was assessed in a standard 4-h 51Cr release assay as previously described (39). Briefly, naive CD8+ T cells were stimulated as described above with tetramers at the concentrations indicated. Following stimulation for 48 h, cells were harvested, washed, and used as effector cells. EL4 target cells (H2b+) were 51Cr labeled, pulsed with different concentrations of peptide for 1 h at 37°C in RPMI with 10% FBS, and washed. After 4-h incubation at 37°C in 5% CO2, with effector cells, the supernatant was harvested on a Packard Cobra Autory Counter (Downers Grove, IL). All assays were performed in triplicate, the percent specific lysis was calculated as follows: [experimental release – spontaneous release]/(maximum release – spontaneous release) × 100. Spontaneous release was defined as counts per minute released from target cells in the absence of effector cells, and maximum release was defined as counts per minute released from target cells lysed with 2.5% Triton X-100.

Flow cytometric analysis

The directly conjugated Abs used for cell surface staining in this study, anti-CD4 (H129.19), anti-CD8 (53-6.7), anti-CD25 (7D4), anti-CD69 (H1.2F3), anti-CD62L (MEL-14), anti-Vα2 (B20.1), and anti-Vß8.1, 8.2 (MR5-2), were purchased from PharMingen (San Diego, CA). Two- and three-color stainings were performed using standard methods. List mode data were collected and analyzed on a FACSan (Becton Dickinson, Mountain View, CA) using Cyclosoft software (Cytometry, Ft. Collins, CO).

Cytokine production

Naive CD8+ T cells were stimulated with various concentrations of tetramers as described above. Supernatants were taken at 40 h, and the production of IL-2 and IFN-γ was measured. IL-2 was measured using [3H]hypoxanthine uptake by the IL-2-dependent cell line CTLL-2. The assay was standardized using recombinant murine IL-2 (PharMingen). IFN-γ levels in supernatants were determined using an ELISA kit (R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions.

Costimulation assay

CD4+ and CD8+ T cells from the spleens of C57BL/6 mice and TCR transgenic CD8+ T cells from the spleens of P14 TCR transgenic mice were purified by negative selection as described above. The cells were resuspended at a concentration of 2 × 105 cells/ml in RPMI 1640 with 10% FBS, antibiotics, glutamine, and 50 μM 2-ME. For costimulation experiments, 96-well flat-bottom microtiter plates were coated with 10 μg/ml of anti-CD28 mAb (37;51; Pharmingen) alone or together with the indicated concentrations of anti-CD3 mAbs (145-2C11; Pharmingen) in 50 μl of sodium bicarbonate buffer (0.1 M NaHCO3, pH 8.2) at 37°C for 2 h. Hamster IgG was used as control Ab. Following incubation, the plates were washed twice with PBS and blocked with complete RPMI 1640 with 10% FBS. Cells (2 × 105) were added to each well in triplicate. The gp33ΔD tetramers at various concentrations were added to stimulate TCR transgenic CD8+ cells along with anti-CD28. Cells were incubated at 37°C in 5% CO2 for 48 h. [3H]hypoxanthine (1 μCi) was added to each well for the final 10 h of culture. Cells were harvested and counted as described above.

Results

CD8+ T cells isolated from P14 TCR tg mice display a naive phenotype

To investigate the requirement of costimulation for the activation of naive CD8+ T cells, mice transgenic for either a TCR specific
for LCMVgp33 or the HY male Ag were used as source of naive CD8$^+$ T cells. CD4$^+$ and class II-expressing cells were depleted with microbeads conjugated to anti-CD4 and anti-class II mAbs. The resulting cells were analyzed by two-color staining with mAbs specific for CD8 and MHC class II to determine the purity. As shown in Fig. 1A, $>93\%$ of cells were CD8$^+$ and MHC class II negative. To determine the activation status of the purified T cells, expression levels of the early activation markers, CD25 and CD69, were assessed by flow cytometry. Following stimulation, CD8$^+$ T cells were negative for CD69 and CD25 and expressed a low level of CD44, consistent with a naive phenotype (Fig. 2). Specific lysis of EL4 cells by spleen cells from noninfected animals (C) or, as a positive control, spleen cells from a P14 TCR tg mouse infected with LCMV 7 days previously (A) is shown.

