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CpG-DNA Activates In Vivo T Cell Epitope Presenting Dendritic Cells to Trigger Protective Antiviral Cytotoxic T Cell Responses

Ramunas M. Vabulas,*† Hanspeter Pircher,‡ Grayson B. Lipford,* Hans Häcker,* and Hermann Wagner‡*

MHC class I-restricted T cell epitopes lack immunogenicity unless aided by IFA or CFA. In an attempt to circumvent the known inflammatory side effects of IFA and CFA, we analyzed the ability of immunostimulatory CpG-DNA to act as an adjuvant for MHC class I-restricted peptide epitopes. Using the immunodominant CD8 T cell epitopes, SIINFEKL from ovalbumin (OVA) or KAVYNFATM (gp33) from lymphocytic choriomeningitis virus glycoprotein, we observed that CpG-DNA conveyed immunogenicity to these epitopes leading to primary induction of peptide-specific CTL. Furthermore, vaccination with the lymphocytic choriomeningitis virus gp33 peptide triggered not only CTL but also protective antiviral defense. We also showed that MHC class I-restricted peptides are constitutively presented by immature dendritic cells (DC) within the draining lymph nodes but failed to induce CTL responses. The use of CpG-DNA as an adjuvant, however, initiated peptide presenting immature DC progression to professional licensed APC. Activated DC induced cytolytic CD8 T cells in wild-type mice and also mice deficient of Th cells or CD40 ligand. CpG-DNA thus incites CTL responses toward MHC class I-restricted T cell epitopes in a Th cell-independent manner. Overall, these results provide new insights into CpG-DNA-mediated adjuvanticity and may influence future vaccination strategies for infectious and perhaps tumor diseases. The Journal of Immunology, 2000, 164: 2372–2378.

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1 Abbreviations used in this paper: DC, dendritic cells; CD40L, CD40 ligand; B3Z, the SIINFEKL-specific T cell hybridoma; LCMV, lymphocytic choriomeningitis virus; gp33, LCMV peptide KAVYNFATM; LN, lymph nodes; ODN, oligodeoxy-nucleotides.

allows Th cell-independent CTL responses to MHC class I-restricted T cell epitopes.

Materials and Methods

**Reagents**

1-Phosphatidylcholine (type XI-E), 1-phosphatidyl-d-t-ceramide, cholesterol, and IFA were purchased from Sigma (Munich, Germany). OVA peptide 257–264 (SIINFEKL) and LCMV glycoprotein 33–41 peptide (KAVYNFATM = gp33) were custom synthesized by Research Genetics (Huntsville, AL). For technical reasons (to prevent dimer formation), the original cysteine at the anchor position 41 in the gp33 had been replaced by methionine. Phosphorothioate-stabilised oligodeoxyribonucleotides (ODN) were synthesized by TibaMolBiol (Berlin, Germany). CpG-ODN (═ ODN 1668, containing a CG-motif marked with bold letters: 5′-TCC-ATG-AHG-CTG-ATG-CT) and control GpC-ODN (inverted CG = ODN 1720: 5′-TCC-ATG-AGC-CTG-ATG-CT) were taken from Ref. 24.

**Mice**

Female C57BL/6 mice were purchased from Harlan Winkelmann (Borchsen, Germany). CD40L knockout mice (C57BL/6-J-Tnfsf5-tm1Imx♂♀) were bought from The Jackson Laboratory (Bar Harbor, ME) and MHC class II-deficient (B6 Adζ/Adζ) mice (25) were kindly provided by Dr. I. Förster (Technical University Munich, Munich, Germany). All animals were housed under specific pathogen-free conditions and were used at 8–12 wk of age.

**Cell lines and in vitro culture medium**

EL-4 (H-2b) thymoma cells were purchased from American Type Culture Collection (Rockville, MD). B3Z, a somatic T cell hybrid generated by fusing the OVA/Kb-specific cytotoxic clone, B3, with a lacZ-inducible derivative of BW5147 fusion partner (26), was kindly provided by Dr. B. L. Kelsall (National Institutes of Health, Bethesda, MD). Cells were cultured in Clicks/RPMI (Biochrom, Berlin, Germany) supplemented with 10% (v/v) heat-inactivated FCS (Biochrom), 5 × 10−5 M 2-ME, and antibiotics (100 U/ml penicillin G and 100 U/ml streptomycin sulfate) at 37°C and 5% CO2.

