Purified MHC Class I and Peptide Complexes Activate Naive CD8+ T Cells Independently of the CD28/B7 and LFA-1/ICAM-1 Costimulatory Interactions

Julia S. Goldstein, Trina Chen, Mark Brunswick, Howard Mostowsky and Steven Kozlowski

*J Immunol* 1998; 160:3180-3187; ;
http://www.jimmunol.org/content/160/7/3180

Why *The JI*?

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**References**  This article cites 64 articles, 35 of which you can access for free at:
http://www.jimmunol.org/content/160/7/3180.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
Purified MHC Class I and Peptide Complexes Activate Naive CD8⁺ T Cells Independently of the CD28/B7 and LFA-1/ICAM-1 Costimulatory Interactions

Julia S. Goldstein,* Trina Chen,* Mark Brunswick,* Howard Mostowsky,† and Steven Kozlowski*

T cells play a central role in the initiation, maintenance, and regulation of the immune response. Effector responses of T cells are controlled by complex combinations of lymphokines and adhesion/costimulatory molecule signals. To isolate the effects of specific adhesion/costimulatory molecules and to define the minimal molecular requirements of naive CD8⁺ T cell activation, we have developed an APC-free system for stimulation of naive CD8⁺ T cells. In this report, we demonstrate that immobilized MHC class I-peptide complexes can activate naive CD8⁺ T cells from TCR transgenic mice at low cell densities. The CD8⁺ T cells were stimulated to proliferate and secrete IL-2 independently of the molecular interactions between CD28/B7.1-B7.2 or LFA-1/ICAM-1 surface receptors. Previous reports have shown that CD28 ligation is necessary for late T cell survival of APC-stimulated naive CD8⁺ T cells. Our data suggest that under certain specific conditions of high intensity T cell signaling, early activation and late cell proliferation can occur independently of APC-derived costimulatory signals. The Journal of Immunology, 1998, 160: 3180–3187.

...
mice provide a source of unprimed naive CD8\(^+\) T cells with known specificity. The 2C TCR recognizes p2C peptides in the context of an H-2L\(^d\) allos-MHC molecule. Using immobilized H-2L\(^d\) and p2C peptides, we have previously shown that naive CD8\(^-\) T cells proliferate, produce IL-2, and mature into cytotoxic effector cells at high T cell densities (34).

In this study, we demonstrate that optimally loaded purified MHC-peptide complexes can stimulate a high percentage of naive CD8\(^+\) T cells at low cell densities. In addition, H-2L\(^d\)-peptide-induced CD8\(^+\) T cell activation failed to be blocked with Abs to the LFA-1 or B7 molecules, thus ruling out T-T cell costimulation through these molecules. Finally, purified MHC-peptide complexes can generate a proliferative response that remained elevated at 96 h poststimulation.

**Materials and Methods**

**Peptides**

Peptides were obtained from the Center for Biologics Evaluation and Research Facility for Biotechnology Resources (Bethesda, MD). Peptides were synthesized on an ABI 433 peptide synthesizer (Applied Biosystems, Foster City, CA), and purity was determined by capillary electrophoresis (PAGE 5000, Beckman, Palo Alto, CA). The peptide purity was determined to be $>99.5\%$. The following peptides were used in these assays: p2Ca, LSPFDPDL; p2C-Y4, LSPYPDLD; and p2C-QY5, QLSPYPFDL. Peptides were diluted in water and filter sterilized before use.

**Expression of soluble H-2L\(^d\) MHC class I**

Generation of the soluble H-2L\(^d\) MHC class I molecule was previously published (34). Briefly, the soluble H-2L\(^d\) construct was generated by the ligation of an XhoI fragment of the genomic H-2L\(^d\) gene containing the α1 and α2 domains to the α3 domain of H-2D\(^\text{d}\) and the Q10b tail. The H-2L\(^d\)-soluble construct was cotransfected with pSV2neo into DAP3 cells using lipofectamin (Life Technologies, Grand Island, NY). High producer clones of soluble H-2L\(^d\) protein were expanded. H-2L\(^d\) was affinity purified on an anti-H-2Ld (30.5.7) (35) sepharose column from cell culture supernatants. The purified peptide was dialyzed against PBS and filter sterilized before use. Variation in the H-2L\(^d\) preparations had been minimal, as determined by their similar binding to immobilized 30.5.7 by ELISA assay and the SDS-PAGE gel pattern.