**Activation of naive CD8$^+$ T cells in the absence of costimulation**

To assess whether the activation of naive CD8$^+$ T cells requires a costimulatory signal, naive CD8$^+$ T cells from either P14 or HY TCR tg mice were stimulated in vitro with H2D$^b$ tetramers folded with gp33 peptide (gp33/D$^b$) or a peptide derived from HY male Ag (HY/D$^b$). CD8$^+$ T cells proliferated vigorously in a dose-dependent manner when stimulated with the appropriate tetramers (Fig. 2). The proliferation, however, was not observed when the same CD8$^+$ T cells were stimulated with the D$^b$ tetramers bound to a control peptide. These results indicate that the interaction between TCRs and soluble peptide/MHC tetramers can induce naive CD8$^+$ T cells to proliferate in a peptide-specific manner. Furthermore, these results demonstrate that preparations of different tetramers do not contain contaminants that nonspecifically activate T cells.

To stringently test both the lack of costimulation and the absence of requirements for other cells, we stimulated single purified CD8$^+$ T cells (purified by negative selection as described above) with tetramers. Purified T cells were distributed into wells of microculture (Terasaki) plates, and each well was examined. Only wells with a single cell immediately after distribution were analyzed. P14 cells were stimulated with either gp33/D$^b$ or HY/D$^b$ tetramers. Fig. 3 shows the pooled results of two such experiments. After 48 h 48% of the P14 single cells stimulated with gp33/D$^b$ had divided at least once, while only 4% of the P14 T cells stimulated with HY/D$^b$ had divided ($p < 10^{-5}$). This experiment demonstrates that nothing more than the T cell itself and the class I tetramer are required for the induction of cell division.

![FIGURE 1. CD8$^+$ T cells purified from P14 TCR tg mice display a naive phenotype. (A) and (B) Spleen cells from P14 TCR tg mice were depleted of CD4$^+$ and MHC class II$^+$ cells using magnetic microbeads conjugated with anti-CD4 (GK1.5) and anti-MHC class II (M5/114.15.2). Purified cells were stained with mAbs anti-IA$^b$ and anti-CD8 to show purity (A) and with anti-CD69 and anti-CD62L to show lack of activation markers (B). C, CTL activity of spleen cells from P14 TCR tg mice was assessed in a standard 4-h $^{3}H$ release assay at different E:T cell ratios. EL4 cells were pulsed with 10 $\mu$g gp33 peptide and used as target cells. Specific lysis of EL4 cells by spleen cells from noninfected animals (C) or, as a positive control, spleen cells from a P14 TCR tg mouse infected with LCMV 7 days previously (A) is shown.](http://www.jimmunol.org/)

![FIGURE 2. Tetramer stimulation induces proliferation of naive CD8$^+$ T cells. Naive CD8$^+$ T cells purified from P14 TCR tg (A) and HY TCR tg (B) mice were stimulated with gp33/D$^b$ (●) or HY/D$^b$ (○) tetramers at the indicated concentrations for 48 h. Cells were incubated with 1 $\mu$Ci of $[^{3}H]$thymidine for the last 10 h of culture. Incorporation of $[^{3}H]$thymidine was measured, and the average counts per minute of duplicates is shown.](http://www.jimmunol.org/)

![FIGURE 3. The gp33 tetramer can stimulate the proliferation of purified single gp33-reactive CD8$^+$ T cells in the absence of accessory cells. CD8$^+$ T cells were purified from P14 spleens and plated in Terasaki plates at an average distribution of 0.4 cells/well. Wells containing single cells were marked. After incubation with gp33 tetramer or irrelevant HY tetramer for 48 h, cells in the marked wells were recounted. The percentage of marked wells containing more than a single cell was calculated. Actual numbers were gp33/D$^b$ tetramer, 36/76; and HY/D$^b$ tetramer, 2/53.](http://www.jimmunol.org/)
expression of TG TCR on the CD8+ forward scatter, and the right panel shows the expression of CD69. B. The expression of TG TCR on the CD8+ T cells was evaluated after stimulation. Cells were stained with mAbs specific for the TCR TG Vα-chain and CD8. Events shown were gated on CD8+ cells.

left panels). They also increased cell surface expression of CD69 (Fig. 4A, right panels) and CD25 and CD44 as well as reduced expression of CD62L (data not shown). Incubation with the HY/Db tetramers did not induce blast formation of the T cells specific for gp33 or increase the expression of these activation markers.

