In Vivo Generation of Cytotoxic T Cells from Epitopes Displayed on Peptide-Based Delivery Vehicles

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In Vivo Generation of Cytotoxic T Cells from Epitopes Displayed on Peptide-Based Delivery Vehicles


The development of nonviral, peptide-based constructs able to elicit protective in vivo CTL responses represents a major challenge in the design of future vaccines. We report the design of branched peptide delivery vehicles, termed loligomers, that facilitate the import, processing, and presentation of CTL epitopes onto nascent MHC class I molecules. These complexes are then effectively displayed on the surface of APCs. The intracellular delivery of CTL epitopes by loligomers prolonged the expression of Ag-MHC class I complexes on the surface of APCs in comparison with free CTL epitope alone. Furthermore, the injection of CTL epitope-containing loligomers into mice led to the generation of in vivo CTL responses and the induction of autoimmune disease in an animal model. Synthetic epitope-carrying, peptide-based delivery vehicles may represent useful components to be included in the formulation of future vaccines. The Journal of Immunology, 2002, 168: 5709–5715.

The cellular arm of the immune system relies upon the efficient activation of lymphocytes through the recognition of Ag displayed on MHC molecules. Peptide Ags derived from endogenous protein or viral/mutant gene products are presented on MHC class I molecules and lead to either self-tolerance or the induction of a CTL response (1). Exogenous proteins, however, enter the MHC class II pathway through endocytosis and are degraded in lysosomes such that the resulting peptides generally do not contribute to MHC class I presentation (2, 3). Although cross-priming can result in CTL activation through the presentation of exogenous Ag on MHC class I molecules, it is not considered to be an efficient method of priming CTL responses (4). Surface loading of MHC class I molecules is also possible, but peptide immunization rarely generates a CTL response. Thus, the appropriate delivery of Ag into cells may represent a method of skewing the number of defined epitopes complexed with class I molecules and also increase the length of time these complexes are presented on APCs.

Over the past few years, there has been an increased interest in using various import signals to target CTL epitopes into cells. These import signals include penetratin from the antennapedia homeodomain (5) (residues 43–58 from the third helix) and the basic region of HIV-1 Tat protein (6) (residues 47–57). Despite the apparent abilities of these sequences to cross the cell membrane, the generation of an in vivo CTL response through the administration of peptide with adenovirus has proven inefficient (7–9). Multi-tasking delivery vehicles have recently been designed such that cellular import and nuclear localization signals were introduced into branched peptides to guide their routing into eukaryotic cells (10–12). These structures, termed loligomers, are easily assembled by solid phase approaches. The word loligomer is derived from the merger of two terms; the Latin root loligo, which refers to members of the squid family (tentacles), and the term oligomer, which defines a polymeric assembly of building blocks. The prototypic loligomer displayed an import signal (IS) and a nuclear localization sequence derived from the SV40 large T Ag on each branch of a lysine-based tentacular scaffold, and these were successfully used to target plasmids (15) and drugs into the nucleus of cells (16). The evolution of branched peptide designs into guided peptide constructs suggests that the efficient routing of CTL epitopes into cells could potentially present distinct advantages over surface loading of linear peptides. In this report, we demonstrate that the intracellular delivery of CTL epitopes into APCs enhances the presentation of Ag on MHC class I molecules and leads to the generation of an in vivo CTL response in mice.

Materials and Methods

Cell lines and mice

The EL-4 cell line (American Type Culture Collection, Manassas, VA) and in vitro cultures of murine T cells were grown in α-MEM supplemented with 10% FCS, HEPES (10 mM), 50 μM 2-ME, and antibiotics. All cells were maintained at 37°C in 5% CO₂. Eight-to-12-wk-old female C57BL/6 mice (B6, H-2b) were used in immunization strategies (The Jackson Laboratory, Bar Harbor, ME). P14 TCR transgenic mice that express a TCR specific for the lymphohytic choriomeningitis virus (LCMV) glycoprotein and MHC H-2Dβ and rat insulin promoter (RIP)-gp mouse have been previously described (17, 18).