**2C TCR Tg mice**

Clonotypic 2C TCR Tg mice (36) specific for the endogenous peptide p2Ca derived from the α-ketoglutarate dehydrogenase protein (37) were a generous gift from Dr. Dennis Loh (Nippon Roache Research Center, Kamakura-shi, Japan). These mice were bred back to C57BL/6 mice for more than 5 generations in sterile microisolators and fed sterilized food and water ad libitum. At 3 wk old, mice were screened by PCR for the presence of the transgene from tail DNA treated with proteinase K (0.5 mg/ml). The presence of the transgene was further confirmed by flow cytometry with anti-V8.1.8.2 mAb (MR5-2) (PharMingen, San Diego, CA) before each in vitro assay. All mice used in these studies were to 12 wk old. Female B10.D2 mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in the same pathogen-free conditions. Sentinel mice were consistently negative for pathogens.

**Cell Purification and fluorescence-activated cell sorting**

Commercially available columns (R & D Systems, Minneapolis, MN) were used to purify CD8\(^+\) spleen T cells by negative selection. Briefly, a single cell suspension of mouse spleens (2 × 10\(^7\) cells/column) was incubated with a mixture of mAb for 10 min at room temperature. After washing to remove unbound Ab, the cells were loaded onto the Ig-coated glass beads column and incubated 15 min. The cells were eluted, washed, and resuspended in complete medium. The negatively selected cell population obtained in this manner had the following phenotype: > 99% V\(\text{α}1\); 81.8.2 TCR positive; 10 to 20% CD44\(^+\); < 3% Ia\(^+\); < 5% CD16/32\(^+\); and < 3% CD45R0\(^+\), as assessed by flow cytometry.

For sorting, the column-purified CD8\(^+\) T cells were stained with α-CD8 FITC (53.6.7) (PharMingen) vs α-CD44 phycoerythrin (PE; IM7) using 1 µg of Ab per 10\(^7\) cells. The sorted population (CD8\(^-\) CD44\(^+\) ) was > 99.5\% pure as determined by postsort analysis. Cell sorting was performed with a FACStar\(^{+}\) cell sorter (Becton Dickinson, Mountain View, CA) equipped with an argon laser at 4880 A. Data analysis was performed with the Cell Quest software (version 1.12). The sorter was calibrated for fluorescence channels 1 and 2 with Immunocount beads (Coulter Immunol., Hialeah, FL) and for forward and side scatter with Calibrate beads (Becton Dickinson) for peak separation. The column-purified CD8\(^+\) T cells were gated on forward vs side scatter for size and viability, and the cell population was collected based on fluorescence channel 1 vs 2.

**Proliferation assays**

H-2L\(^d\) (0.7 µg/well) was coated overnight on 96-well microtiter plates (Immulon 4, Dynatech, Chantilly, VA) at 4°C. The plates were washed three times with PBS and blocked with 2% BSA-PBS. After incubation at room temperature for 30 min the wells were washed three times with PBS. To enhance the unloading of endogenous peptide and the loading of the exogenous peptide, wells were incubated in 100 µl of citrate phosphate buffer (pH 6.5), p2C peptide (30 µM), and human β₂-microglobulin (β₂m) (Calbiochem, La Jolla, CA) (1 µg/well) (38). After 2 h at 37°C, the plates were washed three times with PBS. CD8\(^+\) T cells, in a final volume of 0.2 ml of complete medium (RPMI 1640 (BioWhittaker, Walkersville, MD) plus 10% FCS, 100 U/ml penicillin/streptomycin, nonessential amino acids, 2 µg/ml L-glutamine, and 50 µM 2-ME), were added at 3 × 10\(^5\) cells/well unless otherwise noted. Due to the rapid off rate of the p2C peptides, peptide and β₂m were added at 30 µM and 1 µg/well, respectively, at the beginning of the culture, unless otherwise noted. The CD8\(^+\) T cells were incubated at 37°C/5% CO\(_2\), APC were incubated by irradiating (1500 rads) B10.D2 single-cell splenocyte suspensions. For determination of proliferation, cells were pulsed with 1 µCi/well of \(^{3}H\)thymidine (DuPont/NEN Research, Boston, MA), which was added during the last 12 h of the culture period. The cells were harvested onto filter mats, and the incorporated radioactivity was measured in a Betaplate scintillation counter (LK-B Pharmacia, Piscataway, NJ).