We next asked whether TCR down-regulation on the surface of CD8+ T cells occurred following activation with the appropriate tetramers. Purified naive CD8+ T cells were stimulated with gp33/Dβ or HY/Dβ tetramers at different concentrations for 48 h. After stimulation, cells were stained with mAb specific for the Vα-chain of P14 tg TCR. TCR expression was decreased with increasing concentration of tetramers (Fig. 4B). These results indicate that cells are activated in a manner identical with cells activated with peptide-pulsed APC (not shown).

Costimulatory molecules are not required for naive CD8+ T cells to differentiate into cytotoxic effector cells

The fact that incubation with the appropriate peptide/MHC tetramers results in increased expression of activation molecules, decreased expression of TCR, and robust proliferation suggests that naive CD8+ T cells can be activated without the involvement of costimulatory molecules. We asked whether tetramer stimulation induces naive CD8+ T cells to differentiate into functional effector cells. Purified CD8+ T cells from either P14 or HY TCR tg mice were stimulated in vitro with gp33/Dβ or HY/Dβ tetramers for 2 days, and CTL activity was determined using a standard 51Cr release assay. Fig. 5 shows that CD8+ T cells activated by the cognate tetramer could efficiently lyse target cells pulsed with the corresponding peptide, but not those pulsed with the incorrect peptide. The CTL activity of purified CD8+ T cells induced by tetramer stimulation is comparable to that of peptide-stimulated whole spleen cells in which professional APCs were present during activation (data not shown). Similar lysis was seen in splenocytes from mice infected with LCMV for 7 days (data not shown).

Differentiation of naive CD8+ T cells into effector cells is CD8 coreceptor dependent

We next tested whether the CD8 coreceptor is necessary for activation of naive CD8+ T cells stimulated with the gp33/Dβ tetramers. CD8+ T cells isolated from P14 TG TCR tg mice were stimulated with the gp33/Dβ and HY/Dβ tetramers in the presence of anti-CD8 mAb, and proliferation was assayed. Addition of anti-CD8 mAb inhibited the proliferation of naive CD8+ T cells stimulated with the gp33/Dβ tetramers in a dose-dependent manner (Fig. 6A). In addition, the requirement for CD8 coreceptor by activated CD8+ T cells for cytotoxic function was assessed. As shown in Fig. 6B, the CTL activity of T cells was inhibited by the addition of anti-CD8 mAb, but not by isotype control mAbs. Our results indicate that the CD8 coreceptor is required not only for activation of naive CD8+ T cells but also for the CTL function of effector CD8+ T cells.

T cell activation by a low affinity interaction between class I/peptide complex and TCR is not dependent on costimulation

It has been speculated that a high affinity interaction between TCR and the peptide/MHC complex may abolish the need for a costimulatory signal for T cell activation. It has been asserted that substitution of tyrosine by alanine at position 4 (Y4A) in gp33–41 decreases the affinity of TCR to the Y4A/Dβ complex without decreasing the peptide affinity to Dβ (40–42). We therefore determined whether Y4A/Dβ tetramers could activate the naive CD8+ T cells. Y4A/Dβ tetramers were able to induce the proliferation of naive CD8+ TCR tg T cells, but required a 10-fold higher concentration of the tetramer compared with gp33/Dβ (Fig. 7A).
CD8 coreceptor is required for activation and effector function of CD8\(^{+}\) T cells. A, Naive CD8\(^{+}\) T cells (4 \times 10\(^{5}\)) prepared from P14 TCR tg mice were stimulated with 0.5 \(\mu\)g/ml of gp33/D\(^{b}\) tetramers for 48 h in the presence of different concentrations of anti-CD8 mAbs (○) or isotype control mAbs (●). Cultures were pulsed with 1 \(\mu\)Ci of \([\text{H}]\)thymidine for the final 10 h, and incorporation of \([\text{H}]\)thymidine was measured. Each data point represents the average counts per minute of duplicate determinations. B, Naive CD8\(^{+}\) T cells purified from P14 TCR tg mice were stimulated as described in Materials and Methods. EL4 target cells were pulsed with different concentrations of gp33 peptide. CTL assays were performed in the presence of 2.5 \(\mu\)g/ml of anti-CD8 or isotype control mAbs.

FIGURE 6.