Peptide synthesis and purification

Branched peptides were synthesized by classical solid phase peptide synthesis using tert-butoxycarbonyl chemistry and phenylaceticdiphenylmethyl resin supports (Novabiochem, San Diego, CA) on an Applied Biosystems 430A synthesizer (Foster City, CA). The synthesis, coupling efficiencies, purification, and characterization of loligomers were previously described (11). Linear peptides were synthesized on an Applied Biosystems 431A
synthesize using 9-fluorenylmethoxycarbonyl (Fmoc) chemistry and p-hydroxyethyl phenoxymethyl poly styrene resin supports (Applied Bio- systems, Mississauga, Canada). Peptides were dissolved in water containing 0.1% (v/v) trifluoroacetic acid and loaded on a C4 reverse phase column (Waters, Milford, MA) equilibrated in the same mobile phase. The peptides were eluted using an acetonitrile gradient from 20 to 60% aceto- nitrite in water in 30 min. Mass spectrometry and amino acid analyses were performed to verify the mass and amino acid composition of each peptide.

Derivatization of peptides with fluorescent probes

The single thiol group (cysteine residue) introduced in the C-terminal arm of peptides was derivatized with 5-maleimido forms of rhodamine, biotin, fluorescein, or Texas Red (Molecular Probes, Eugene, OR). Briefly, 3 mg of each peptide was dissolved in 1 ml of 0.1 M sodium citrate buffer (pH 6.5). The thiol-reactive moiety (0.3 mg) was then added as a solid to the peptide solution, and the resulting solution was mixed for 4 h at 4 °C in the dark. Labelled peptides were desalted on a Sephadex G-25 column (3 x 2cm; Pharmacia, Baie d’Urfé, Canada) equilibrated with 50 mM ammonium bicarbonate, lyophilized, and stored at −70 °C until used.

Confocal and electron microscopy

Peptides used for confocal microscopy were conjugated with fluorescein, Texas Red, or both chromophores. Biotin-labeled peptides (on the C-terminal arm) were used for electron microscopy. Briefly, EL-4 cells (5 x 10⁶ cells) were incubated with 0.5 μM peptide (loligomer SIINFEKL-15S, loligomeSIINFEKL, loligomeAβ, or linearSIINFEKL-15S) for various lengths of time. Cells were centrifuged, and the resulting pellets were washed with PBS. For confocal slides, a drop of resuspended cells was placed onto a glass slide. Slides were immediately examined on a Zeiss LSM 510 con- focal microscope (argon-krypton laser). For electron microscopy, cells were fixed with 0.2% paraformaldehyde in PBS, embedded in LRWhite resin (Marivac, Halifax, Canada) and cut to a thickness of 0.2 μm. Grids were then labeled with streptavidin-gold particles (15-nm diameter; Chek monitor from Roche (Indianapolis, IN).

Internalization and retention of peptides by EL-4 cells

EL-4 cells (5 x 10⁶ cells/ml) were incubated at 4 or 37°C with increasing amounts of fluorescein-labeled peptide for 4 h (concentration study) or with 0.5 μM peptide over a period of 6 h (time study). Cells were subse- quently recovered by centrifugation (1000 rpm), washed with PBS to remove excess peptide, resuspended in 0.5 ml 1% BSA in PBS with 5 μl 7-amino-actinomycin D (7-AAD) (0.1 mg/ml; Molecular Probes), and placed on ice for 10 min. The fluorescence intensities of viable cell pop- ulations were recorded by flow cytometry and represented a measure of the amount of peptide internalized by EL-4 cells (Fig. 1B).

Expression of SIINFEKL-Kb complexes by EL-4 cells

EL-4 cells were incubated with either 0.5 μM SIINFEKL or loligomerSIINFEKL-15S at 37°C for increasing amounts of time. At each time point, cells were spun down, washed with peptide-free medium, and resus- pended in 1% BSA in PBS (100 μl) with fluorescein-labeled mAb 25D1.16.30 (1 μl). Samples were kept on ice for 30 min, washed twice with PBS, and subsequently resuspended in 0.5 ml 1% BSA in PBS, 7-AAD (5 μl) was added to each sample, and the fluorescence signal was measured by flow cytometry.