All mAbs (FITC, PE, or unlabeled) used in our studies were purchased from PharMingen. In the blocking experiments, purified mAbs (no azide, low endotoxin) α-LFA-1 (M17/4), α-C80 (1G10), α-CD86 (GL1), and rat IgG2a (isotype control) were added at 10 µg/ml at the beginning of culture. The percentage of inhibition was calculated as follows: 1 – (sample + Ab cpm/sample − Ab cpm) × 100. The background cpm were less than 500 cpm.

**FACS analysis**

Column-purified cells were stimulated on immobilized MHC and peptide. After 4 h of incubation at 37°C/5% CO\(_2\), cells were washed twice with PBS and incubated for 15 min in 0.5 µM EDTA at 37°C/5% CO\(_2\). Recovered cells were washed in complete medium and resuspended in 100 µl of FACS buffer (PBS, 5% FCS, and 0.1% NaN\(_3\)). Staining was performed using 1 µg/10\(^6\) cells and incubating with α-CD69 FITC (H1.2F3), α-CD8 PE (53.6.7), and α-CD3 FITC (145-2C11) for 30 min in ice. A total of 10\(^4\) events per sample were acquired using a FACSscan (Becton Dickinson) flow cytometer. Data following analysis was performed using the Cell Quest software. Fluorochrome-conjugated isotype-matched rat mAbs were used as negative controls.

**IL-2 ELISA**

For IL-2 determination, 100 µl of 48-h culture supernatants were analyzed with a commercially available kit (Endogen, Boston, MA) as per manufacturer specifications. Horseradish peroxidase-conjugated streptavidin (Zymed, San Francisco, CA) and tetramethylbenzidine (DAKO, Carpinteria, CA) were used as developers. The absorbance was read on a Bio-Rad (Hercules, CA) model 3550 microplate reader at 655 nm or 450 nm after addition of 0.18 M sulfuric acid stop solution with background subtraction.

**Results**

**Naive CD8\(^+\) T cells can proliferate at low cell densities**

Previous studies regarding the activation of naive CD8\(^+\) T cells have been hampered by the inability to examine the consequences of Ag-specific stimulation in the absence of APC interactions. We developed a system (34) in which we can induce activation of naive CD8\(^+\) T cells in response to purified MHC and peptide in the absence of APC. The 2C Tg naive CD8\(^+\) T cells respond specifically to purified H-2L\(^d\) and peptide by proliferating, secreting IL-2, and becoming lytic effectors in the absence of APC. High cell densities (1–3 × 10\(^5\) cells/well) were required to detect a response. It was conceivable that the need for high cell density was due to T-T cell interactions. Although the 2C Tg cells do not
express the H-2Ld molecule, T-T cell costimulation in trans might have occurred. Therefore, it was important to exclude the possibility of these interactions and the potential presence of a rare contaminant syngeneic APC in the cultures. We suspected that the relatively low response observed in our APC-free system might have been due to low level recruitment of responder cells by the immobilized MHC-peptide complexes. This, in turn, might have been due to incomplete peptide and/or β2m loading of the recombinant MHC molecules under the conditions used. On the basis of a recent study regarding the role of the pH and β2m excess in facilitating MHC class I binding sites (38), we modified our strategy to increase the unloading of endogenous peptide and improve exogenous peptide loading. As shown in Fig. 1A, citrate buffer treatment (pH 6.5) in the presence of exogenous peptide and β2m produced a strong proliferative response even at 3 × 10^4 cells/well when the sorted CD8+CD44+ T cells were stimulated with immobilized H-2L^d-p2C-QY5 complexes. At high cell densities (3 × 10^5/well), naive CD8+ T cells proliferate with similar intensities independently of the H-2L^d pH treatment. Only the pH-treated H-2L^d molecules were able to support proliferation at lower cell densities. In addition, the modified loading protocol decreased the peptide and MHC concentrations required for T cell activation (data not shown). We next compared the effect of the modified peptide loading protocol on the different p2C peptides in a proliferation assay (Fig. 1B). Although at lower cell densities naive CD8+ T cells had a higher proliferative response to the more potent peptide (p2C-QY5), the weaker peptides, p2Ca and p2C-Y4, elicited strong proliferative responses when the H-2L^d was pH treated. We further evaluated the responses of serially diluted sorted CD8+ T cells after stimulation with either APCs or with plate-bound MHC class I + p2C-QY5. As seen in Fig. 1C, the proliferative responses induced by either stimulus were similar. Interestingly, immobilized purified MHC class I-p2C-QY5 complexes were able to elicit a proliferative response even at the lower cell densities. In conclusion, the modified loading protocol, which presumably increases the number of peptide-MHC complexes available for TCR-CD3 engagement, allows for a response of the 2C naive CD8+ T cells at low cell densities. Since a proliferative response is detected at lower cell densities, the likelihood of costimulatory interactions between T cells or with a rare contaminant APC is diminished.