To examine the ability of lower affinity ligands to induce cytotoxic T cell stimulation, we purified CD8\(^{+}\) T cells from P14 mice with 0.5 \(\mu\)g of gp33/D\(^{b}\) or Y4A/D\(^{b}\) tetramers. The CTL resulting from both Y4A/D\(^{b}\) and gp33/D\(^{b}\) were identical (Fig. 7). If the lower affinity of the receptor required costimulation, then increasing the concentration alone should not have abolished that requirement, because it would only increase the fraction of receptor occupied, not the average time of occupancy. Thus, these data do not support a qualitative difference in the requirement for costimulation based on affinity.

We were interested in determining the cytokines produced, since perhaps only some of the functions of CD8\(^{+}\) effector cells were induced by tetramers. We expected production of IFN-\(\gamma\), but wondered whether there was autocrine production of IL-2. We tested supernatants of CD8\(^{+}\) T cells stimulated with wild-type gp33/D\(^{b}\) or Y4A/D\(^{b}\) tetramers. As shown in Fig. 8, both gp33/D\(^{b}\) and Y4A/D\(^{b}\) tetramers induced significant amounts of both IFN-\(\gamma\) and IL-2 production by CD8\(^{+}\) T cells from P14 tg mice. IL-4 was not detectable by intracellular staining (not shown). The secretion of IL-2 and IFN-\(\gamma\) correlated with both the proliferation and the CTL activity of CD8\(^{+}\) T cells induced by both types of tetramers (Fig. 8), although, again, more Y4A/D\(^{b}\) tetramers were needed. These results demonstrate that naive CD8\(^{+}\) T cells from P14 TCR tg mice can be efficiently activated by both wild-type gp33/D\(^{b}\) and Y4A/D\(^{b}\) to produce needed cytokines in the absence of apparent costimulation.

Activation of naive CD8\(^{+}\) T cells induced with tetramers is not enhanced by costimulatory signal

The data presented above clearly show that naive CD8\(^{+}\) T cells can differentiate into effector cells following activation by tetramers without involvement with costimulatory signal. It is important to determine whether provision of costimulation would enhance the response to tetramers, either by shifting the dose response to lower levels of tetramer or by increasing the maximum response. It has been shown that cross-linking of CD28 on T cells by specific mAb can trigger a costimulatory signal and results in an optimal T cell proliferation (12). We examined the role of costimulation in tetramer-induced activation of naive CD8\(^{+}\) T cells. As reported previously, the presence of costimulation provided by immobilized anti-CD28 mAb significantly increased the proliferation of not only CD4\(^{+}\) (Fig. 9A) and CD8\(^{+}\) (Fig. 9B) T cells from B6 mice, but also P14 transgenic CD8\(^{+}\) T cells (Fig. 9C) T cells, when stimulated with anti-CD3 mAb. This is evidenced by a shift to lower anti-CD3 doses. Strikingly, the enhanced proliferation by anti-CD28 mAb costimulation was not observed when the same TCR transgenic CD8\(^{+}\) T cells (Fig. 9D) were stimulated with tetramers. This further demonstrates that in vitro tetramer-induced activation of naive CD8\(^{+}\) T cells does not depend on costimulation, and further, that it differs from anti-CD3e-induced activation.

FIGURE 8.

Discussion

In this study we examined the ability of soluble peptide/MHC tetramers to activate naive CD8\(^{+}\) T cells in vitro from two different TCR tg mice, in the absence of apparent costimulation or exogenous growth factors. The data demonstrate that naive CD8\(^{+}\) T cells can be fully activated and differentiate into cytotoxic CD8\(^{+}\) T cells without costimulation. When CD8\(^{+}\) T cells were stimulated with our tetramers in the presence of anti-CD28 mAbs, no enhancement of proliferation (Fig. 9) or CTL activity was observed (data not shown).
Single, naive CD8\(^+\) T cells from TCR tg mice were activated with soluble peptide/MHC tetramers, and therefore, involvement of any other costimulation was excluded. In support of our data, it has been shown that CD8\(^+\) T cells can be activated by fibroblasts, which express the appropriate Ag for T cells, but not CD80/CD86 molecules (43, 44). In addition, mice deficient for the CD28 gene are able to mount an efficient CD8\(^+\) T cell-mediated cytotoxic response following LCMV infection (17) despite the fact that CD4\(^+\) helper function and Ab class switching are severely impaired in these mice. However, the findings from these studies could not rule out the possibility that unknown molecules might act as costimulators in the absence of CD28 engagement (11). Cell surface expression, cytokine secretion, and cytotoxic T cell assays all show that naive CD8\(^+\) TCR tg T cells activated by tetramers are indistinguishable from T cells activated by peptide-pulsed APC.