Immunization of mice and in vitro restimulation

Peptides (500 μg) were dissolved in 100 μl Quil A (100 μg/ml; Cedarlane) and injected s.c. into the hind leg of C57BL/6. Mice were sacrificed 11 days later, and their spleens were resected. Three days before restimulation B6 spleen cells were activated with Con A (2 mg/ml; ICN Pharmaceuticals Canada, Montréal, Canada), and peptides (loligomerSIINFEKL-15S and SIINFEKL-0.5 μm) were added to the culture the night before restimulation. Con A blasts were harvested using Lymphocyte M (Cedarlane, Hornby, Canada), irradiated (50 Gy), and used as stimulators for the lymphocytes obtained from the immunized mice at a ratio of 7 x 10⁶ spleen cells to 7 x 10⁶ stimulators. After 5 days CTLs were harvested with Lympholyte M and used in a standard 51Cr release assay at ratios of 100:1, 30:1, and 10:1 against target cells.

Chromium 51 release assay

EL-4 cells were preincubated with 0.5 μM peptide (loligomerSIINFEKL-15S, SIINFEKL, or loligomerSIINFEKL-15S) overnight (18 h before assay) as well as during the labeling procedure with sodium [51Cr]chromate for 1 h. The isotope (Na)²⁰CrO₄ (360 μCi; NEN Life Science, Boston, MA) was added to −5 x 10⁶ target cells suspended in 100 μl FCS. The cells were counted after three washes with medium containing 1% FCS. Target cells (2 x 10⁶; 100 μl) were dispensed with CTLs (100 μl) into wells of 96-well plates and incubated at 37°C for 4 h. Cells were centrifuged (800 rpm) for 5 min, and 100 μl of each supernatant was collected for radioactivity measurements. Specific lysis was calculated as: % specific lysis = (release by CTL – spontaneous release)/(total release – spontaneous release) x 100.

Induction of diabetes

Double-transgenic RIP-gp/P4 mice were immunized with 20 μg loligomerKAVYNFATM-15S and 100 μg anti-CD40 (FGK45) i.v. (19). The loligomer activates the transgenic P14 T cells, while the anti-CD40 acti- vates APCs (20). Blood glucose was monitored regularly using an Accu- Chek monitor from Roche (Indianapolis, IN).

Results

Design and preparation of peptide and loligomer constructs harboring CTL epitopes

Branch peptide delivery vehicles (CTL loligomers) were de- signed to carry a CTL epitope, either SIINFEKL (H-2Kb re- stricted epitope from chicken OVA (21); residues 257–264) or KAVYNFATM (H-2Db restricted epitope derived from the LCMV glycoprotein (22), p33; residues 33–41) placed on the N terminus of a pentalysine IS (Fig. 1). Cleavage within the IS do- main by resident endoplasmic reticulum (ER)/Golgi proteases would then release the epitope from the delivery vehicle. Various control peptides were also constructed to demonstrate the necessity of the IS for efficient cellular entry and the advantage of displaying multiple epitopes on one delivery vehicle compared with a linear peptide carrying the same domains (Fig. 1B).