A large percentage of the naive 2C CD8+ T cells are activated by purified MHC and antigenic peptides

Even though the efficient peptide loading of the immobilized MHC molecules allowed for the activation of 2C CD8+ T cells at low cell densities, determining the percentage of the cell population that was activated is critical to the understanding of our system. Early activation events include the modulation of certain surface molecules. Among these molecules, the CD69 molecule (VEA) is up-regulated at the cell surface (39, 40), and it was recently shown (41) that down-regulation of the expression of the CD3 surface molecule follows CD8+ T cell TCR engagement and cell activation. Our previous studies had shown that at 48 h poststimulation, >95% of the naive 2C Tg CD8+ T cells up-regulated surface expression of the CD69 and the CD25 (IL-2Ra) molecules after activation with all the p2C peptides tested (34). We determined surface expression of CD69 and of CD3 at 4 h poststimulation to assess the percentage of cells that were receiving the activation signal and to rule out that a subpopulation of cells was being selected. Our results demonstrate (Fig. 2A) that 40% of the gated CD8+ T cells up-regulate surface expression of the CD69 Ag and that >95% of the cells down-regulate the expression of the surface CD3 molecule. The reason that only 40% of the CD8+ T cells express CD69 is probably the early time point evaluated.

![FIGURE 1. Treatment of H-2L^d with citrate phosphate buffer (pH 6.5) and β2m allow for proliferation at low cell densities.](http://www.jimmunol.org/)

**A.** Comparison of different H-2L^d loading protocols. Plates coated with H-2L^d were either incubated for 2 h at 37°C/5%CO2 or treated with citrate phosphate buffer (pH 6.5), as described in Materials and Methods. Sorted CD8+CD44+ T cells were incubated with p2Ca (30 μM; ■) or p2Ca and β2m (1 μg/ml) on untreated (▲) or pH-treated (●) H-2L^d. SDs are representative of triplicate samples. **B.** Comparison of the proliferative responses of naive CD8+ T cells induced by the different p2C peptides on pH-treated H-2L^d. Sorted naive CD8+CD44+ T cells were incubated with p2Ca (30 μM; ■) or p2Ca and β2m (1 μg/ml) on untreated (▲) or pH-treated (●) immobilized H-2L^d. p2Ca (▲) and p2C-Y4 (■) were added at 30 μM during the peptide loading and culture. p2C-QY5 (●) was added at 30 μM during the peptide loading and at 10 μM during the culture period. β2m was added at 1 μg/ml during loading and culture. SDs are representative of triplicate samples. **C.** The proliferative response of naive CD8+ T cells induced by purified protein and peptide is comparable to the response induced by allogeneic APCs. Sorted naive CD8+CD44+ T cells were incubated with pH-treated H-2L^d, p2C-QY5, and β2m (●) or irradiated B10.D2 (▲) (3 × 10^4/well). SDs are representative of triplicate samples. These results are representative of three independent experiments.
H-2L\textsuperscript{d}-p2C-QY5 complexes, blockade of the B7.1, B7.2, or LFA-1 molecules with specific mAbs was unable to decrease the proliferative response of naive CD8\textsuperscript{+} T cells to allogeneic APC stimulation. These results show that these two costimulatory pathways are not an absolute requirement in the activation of 2C Tg naive CD8\textsuperscript{+} T cells when a potent TCR stimulus is used. The inability of these Abs to block the proliferative response to purified MHC class I and peptide suggests that T-T cell interactions and/or potentially contaminating presenting cells do not participate in the response.