**Peptide-liposome formation**

Liposomes were manufactured by a rehydration entrapment method (27). Briefly, 18 mg phosphatidylcholine, 2 mg phosphatidylglycerol, and 5 mg cholesterol (2:0:2) were suspended in 5 ml chloroform in a 250-ml round bottom flask. The mixture was rotary evaporated under reduced pressure until a fine film formed on the flask wall. Residual chloroform was removed by vacuum desiccation. The peptide (3 mg) was solubilized in a minimal volume of water and diluted to 1 ml with PBS. This solution was slowly added to the dried lipid and hand shaken until the lipids were re-suspended. The mixture was then filter extruded through a 0.2-μm pore size Anotop 10 Plus syringe mount filter (Whatman, Maidstone, England).

**Immunization and 51Cr release assay**

For priming of cytotoxic T cells, 0.3 mg of respective peptide entrapped in liposomes or solubilized in PBS were injected into both fore footpads of mice. As an adjuvant CpG-ODN was coJECTED (10 nmol/mouse). After 4–5 days, draining LN were removed and a single-cell suspension was prepared by pressing the LN through a screen. LN cells were cultured at 1–2 × 107/ml in 24-well plates in medium conditioned with 10 U/ml IL-2 for additional 4–5 days, and then 51Cr release assay was performed. For in vitro restimulation of primed lymphocytes 2 wk after immunization, spleen cell suspensions were prepared (5 × 106/ml) and cocultured with SIINFEKL-labeled (0.5 μM) irradiated (15 Gy) spleen cells (2 × 106/ml) with 10 U/ml IL-2 for 2 days, and then 51Cr release assay was performed. In some experiments, CD4- or CD8-positive T cells were depleted from the expanded lymphocyte population before measuring lytic activity. This was done by labeling lymphocytes with magnetic beads coated with the anti-CD4 (clone DB4) or anti-CD8 (Dynal, Hamburg, Germany) allowing negative selection of the CD4+ or CD8+ T cell subset, respectively. The efficiency of negative selection was >90% as judged by FACS analysis. The 51Cr release assay was performed as follows. EL-4 target cells (2 × 105) were labeled with 200 μCi Na111CrO4 (Amersham Pharmacia Biotech, Freiburg, Germany) for 1 h at 37°C and washed; one-half of 51Cr-labeled cells was incubated with peptide solution (0.1 μM SIINFEKL or 0.1 μM KAVYNFATM) for an additional 30 min. Peptide-unlabeled cells served as a specificity control. After extensive washing, 100 μl (104 cells) of target cells were added to the same volume of replicative serial dilutions of effector CTL.

After 4 h of incubation at 37°C and 5% CO2, 100 μl of culture supernatant were removed from each well and γ-irradiation was measured. Specific lysis was calculated according to the formula: % specific lysis = [cpm (sample) − cpm (spontaneous release)]/cpm (maximal release) − cpm (spontaneous release) × 100. Spontaneous release ranged between 5 and 15%.

**Preparation of DC**

For preparation of DC, draining LN were removed and collected into ice-cold HBSS (Life Technologies, Karlsruhe, Germany). LN were digested for 1 h at 37°C using collagenase type I purchased from Sigma. Single-cell suspensions were prepared, and clumps were removed using a 100-μm pore size filter (Becton Dickinson, Heidelberg, Germany). Immediately after collagenase treatment LN cells were washed in Ca++-free HBSS, from this point onwards the cell suspension was always handled in buffers containing 2 mM EDTA. For positive selection of dendritic cells, single-cell suspensions were incubated with magnetic beads coated with the anti-CD11c Ab (Miltenyi, Bergisch Gladbach, Germany). Selection of DC was performed using MS separation columns and MiniMACS according to the protocol supplied by the manufacturer (Miltenyi). The selection ensued >80% pure CD11c+ cell population as judged by FACS analysis.

**Presentation assay**

Presentation of SIINFEKL was assayed by measurement of lucZ activity in SIINFEKL/Kb-specific T cell hybridoma (B3Z) transfected with a LacZ reporter under the transcriptional control of IL-2 gene promoter elements (26) after coinoculation with subpopulations of LN cells. Briefly, 12 and 24 h after SIINFEKL injection, the draining LN were harvested, and LN cells were separated into CD11c+ and CD11c− fractions as described above. Graded numbers of fractionated cells were incubated with 105 B3Z cells/well in 96-well plate at 37°C/5% CO2 overnight. On the next day, individual cultures were washed with 100 μl PBS and lysed by addition of 100 μl Z-buffer (100 mM NaCl, 2 mM Na2HPO4, 1.5 mM NaH2PO4, 2 mM KCl, 0.5 mM MgCl2, 0.125% Nonidet P-40, 0.15 mM chloroform red β-galactoside (Calbiochem, San Diego, CA) in PBS). After 4 h incubation at 37°C, 50 μl stop buffer (300 mM glycine and 15 mM EDTA in water) were added to each well, and absorption of individual cultures was read using a 96-well Emex plate reader (Molecular Devices, Sunnyvale, CA). The absorption wavelength was 570 nm, with 650 nm as the reference wavelength. Induction index was calculated by dividing induced activity by background.