Internalization and processing of CTL loligomers by EL-4 cells

The internalization of CTL-containing loligomers by cells had to be established before demonstrating their utility as intracellular Ag delivery vehicles. EL-4 cells were treated with increasing concentrations of fluorescein-labeled loligomers. The mean fluorescence intensity of cells exposed to the labeled constructs was analyzed by flow cytometry and represented a measure of the amount of peptide internalized by EL-4 cells (Fig. 2). Both loligomerSIINFEKL-15S and loligomerAβ were taken up into EL-4 cells at 37°C in a time- and peptide-concentration-dependent manner (Fig. 2, A and B). Fluorescence signal inside cells was not detected for cells incubated with these peptides at 4°C (Fig. 2, A and B), which verified the mechanism of internalization (absorptive endocytosis) observed for previous constructs (11). The uptake of loligomers reached satu- ration well before 4 h, and this time point was used in subsequent experiments to maximize the amount of peptide within the cell. The addition of the epitope onto the N-terminal end of the molecule de- creased the efficiency of import of loligomerSIINFEKL-15S in relation to loligomerAβ alone (Fig. 2, A and B). However, adequate internalization levels were still achieved as observed by confocal microscopy (Fig. 3). The minimal uptake of loligomerSIINFEKL at either 4 or 37°C (Fig. 2C) demonstrated the necessity for the IS domain to cross the cell membrane, while the exclusion of linearSIINFEKL-15S illustrated the need for multiple targeting domains (Fig. 2C). The increased internal- ization of loligomerSIINFEKL-15S and loligomerAβ (Fig. 2D) within
the 0–0.5 μM range also illustrates that uptake remains a concentration-dependent event. Cationic peptides are moderately cytotoxic (lolligomer SIINFEKL/H9262 M has a C50 value of 8 μM; data not shown), and for this reason a concentration of 0.5 μM (Fig. 2, A–C) and an incubation time of 4 h were used in assays to ensure the optimal loading of EL-4 cells with minimal toxicity.

The proper routing of CTL loligomers within cells is necessary for the success of this intracellular Ag delivery strategy. EL-4 cells (murine thymoma cells) were used as APCs in the context of our experiments, as previous studies have shown that they effectively present both SIINFEKL and KAVYNFATM epitopes (23). Biotinylated or fluorescently labeled peptides were used to establish the internalization of various peptides into EL-4 cells. Confocal and electron microscopy results (Fig. 3) illustrate that loligomer SIINFEKL/H9262 was readily imported and processed by EL-4 cells, events that were not observed for the linear SIINFEKL peptide. Colocalization of DiOC6 (a fluorescent label for ER and Golgi compartments, green) with Texas Red-labeled peptides (red) suggested the partial routing of peptide constructs to the Golgi and ER vesicular compartments within 4 h (Fig. 3B). Electron microscopy further revealed the internalization and compartmentalization of loligomer SIINFEKL/H9262. A 4-h incubation of EL-4 cells with CTL loligomers and subsequent visualization with streptavidin gold particles localized the peptides to vesicles found close to the cell membrane (Fig. 3E). Ten hours later, CTL loligomers were present in both the cytoplasm and within vesicles (Fig. 3F). Evidence of loligomer SIINFEKL/H9262 within the ER lumen of cells was not conclusive by this method of staining.

Appropriate length of peptide is essential for Ag binding to the MHC class I groove. The cleavage of SIINFEKL from the branched carrier was visualized through the use of a dual-labeled loligomer SIINFEKL/H9262 (Fig. 3, C and D). A Texas Red chromophore (red) was introduced at the C-terminal arm of this construct, while the ε-amino group of the unique lysine residue on each SIINFEKL sequence was derivatized with a fluorescein probe (Fig. 1, green). Areas of yellow fluorescence indicated the presence of intact or partially processed loligomers after 4 h (Fig. 3D, bottom cell), while distinct regions of both red and green fluorescence (observed in both cells) illustrated the cleavage of epitopes from the branched vehicle. The shuttling of the epitope into different cellular compartments from the branched core suggests that one or more host proteases selective for Arg/Lys dipeptide sequences or proteasomes associated with the cytosolic face of vesicular membranes are able to process the pentalysine import sequences, thus leading to the release of the CTL epitope. The phase contrast image confirmed the cytosolic location of the loligomers with no nuclear staining visible (Fig. 3C). Images depict representative events observed for all cells in the field of view.