**Purified MHC and peptide induce IL-2 secretion at low cell densities and in the absence of CD28/B7.1 + B7.2 or LFA-1/ICAM-1 costimulation**

It has been shown that ligation of the CD28 costimulatory molecule stabilizes IL-2 mRNA (42) and increases IL-2 secretion (43) and CD8\textsuperscript{+} T cell survival (25). In Tg naive CD4\textsuperscript{+} T cells, IL-2 secretion was shown to be dependent on costimulation (14). To further demonstrate that a potent signal delivered through the TCR can drive T cells to full activation, we determined the levels of secreted IL-2 in the supernatants of 48-h cultures. Naive CD8\textsuperscript{+} T cells stimulated with either purified MHC and peptide or allogeneic APC (Fig. 4A) produced nanogram amounts of IL-2 even at low cell densities. When cells were stimulated with H-2L\textsuperscript{d} and p2C-QY5, CD28 engagement was not an absolute requirement for induction of IL-2 production. The presence of IL-2 in the cultures of cells seeded at low cell densities indicates that cell-cell interaction was not a determinant factor in induction of IL-2 secretion. The lower amount of IL-2 generated by APC-stimulated T cells at high T cell densities may be due to a lower T cell:APC ratio with high T cell numbers. Furthermore, blockade of the B7.1, B7.2, or LFA-1 molecules (Fig. 4, B and C) decreased IL-2 secretion only when the cells were stimulated with APC. When cells were stimulated with H-2L\textsuperscript{d} and p2C-QY5 in the presence of costimulatory molecule blockade, the levels of IL-2 detected were found to be similar to that of control mAb. These results indicate that stimulation of naive CD8\textsuperscript{+} T cells with purified MHC class I and peptide can support IL-2 secretion independently of two major T cell costimulatory pathways.

**Late proliferation of 2C-Tg CD8\textsuperscript{+} T cells in response to purified protein and peptide is not decreased**

Recently, studies of naive 2C-Tg T cells activated with allogeneic APCs demonstrated that the role of CD28 signaling is to sustain the late proliferative response and enhance long term cell survival.
Those studies demonstrated that Tg T cells activated with a high TCR stimulus (addition of exogenous p2Ca to the APC) cannot overcome the need for CD28 costimulation. The APC-free system provides a unique method to discriminate the need for costimulatory molecules during T cell activation. We evaluated the requirements for costimulation during early and late proliferation in a time course study (Fig. 5). Naive CD8\(^+\) T cells were stimulated with APCs, using a high APC:CD8\(^+\) ratio (4:1) to ensure a high level of costimulation. The response was compared with that of cells stimulated with immobilized H-2L\(^d\) and p2C-QY5. The proliferative responses were assessed at 24, 48, 72, and 96 h. As expected, the proliferative response of 2C CD8\(^+\) T cells induced with APC was maximal at 72 h. MHC and peptide also induced a strong proliferative response at 48 and 72 h. Interestingly, the response of cells stimulated with MHC class I and p2C-QY5 complexes was not decreased at the 96-h time point. Our data show that naive CD8\(^+\) T cells stimulated with purified protein and peptide complexes can sustain proliferation up to 96 h and that a potent TCR signal can bypass the need for costimulation.