**Flow cytometry**

Cells were washed in PBS containing 2% FCS (PBS/FCS) and first incubated for 10 min at 4°C with anti-FcγRI/IIIAb to block unspecific binding of the following Ab reagents. All mAbs used were purchased from PharMingen (Hamburg, Germany). FITC- and PE-labeled mAbs (used at 4–20 μg/ml) included Abs against MHC class II (clone 2G9), CD11c (clone N418), CD80/B7-1 (clone 1G10), CD69/B7-2 (clone GL1), and CD40 (clone 3/23). Isotype controls included purified rat IgG2a and hamster IgG. After incubation with mAbs for 30 min at 4°C, cells were washed with PBS/FCS. FACS analysis was performed on a flow cytometer FACScalibur (Becton Dickinson), acquiring 10,000 events. FACS data were analyzed using CellQuest FACS software.

**Protection of mice from replication of LCMV**

Mice were immunized once s.c. at the tail base with 0.3 mg gp33 plus 10 nmol CpG-ODN 1668, gp33 emulsified in IFA, CpG-ODN 1660 alone, or gp33 alone or were mock treated. Two weeks later, mice were infected i.v. with 200 PFU of LCMV, and viral titers in the spleens were determined after 4 days in a virus plaque assay, as described previously (28). LCMV was quantified with an immunological focus assay in 24- or 96-well plates. The LCMV-WE virus strain used in this study was originally obtained from Rolf Zinkernagel (University Hospital Zurich, Zurich, Switzerland) and was grown on L929 fibroblast cells with a low multiplicity of infection.

**Results**

**CpG-ODN renders CD8 T cell peptides immunogenic**

The use of CpG-ODN as an adjuvant appears to function by virtue of its direct DC-activating qualities (22, 23). We were interested to determine whether CpG-ODN-mediated activation of DC in vivo would render MHC class I-restricted peptides immunogenic for CD8 CTL precursors. C57BL/6 mice were challenged with SIINFEKL, either encapsulated in liposomes or as an aqueous solution,
ODN 1668. SIINFEKL-specific CTL could be generated in the class I peptide from LCMV (see below). To probe for the generation of primed T cells in the spleen, we selected peptide-specific CTL which could be detected after in vitro IL-2-driven expansion. Activated CTL precursors were present in draining LN for up to 9 days. Based on comparison of lytic units generated per culture, activation of CTL precursors peaked at days 3–4 (data not shown).

FIGURE 1. Priming of peptide-specific CD8\(^+\) CTL. A, C57BL/6 mice were immunized in the fore footpads with 0.3 mg SIINFEKL either entrapped in liposomes (a and b) or free in PBS (c–e). In a and c, 10 nmol CpG-ODN 1668 was admixed as adjuvant. In b and d, SIINFEKL alone was used for immunization. In e, 10 nmol nonimmunostimulatory GpC-ODN 1720 were used. After 4 days, lymphocytes from the draining LN were harvested and cultured in vitro with 10 U/ml rIL-2 for an additional 4 days. Thereafter, lytic activity against EL-4 cells (○, ▽ or EL-4 cells pulsed with the SIINFEKL (●, ▼) was scored in a standard \(^{51}\)Cr release assay. Symbols represent CTL derived from individual mice. B. Before testing lytic activity in \(^{51}\)Cr release assay, SIINFEKL plus CpG-ODN-induced CTL were depleted of CD4\(^+\) or CD8\(^+\) cells. Targets were EL-4 cells (○, ▽) or EL-4 cells pulsed with the SIINFEKL (●, ▼). Symbols represent CTL derived from individual mice. A is representative of at least four experiments performed; B is representative of two experiments.

Next we analyzed the time window required to activate peptide-specific CTL precursors in vivo. Activation of CTL precursors within draining LN was scored over a period from day 1 until day 9 postchallenge with SIINFEKL plus CpG-ODN 1668. As shown in Fig. 2A, within 24 h LN draining the injection site developed peptide-specific CTL which could be detected after in vitro IL-2-driven expansion. Activated CTL precursors were present in draining LN for up to 9 days. Based on comparison of lytic units generated per culture, activation of CTL precursors peaked at days 3–4 (data not shown).