**FIGURE 1.** A. Structure of a CTL loligomer that carries the chicken OVA CTL epitope, SIINFEKL. Peptides and loligomer constructs harboring the p33 epitope (KAVYNFATM) were also prepared for this study. Fluorescein, Texas Red, and biotin were covalently attached to the peptide using the single thiol group (cysteine side chain labeled with an asterisk) present within the C-terminal analytical arm of peptides. For dual-labeling experiments, fluorescein was introduced at the ε-amino group of lysine 263 (labeled Fl) of SIINFEKL during solid phase synthesis. B. List of synthetic peptides and associated acronyms used in the present study.
Internalization of loligomers by EL-4 cells is dependent upon time and concentration. EL-4 cells were incubated with 0.5 μM fluorescein-labeled loligromer<sub>SINFEKL</sub><sup>1-15</sup> (A) at 37°C (■) and 4°C (□), 0.5 μM fluorescein-labeled loligromer<sub>SINFEKL</sub><sup>1-15</sup> (B) at 37°C (●) and 4°C (○), 0.5 μM fluorescein-labeled loligromer<sub>SINFEKL</sub><sup>1-15</sup> (C) at 37°C (▲) and 4°C (△), or 0.5 μM fluorescein-labeled linear<sub>SINFEKL</sub><sup>1-15</sup> (D) 37°C (▲) and 4°C (○) as a function of time. Autofluorescence was minimal for EL-4 cells (△, all panels). Relative mean fluorescence intensities were measured by flow cytometry. D. Cells were incubated with increasing concentrations of peptide (loligromer<sub>SINFEKL</sub><sup>1-15</sup> (■), loligromer<sub>SINFEKL</sub><sup>1-15</sup> (▲), and linear<sub>SINFEKL</sub><sup>1-15</sup> (△)) for 4 h at 37°C before flow cytometry measurements. Each symbol represents the average mean fluorescence signals derived from three separate experiments.

**Extending the cellular half-life and presentation of CTL epitopes by APCs**

The duration of antigenic stimulation is an important factor in committing T cells to activation (24). Thus, CTL loligomers were evaluated for their ability to 1) extend the biological half-life of the epitopes within cells, and 2) lengthen and/or skew the processing and presentation of CTL epitopes by APCs. The incubation of EL-4 cells with loligromer<sub>SINFEKL</sub><sup>1-15</sup> for 4 h and subsequent removal of peptide from the medium illustrated the ability of cells to retain CTL loligomers while the initial uptake of linear<sub>SINFEKL</sub><sup>1-15</sup> was minimal and therefore resulted in very little retention of this peptide within cells (Fig. 4). Confocal images also verified the presence of CTL loligomers within EL-4 cells >20 h after its removal from the medium (Fig. 4B).

We then looked at the expression time of SIINFEKL-K<sup>b</sup> complexes on the cell surface using a fluorescein-labeled mAb (25) (25D1.16.30, Fig. 5A) that specifically recognized such complexes. Both SIINFEKL and loligromer<sub>SINFEKL</sub><sup>1-15</sup> led to the presence of surface SIINFEKL-K<sup>b</sup> molecules after incubation of EL-4 cells with peptide. However, the initial generation of complexes by SIINFEKL alone decreased more rapidly than loligromer<sub>SINFEKL</sub><sup>1-15</sup>-generated complexes as a function of time.

To determine whether this prolonged expression was still biologically relevant, a chromium release assay was performed on target EL-4 cells that had been incubated with peptide either once (18 h before the assay was performed) or twice (18 h before the assay and during the labeling procedure). CTLs were capable of killing EL-4 cells that had been incubated with SIINFEKL-containing peptides (Fig. 5B). More importantly, recognition of loligromer-loaded EL-4 cells (loaded once or twice) was still greater than that of EL-4 cells that had been loaded twice with SIINFEKL. Unexpectedly, SIINFEKL-loaded K<sup>b</sup> complexes (loaded once) were still weakly recognized after 18 h, which was attributed to either the high concentration of peptide used to load cells (0.5 μM) or the longer half-life of the Ag on K<sup>b</sup> molecules. EL-4 cells in the
Thus, CTL-epitope containing loligomers are able to produce a more robust T cell response than SIINFEKL-raised T cells (data not shown).