Recently, it was shown that TCR ligation with Ab in the absence of CD28 ligation results in activation-induced cell death, but in these experiments, anti-CD28 prevents cell death (45). Thus, although we do not see enhancement of proliferation, it is possible that we might see inhibition of cell death. Preliminary experiments suggest that most cells stimulated by tetramers undergo apoptosis. In this case ligation with anti-CD28 might block cell death without enhancing proliferation.

Delon et al. (46) have demonstrated that Ca\(^{2+}\) mobilization in CD8\(^+\) T cells can be triggered with soluble peptide/MHC class I complex in the absence of a costimulatory signal, and Goldstein et al. showed proliferation of ZC allospecific tg mice with soluble secreted L\(^1\). However, this study did not address the differentiation of naive CD8\(^+\) T cells into effector cells. Two other studies have also demonstrated that CD8\(^+\) T cells from other TCR tg strains are activated and differentiate into functional cytotoxic T cells when stimulated with agonist peptide/MHC complex without costimulation. However, in these studies, exogenous IL-2 was required (47, 48). In our study, no exogenous growth factors were needed, and our CD8\(^+\) T cells produced significant amounts of IL-2 as well as IFN-\(\gamma\) (Fig. 8). One possible explanation for the discrepancy between the previous study and ours is that CD8\(^+\) T cells were positively purified with anti-CD8 mAbs in the former study. We have found that CD8\(^+\) T cells positively selected with anti-CD8 mAb-coupled beads do not proliferate when stimulated with tetramers. This is probably due to the blockade of the interaction between MHC and CD8 coreceptor (Fig. 6) during culture.

It has been proposed that the affinity between the TCR and the peptide/MHC complex determines the requirement for a costimulatory signal in activation of CD8\(^+\) T cells (42). Using CD8\(^+\) T cells from CD8-deficient mice, Bachmann et al. (41) have reported that decreasing the affinity of interaction between the TCR and the peptide/MHC complex results in an increasing dependence on costimulation for CD8\(^+\) T cell activation. We have several lines of evidence that argue against this interpretation. In our experiments, Y4A/D\(^\alpha\) tetramers caused the equivalent proliferation and differentiation of CD8\(^+\) T cells, although Y4A/D\(^\alpha\) required a 10-fold higher concentration. No added IL-2 was needed, and Y4A induced the production of both IFN-\(\gamma\) and IL-2 from P14 cells, which differs from the findings of Bachmann (41). In addition, our data (R. Maile and J. A. Frelinger, unpublished observations) show that the HY/D\(^\alpha\) complex has a low affinity for the transgene-encoded TCR. In this paper we show that the HY/D\(^\alpha\) tetramer was effective at stimulating the T cells, although a higher concentration was required than for gp33/D\(^\alpha\) tetramers. Thus, our in vitro results argue that the dependence on costimulation is not determined by the affinity of TCR for its ligand.

Additionally, it is important to note that the use of tetramers for the purification of peptide-specific T cells has been proposed. Our studies suggest that the binding of the tetramers to T cells is not a neutral event, and that investigators should be aware of the potential activation of T cells by their binding.

We have been able to clearly show that naive CD8\(^+\) T cells are able to be completely stimulated and differentiate into cytolytic effectors with only the signal generated from the interaction between TCR and peptide/MHC class I complex without apparent involvement with costimulatory molecules. This suggests that the rules for CD4\(^+\) and CD8\(^+\) T cell activation may indeed be different. The report that a monomeric form of peptide/MHC complex can activate CD8\(^+\), but not CD4\(^+\), T cells (46, 49) (our unpublished observations) supports this idea. This makes some sense given the role of CD8\(^+\) CTL in infectious disease, where the ability to respond to infected cells, which are typically not professional APC, might well provide an advantage.

Acknowledgments

We thank Katherine Midkiff, Timothy Broderick, and Brian Cox for technical assistance. We thank Dr. Roland Tisch for critical review of the manuscript, and the members of the Frelinger and Collins laboratories for many helpful discussions.

References