We also tested whether activation of CTL precursors is restricted to the draining LNs or becomes disseminated to a systemic level. To probe for the generation of primed T cells in the spleen, mice were challenged in the fore footpads with SIINFEKL (Ag) plus CpG-ODN 1668 (adjuvants). After 2 wk, their splenocytes were prepared and restimulated in vitro with the peptide SIINFEKL. As shown in Fig. 2B, strong SIINFEKL-specific lytic activity was generated, implying systemic dissemination of activated CTL. This conclusion is also supported by the induction of protective immunity toward LCMV after immunization with an MHC class I peptide from LCMV (see below).

Finally, we tested the effective dose range of the adjuvant CpG-ODN 1668. SIINFEKL-specific CTL could be generated in the draining LN in mice challenged locally with Ag plus 10–1 nmol CpG-ODN 1668 (data not shown).

Immature DC constitutively present SIINFEKL whereas CpG-DNA activates Ag-presenting DC

In situ, DC are believed to initiate primary CTL responses in a two-step process. After sampling and processing Ag as immature DC, they must subsequently mature into professional APC via activation (7). DC activation may be brought about by engagement with CD40L-positive Th cells (8–10) or via direct recognition of
We analyzed whether immature DC within draining LN take up SIINFEKL and present the peptide in a H-2Kb-restricted fashion. CD11c+ DC from LN draining a local site of SIINFEKL challenge were positively selected and probed for H-2Kb-restricted peptide presentation. This was accomplished by using the SIINFEKL/Kb-reactive T cell hybridoma B3Z, which is transfected with a LacZ reporter under the transcriptional control of IL-2 gene promoter elements (26). Within 12 h after s.c. challenge with soluble SIINFEKL, positively selected DC from the draining LN were harvested and digested with collagenase for 1 h, and DC were isolated using CD11c-MicroBeads. CD11c-positive and CD11c-negative cell populations were serially diluted and tested for SIINFEKL presentation by incubating them overnight with 10⁶ B3Z cells (the SIINFEKL/Kb complex-specific T cell hybridoma transfected with NFAT-LacZ reporter). Induced β-galactosidase activity was measured in duplicates with CPRG substrate as described in Materials and Methods. Induction index was calculated by dividing induced activity through background. Pooled cells from two mice per group were used. Individual points represent means from duplicate wells ± SD. Data are representative of three experiments performed.

In a second step, the activation phenotype of the CD11c+ DC in LN was scored by FACS analysis. Costimulatory molecules (CD80, CD86, CD40) and MHC class II expression by DC from LN draining a SIINFEKL challenge remained immature and were indistinguishable from mock challenged DC (data not shown). Challenge with a mixture of SIINFEKL plus CpG-ODN, however, greatly augmented DC surface expression of costimulatory molecules and MHC class II (Fig. 4). DC activation is CpG-motif dependent, because ODN lacking a CpG-motif fail to activate DC (22, 23). Together, these data suggested that within the draining LN immature DC constitutively take up and present SIINFEKL. It is the CpG-DNA inoculation, however, that causes their activation and transit to professional APC.

CpG-DNA bypasses the requirement for T help in generating CTL responses to T cell peptides

Activation of Ag-presenting immature DC can be brought about by CD40L+ Th cells or by direct DC recognition of pathogen derived ligands. We analyzed whether CTL induction by peptides plus CpG-ODN was independent of T cell help. CTL induction by SIINFEKL plus CpG-ODN 1668 was tested in wild-type C57BL/6 mice and compared with that of haploype matched CD40L−/− mice or Th cell-deficient MHC class II−/− mice. Fig. 5 shows that the magnitudes of SIINFEKL specific CTL responses in CD40L−/− and MHC class II−/− mice were similar to those in H-2Kb wild-type mice. These data suggests that CpG-DNA

FIGURE 3. SIINFEKL is presented in vivo by DC with similar efficiency in CpG-ODN-treated and untreated mice. C57BL/6 mice were injected in the footpads with 0.15 mg SIINFEKL free in PBS (○, ●), per footpad, SIINFEKL plus 5 nmol CpG-ODN 1668 (△, ▼) per footpad, or CpG-ODN 1668 alone (□, ■); 12 and 24 h after injection draining LN were harvested and digested with collagenase for 1 h, and DC were isolated using CD11c-MicroBeads. CD11c-positive (○, △) and CD11c-negative (●, ▼, ■) cell populations were serially diluted and tested for SIINFEKL presentation by incubating them overnight with 10⁶ B3Z cells (the SIINFEKL/Kb complex-specific T cell hybridoma transfected with NFAT-LacZ reporter). Induced β-galactosidase activity was measured in duplicates with CPRG substrate as described in Materials and Methods. Induction index was calculated by dividing induced activity through background. Pooled cells from two mice per group were used. Individual points represent means from duplicate wells ± SD. Data are representative of two experiments performed.