The demonstration that loligomers can prolong the expression of antigen on MHC class I complexes on APCs led us to test its ability to induce effective CTL function in vivo. To determine whether loligomers were capable of inducing effective CTL function in vivo, we examined its ability to induce an in vivo CTL response to different epitopes could potentially lead to a generalized model of Ag delivery by branched peptide delivery vehicles.

In vivo priming of CTLs by loligomers

The demonstration that loligomers can prolong the expression of peptide:MHC class I complexes on APCs led us to test its ability to stimulate in vivo CTL responses. Administration of loligomer or SIINFEKL (500 μg in Quil A) to mice led to the priming of CTLs that recognized EL-4 targets that had been loaded once with KAVYNFATM or KAVYNFATM and EL-4 cells that had been pulsed only once with loligomer KAVYNFATM. EL-4 target cells pulsed once with either loligomer or KAVYNFATM and EL-4 cells that had been pulsed only once with loligomer KAVYNFATM to the same extent. Thus, loligomers were, in fact, capable of prolonging the expression of peptide:MHC complexes on the cell surface, and this phenomenon was not restricted to one epitope. The half-life of peptide:MHC class I complexes on the surface of EL-4 cells was significantly shorter when these APCs were exposed to the free peptide KAVYNFATM instead of SIINFEKL.

In vivo immune response that was better than immunization with the linear SIINFEKL peptide alone. The results obtained using the CTL epitope, KAVYNFATM, led to a similar outcome in which CTLs generated from loligomer KAVYNFATM-injected mice resulted in a higher specific lysis of targets compared with KAVYNFATM-raised CTLs (Fig. 6B). Therefore, the ability of loligomers to generate an in vivo CTL response to different epitopes could potentially lead to a generalized model of Ag delivery by branched peptide delivery vehicles.

To determine whether loligomers were capable of inducing effective CTL function in vivo, we examined its ability to induce autoimmunity in a transgenic animal model. RIP-gp transgenic mice express the LCMV glycoprotein in the β-islet cells of the pancreas under the direction of the rat insulin promoter. LCMV-specific T cells remain in the T cell repertoire and upon infection with LCMV become activated to destroy the islet cells expressing the LCMV-gp, leading to diabetes. The administration of peptides
A miuim release assay was performed. B Were also restimulated in the absence of peptide to serve as controls. C and anti-CD40 and monitored for hyperglycemia (Fig. 6. The results obtained using the CTL epitope, KAVYNFATM, led to the presence of SIINFEKL:Kb molecules after incubation of EL-4 cells with peptide. However, the initial generation of complexes by SIINFEKL alone decreased more rapidly than that of loligomerSIINFEKL + IS-generated complexes as a function of time. Furthermore, lysis of peptide-loaded target cells by epitope-specific CTLs verified that the surface complexes were still biologically relevant. Results from chromium release assays indicated that recognition of loligomer-loaded targets (loaded once or twice) was greater than that of EL-4 cells that had been loaded twice with SIINFEKL (Fig. 5B). This time-dependent event was even more dramatic for the p33 epitope (Fig. 5C). Recognition of complexes generated by EL-4 cells loaded once with loligomerKAVYNFATM + IS (18 h before assay) by peptide-specific CTLs was equivalent to that of EL-4 cells that had been incubated either twice with the same CTL loligomer or twice with KAVYNFATM. This demonstrated that a significant number of KAVYNFATM:D0 complexes were still present on the cell surface 18 h after exposure to loligomers, whereas the number of complexes present on targets loaded once with KAVYNFATM had diminished to background levels (Fig. 5C). The half-life of peptide:MHC class I complexes on the surface of EL-4 cells was significantly shorter when these APCs were exposed to the free peptide KAVYNFATM instead of SIINFEKL. Stability differences associated with selected Ags and their complexes with class I molecules may explain temporal variations in terms of target recognition by CTLs. The generation of a functional T cell response in the absence of CD28 (27) and the commitment of naive T cells to proliferation (24) are events that are regulated by the prolongation of a CTL response. This hypothesis was proven by the ability of EL-4 cells to retain loligomerSIINFEKL + IS (Fig. 4) and extend the expression time of SIINFEKL:Kb complexes on the cell surface, as detected by a specific fluorescein-labeled mAb (25). Both SIINFEKL and loligomerSIINFEKL + IS led to the presence of SIINFEKL:Kb molecules after incubation of EL-4 cells with peptide. However, the initial generation of complexes by SIINFEKL alone decreased more rapidly than that of loligomerSIINFEKL + IS-generated complexes as a function of time. Furthermore, lysis of peptide-loaded target cells by epitope-specific CTLs verified that the surface complexes were still biologically relevant. Results from chromium release assays indicated that recognition of loligomer-loaded targets (loaded once or twice) was greater than that of EL-4 cells that had been loaded twice with SIINFEKL (Fig. 5B). This time-dependent event was even more dramatic for the p33 epitope (Fig. 5C). Recognition of complexes generated by EL-4 cells loaded once with loligomerKAVYNFATM + IS (18 h before assay) by peptide-specific CTLs was equivalent to that of EL-4 cells that had been incubated either twice with the same CTL loligomer or twice with KAVYNFATM. This demonstrated that a significant number of KAVYNFATM:D0 complexes were still present on the cell surface 18 h after exposure to loligomers, whereas the number of complexes present on targets loaded once with KAVYNFATM had diminished to background levels (Fig. 5C). The half-life of peptide:MHC class I complexes on the surface of EL-4 cells was significantly shorter when these APCs were exposed to the free peptide KAVYNFATM instead of SIINFEKL. Stability differences associated with selected Ags and their complexes with class I molecules may explain temporal variations in terms of target recognition by CTLs. The generation of a functional T cell response in the absence of CD28 (27) and the commitment of naive T cells to proliferation (24) are events that are regulated by the prolongation of peptide:MHC class I complexes on the surface of APCs.