Discussion
In our studies, we have used splenic CD8\(^+\) T cells sorted for the CD44\(^2\) population to study the costimulatory requirements of naive CD8\(^+\) T cells (44). In the present study, a strategy for efficient MHC class I-peptide loading (38) allowed us to observe significant proliferative and IL-2 responses at low cell densities (\(\geq 3 \times 10^4\) T cells/well). The majority of these phenotypically naive T cells increased their size at 48 h, and a highly significant percentage of the T cells down-regulated the CD3 surface molecule at 4 h poststimulation. In addition, a large percentage of the CD8\(^+\) T cells showed early up-regulation of the surface CD69. This evidence strongly argues against a small subset of the sorted T cells being responsible for the observed costimulatory-independent activation. Furthermore, the responses of naive CD8\(^+\) T cells to H-2L\(^d\)-p2C-QY5 complexes were not blocked by antibodies to the B7 or LFA-1 molecules. This clearly demonstrates the lack of an absolute requirement for B7 or LFA-1 molecules in the activation of naive CD8\(^+\) T cells.
Although we cannot absolutely exclude costimulatory signals involving molecules other than B7 and LFA-1, several facts suggest that costimulation is not an absolute requirement. First, the cell density at which we observe activation of a high percentage of the T cells is at $3 \times 10^6$ cells/well, and at that density few of the added T cells are initially in contact with each other. Second, under physiologic conditions, adhesion/costimulatory molecules need to be present on the same cell surface as the TCR binding complex (45, 46). The 2C T cells used in the APC-free system do not have the antigenic allo-H-2L$^d$ molecule, and we previously have shown that the presentation of peptide antigen on self-H-2$^d$ haplotype molecules does not occur in our system (34). Therefore, effective costimulation from T-T cell interactions is unlikely. Finally, B7 molecules are up-regulated on activated 2C T cells (24), and ICAM-1 is expressed on T cells (47). Therefore, the ligands for the potent T cell costimulatory receptors CD28 and LFA-1 are present. We reasoned that if the TCR signal of the 2C T cells was insufficient for activation without cell-cell interactions, B7 molecules or LFA-1 would play a role. Our studies show that these molecules do not play a role, and consequently, it is highly unlikely that T-T cell costimulatory interactions or rare contaminant presenting cells are driving the T cell responses we observe.

Previous studies have had conflicting results regarding the need for ICAM-1 and B7 costimulation in the 2C system for induction of proliferation at 48 and 72 h (24, 25, 32, 33). Experiments in which transfected Drosophila cells are used as APCs for 2C T cell activation demonstrate a requirement for costimulatory molecules even in the presence of high affinity p2C peptides (32, 33). The same peptides with spleen cells or RMA-S.L$^d$ cells as APCs demonstrate early 2C T cell activation without B7-CD28 or CD8 interactions (24, 25). The difference between these results is most likely due to the differences in the APC. Spleen cells express a number of cell surface molecules, other than B7, that can costimulate, and RMA-S cells have been shown to costimulate (48). Transfected Drosophila cells have only moderate expression of H-2L$^d$, the cells die at 37°C, and insect cell-expressed H-2L$^d$ may have structural differences from mammalian cell-expressed H-2L$^d$. Thus, it is unclear whether the requirement for costimulatory molecules by the Drosophila cells is due to a weak TCR signal or due to the complete lack of any alternative mammalian costimulatory molecules. Our system, which is devoid of APC costimulatory molecules, demonstrates that under conditions of high Ag density, these molecules are not an absolute requirement. This suggests that the Drosophila cell results may be due to a less potent TCR signal or other effects by the dying Drosophila cells. Another factor that could increase the TCR signal in our system is the use of the extremely potent p2C-QY5 peptide.

In the activation of 2C T cells with potent p2C peptides, using either spleen cells or RMA-S.L$^d$-presenting cells, CD8 interactions are unnecessary (24). In our system, anti-CD8 can block 2C T cell activation (data not shown). The combination of high Ag density and affinity with APC costimulatory molecules may be more potent than high density Ag alone. It is certainly possible that addition of costimulatory signals to a very potent TCR signal may lead to CD8 independence. The addition of costimulatory signals to our system may decrease the need for CD8 interactions or increase the response in another way. Notwithstanding this, the response we observe, in the absence of costimulation, is a significant one.