FIGURE 4. Activation of DC by CpG-DNA in draining LN. C57BL/6 mice were injected in the footpads with 0.15 mg SIINFEKL free in PBS (solid histograms) per footpad or SIINFEKL plus 5 nmol CpG-ODN 1668 per footpad (thick lines); 20 h after injection DC from draining LN were isolated using CD11c-MicroBeads, stained for CD40, B7.1 (CD80), B7.2 (CD86), and MHC class II molecules. The expression of surface markers were analyzed with FACS acquiring 10,000 events. Thin lines indicate isotype controls. Data are representative of two experiments performed.
allowed induction of Th cell-independent CTL responses toward MHC class I-restricted T cell peptides.

**CpG-DNA allows T cell peptide to induce protective antiviral CTL responses**

Murine LCMV infections are controlled by LCMV-specific CD8 CTL via perforin-dependent cytolysis of virus-infected cells (30). A major MHC class I-restricted T cell epitope for H-2b mice is the aa 33–41 peptide (gp33) derived from LCMV glycoprotein (31). This T cell epitope injected in IFA has been shown to induce protective immunity (32). We used this model system to determine whether CpG-ODN 1668 renders the MHC class I-restricted peptide gp33 immunogenic for CTL and whether the immunized mice are protected against LCMV infection. Draining LN of C57BL/6 mice challenged s.c. with an aqueous solution of gp33 plus CpG-ODN 1668 generated gp33-specific CTL (Fig. 6). The draining LN of mice immunized with gp33 alone, however, failed to do so.

These data demonstrate that CpG-DNA used as an adjuvant conveyed immunogenicity to the MHC class I-restricted T cell epitope gp33.

**To analyze whether activation of gp33-reactive CTL by CpG-DNA is paralleled by protective immunity, C57BL/6 mice were immunized s.c. at the base of the tail with 0.3 mg gp33 plus 10 nmol CpG-ODN 1668 (n = 6), gp33 emulsified in IFA (n = 6), PBS (n = 3), CpG-ODN 1668 alone (n = 3), and gp33 alone (n = 3). Two weeks after immunization mice were infected i.v. with 200 PFU LCMV, and 4 days later viral titers in spleens were determined. Pooled results for two independent experiments are shown. ⋅, average virus titer in each group. ⋅⋅⋅⋅, virus detection limit in the assay.**

**Discussion**

Adjuvants, such as CFA or IFA allow the induction of protective immunity against infections using defined T cell epitopes as vaccines (32, 34). The severe side effects caused primarily by the paraffin oil contained in these adjuvants precluded a detailed analysis of their mode of action (35) and their use in humans. There is accumulating evidence that CpG-DNA acts as a potent vaccine adjuvant for promoting Th-1-like immune responses (16–19, 36). In contrast to CFA and IFA, the side effects of CpG-DNA, such as toxic cytokine syndrome, appear to be transient (37). We therefore considered CpG-ODN useful as a model to study the mechanisms of adjuvant action. Here we demonstrate that CpG-DNA activates in situ immature Ag-presenting DC to transit to professional APC that are able to activate CTL precursors in the absence of T help.
In the case of LCMV peptide gp33, activation of CTL was paralleled by the induction of protective antiviral immunity. These data implicate CpG-DNA as a potential powerful adjuvant in vaccination protocols toward infectious and perhaps tumor-associated diseases.