It was demonstrated that the administration of loligomers harboring the SIINFEKL epitope into mice elicited a CTL response in the presence of adjuvant (Fig. 6A). The results obtained using the CTL epitope, KAVYNFATM, led to a similar outcome, in which CTLs generated from loligomerKAVYNFATM + IS-injected mice resulted in a higher specific lysis of targets compared with KAVYNFATM-raised CTLs (Fig. 6B). The induction of effective CTL function in vivo was also demonstrated by the ability of the loligomers to induce diabetes in a transgenic model (Fig. 6C). The mouse studies presented in Fig. 6 did not, however, determine whether the higher specific lysis of CTL derived from
loliqomer-treated mice originated from an in vivo immune response or from a potentially more efficient in vitro restimulation event. Therefore, the ability of loligomers to generate an in vivo CTL response to different epitopes could potentially lead to a generalized model of Ag delivery by branched peptide delivery vehicles.

Peptides derived from the third helix of the antennapedia homeodomain and the basic region of HIV Tat (Tat) represent two other peptide-based cellular import signals that have been recently used to deliver CTL epitopes into cells (7–9). The antennapedia peptide constructs have been moderately successful in eliciting in vivo T cell responses requiring that the constructs be injected twice into mice and only yielded a response when SDS was added to the vaccine preparation (9). Studies with Tat fusion peptides indicated that such constructs were not able to produce a T cell response even with the addition of CFA (7). The simple fusion of CTL epitopes to these import signals led to their delivery into cells. However, subsequent steps to enable Ag loading onto MHC molecules were not addressed. For example, peptide length (28–30) as well as C-terminal amino acids of the Ags (31, 32) are important factors for peptide loading onto MHC class I molecules. One would expect that tailoring these vehicles to meet these requirements could potentially enhance their ability to load MHC class I molecules with peptide. The results presented in this study suggest that the incorporation of Ags into loligomer scaffolds sustains the presentation of peptide/MHC I complexes on the surface of cells with a limited requirement for adjuvant. Finally, loligomers are ideal for the incorporation of signals that may enhance other mechanistic arms associated with a productive immune response. For example, the addition of a CpG motif (33) or a lipid tail (34) to these constructs may enhance their ability to target a specific immune response.

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