In addition to early proliferation, we observe high levels of IL-2 production in the APC-free system. Previous investigators have observed that IL-2 secretion can be enhanced by the ligation of CD28 and that IL-2 is an important determinant in the outcome of T cell stimulation (43). It was also described that the lack of IL-2 in the cultures induced a state of anergy that could be reversed by the exogenous addition of this cytokine (15). In the APC-free system, the production of IL-2, which occurred in the absence of CD28 ligation, further demonstrates that the CD28/B7 pathway is not an absolute requirement for successful CD8$^+$ T cell activation.

Some studies suggested that CD28 ligation may have less importance in initial stimulation of T cell proliferation and greater importance in prolonged T cell proliferation (22--25). This effect on late proliferation may relate to the prevention of apoptosis by CD28 ligation (25, 26). These studies have noted greater effects of CD28 on T cell proliferation and survival at a later time point (96 h). Despite the lack of APC-derived costimulatory signals in our system, we observe a strong proliferative response of T cells at 96 h. One possible explanation is that a high affinity TCR ligand delivered at high density can generate late survival signals in the absence of costimulation. Alternatively, the sustained late proliferation we observed could be due to the lack of a negative APC-delivered signal. In such a model, costimulatory signals would be required to overcome APC-derived negative signals. These negative signals are absent in the APC-free system. The decreased late 2C T cell proliferation seen with splenic APC and p2C peptides (24, 25) can be interpreted as resulting from a negative APC signal. CD28-mediated costimulation is then required to overcome the inhibitory signal.

A similar system, using purified class II MHC and peptide to stimulate TCR Tg CD4$^+$ T cells, was unable to show IL-2 production without presenting cells or addition of exogenous signals (49). Three reasons can be postulated to explain the differences between this system and ours: 1) It is possible that CD8$^+$ and CD4$^+$ cells differ in their requirements for costimulation (50). 2) Our system may achieve a higher ligand density. 3)The need for costimulation may be a function of the kinetic or equilibrium binding constants of the MHC-peptide complex for the TCR and the number of available MHC-peptide complexes. This is a reasonable hypothesis, since the measured affinity of certain p2C peptide-H-2L$^d$ complexes for the 2C TCR is about $10^{-7}$ M ($K_d$) (51--54), compared with an affinity of $5 \times 10^{-5}$ M ($K_d$) for the class II MHC-peptide complex binding for the TCR (55, 56) studied in the purified class II MHC model.

The need for a second signal in T cell activation has implications for the maintenance of self-tolerance. It is widely accepted that the presence of an Ag is not sufficient to initiate an immune response and that a second component delivered by an APC is also necessary. Our results are compatible with the “signal intensity” hypothesis (57, 58), which postulates that the requirement for costimulation is dependent on the strength of the TCR signal rather than being an absolute requirement. A potent TCR signal such as the one used in our model system may be quite rare, and generally two signals are required for T cell activation. Nevertheless, rare high affinity-high density T cell ligands that can overcome the requirement for a second signal may play a role in the pathogenesis of autoimmune diseases and graft rejection. Reagents that quantitate peptide-MHC complexes (59--62) will allow evaluation of Ag density effects on requirements for costimulation. Previous studies demonstrating the requirement for costimulation with tissue-specific expression of foreign Ags (63, 64) may not have had sufficient Ag density or affinity to bypass costimulation. The expression level of particular peptide-MHC complexes in physiologic and pathologic states may better define the likelihood of costimulatory independent costimulation. Even if T lymphocytes require costimulation under physiologic conditions, the ability to bypass costimulation with high Ag density will be a useful vaccination strategy for individuals treated with costimulatory blockade for autoimmune disease or graft rejection. Finally, the observation that
a potent TCR signal can bypass the need for a costimulatory signal has important implications for the mechanisms of T cell signaling.

Acknowledgments

We thank Drs. Ezio Bonvini, David H. Margulies, Barbara Rellahan, Kathrym E. Stein, and Melanie S. Vaccio for review and discussion of the manuscript. We thank Dr. Dennis Loh for the 2C Tg mice and Mary Belcher for helping maintain the mouse colony. We acknowledge Drs. Nga Nguyen and Bhaskar Chandrasekhar of the Facility for Biotechnology Resource Sources, Center for Biologics Evaluation and Research, for providing services in peptide synthesis.

References