MHC class I-restricted T cell epitopes (Ag) admixed with CFA or IFA have been shown to prime peptide-specific CTL responses (32, 34), implying presentation of T cell epitopes by APC. To address the question of whether upon s.c. challenge soluble T cell epitopes become presented by DC within draining LN, DC from such LN were scored ex vivo for H-2K^b-restricted peptide presentation. Within 12 h after peptide challenge, positively selected immature DC presented the OVA peptide SIINFEKL, activating the SIINFEKL-specific, H-2Kb-restricted T cell hybridoma, whereas the DC negative population did not fit (Fig. 3). Thus, it appears that uptake and presentation of peptide are constitutive functions of phenotypically immature DC (38). Others have pointed out a MHC class I presentation pathway for cytosolic delivery of exogenous Ags in DC (39–41). Injection of peptide alone failed to induce productive CTL responses, Whether T cell peptides presented by immature DC are ignored by Ag-reactive T cells or induce tolerance (34, 42) needs to be analyzed. In contrast, coinoculation of CpG-DNA activated Ag-presenting immature DC to transit to professional APC as defined by up-regulation of costimulatory molecules (CD80, CD86, CD40) and MHC class II (Fig. 4). The evidence for DC maturation is also supported by priming of peptide-reactive precursor CTL (Fig. 1) and by induction of cytokine production. The effects of CpG-ODN on immature DC within LN were CpG-specific, because control ODN were inactive.

Recently, a pivotal role of CD40 ligation in DC activation has been identified (6, 7, 43). CD40 ligation by CD40L-positive Th cells or cross-linking by anti-CD40 mAb caused Ag-presenting immature DC to transit to professional APC. Once matured DC could activate CTL precursors in a Th cell-independent fashion (8–10). Here we describe that similar to CD40 ligation, CpG-DNA also activates in situ Ag-presenting immature DC to transit to professional APC. There are additional parallels between DC CD40 ligation and pathogen pattern recognition receptor engagement. Pathogen-derived ligands like CpG-DNA and LPS or Th cell-dependent CD40L-CD40 interaction initiate in APC signal transduction via activation of c-Jun NH2-terminal kinase and p38, but not via the extracellular signal-related kinase (44–46). We propose that CD40 or the CpG-DNA receptor, despite being different receptors, trigger similar intracellular signal pathways. These similarities therefore mimic each other in functional outcome, i.e., in the activation/maintenance of immature DC. One would thus predict a number of potential uses for CpG-ODN as adjuvants or immunomodulating agents (47–50). As shown here, CpG-ODN renders T cell epitopes immunogenic by activating Ag-presenting immature DC. Because activation of Ag-presenting DC by CpG-DNA bypasses the need for Th cells, productive CTL responses can be initiated merely by MHC class I-restricted T cell epitopes. This attribute could be helpful in cases where the Th cell compartment is compromised. Additionally, CpG-ODN represents a single, easily produced, and chemically defined reagent.

To determine whether the potential benefits may be of practical significance, we vaccinated H-2^b mice with a mixture of MHC class I-restricted LCMV-derived gp33 peptide plus CpG-ODN. LCMV infections are controlled by LCMV-specific CTL (30). This model system allowed us to analyze whether CpG-ODN not only aids peptide-specific CTL induction but also confers antiviral protection. Indeed, mice challenged once with gp33 peptide admixed with CpG-ODN 1668 generated primary CTL responses and were also protected from a subsequent LCMV infection. During the completion of these studies Oxenius et al. (51) arrived at a similar conclusion by priming and boosting H-2^k mice with CpG-ODN admixed with gp33.

The nature of the Ag is believed to control the induction of CD8 or CD4 T cell immunity, in that endogenous Ags are presented by MHC class I molecules whereas exogenous and endocyted Ags are presented by MHC class II molecules (1). DC, however, have the capacity to generate MHC class I-restricted T cell epitopes from soluble proteins via TAP-dependent pathways. High rate macrophagocytosis and cell type-specific routes may deliver exogenous material to the cytosol and may account, at least in part, for the cross-priming capacity of DC (38–41). Here we show that DC from LN draining a site of soluble SIINFEKL-peptide challenge retain their immature phenotype but effectively present SIINFEKL in a H-2K^b-restricted fashion. DC-depleted LN cells, however, did not present SIINFEKL. The exclusivity of cell type for the ability to present SIINFEKL contradicts a simple loading of empty MHC class I heavy chains. Whether DC indeed are specialized to take up and to sample, via macrophagocytosis, extracellular peptides to deliver them in the MHC class I presentation pathway needs to be clarified.

The data described here define the adjuvanticity of CpG-ODN by their ability to activate Ag-presenting immature DC to transit to professional APC. Operationally, similar to CD40 ligation by Th cells or by anti-CD40 mAb, activated Ag-presenting DC initiate CTL responses in the absence of T help. Our data endorse CpG-DNA as an inexpensive and promising adjuvant for vaccination protocols directed toward infectious and perhaps tumor-associated diseases.

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