Immunization with Human Papillomavirus Type 16 (HPV16) Oncoprotein-loaded Dendritic Cells as well as Protein in Adjuvant Induces MHC Class I-restricted Protection to HPV16-induced Tumor Cells

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ABSTRACT

Human papillomavirus (HPV) E6 and E7 oncoproteins are attractive targets for T-cell-based immunotherapy of cervical cancer. In this study, we demonstrate that dendritic cells (DCs) pulsed with HPV16 E7 protein are not only recognized in vivo by E7-specific CTLs but also elicit E7-specific CTL responses in vivo, associated with protection against a challenge with syngeneic HPV16-induced tumor cells. Vaccination with soluble E7 protein in incomplete Freund’s adjuvant likewise induces E7-specific CTL responses associated with tumor protection. The presence of HPV16 E7-specific CTLs in vivo and the observation that depletion of CD8+ cells completely abolishes tumor protection demonstrate that CTLs are the major effector cells in mediating antitumor activity. The in vivo involvement of DCs in the activation of protective CTLs is suggested by the surface display of E7 peptide-loaded MHC class I molecules on these cells after E7 protein immunization. These data show that HPV16 E7 protein-pulsed DCs, as well as the administration of E7 protein antigen in adjuvant, can effectively stimulate tumor-specific MHC class I-restricted CD8+ T-cell-mediated protective immunity to HPV16-induced cancers.

INTRODUCTION

Progress in the identification of tumor-associated antigens and in defining the specificity of the cellular immune response to cancer has led to the development of vaccine strategies based on the induction of tumor-specific CTL responses. CTLs recognize and kill cells displaying MHC class I molecules complexed with peptides that are derived from endogenous antigens, whereas T helper cells are activated when peptides from extracellular antigens are presented by MHC class II molecules (1). However, the processing pathways leading to peptide presentation by MHC class I and class II molecules are not absolutely separated, because it has been reported that exogenous proteins are also able to reach the class I pathway (2–5). Thus, CTL priming by exogenous protein can be established (6–16), which holds much promise for nonlive vaccines composed of tumor protein antigens.

APCs are required for the MHC-restricted presentation of Ag to T cells. Many cell types expressing the appropriate MHC alleles can function as APCs. However, DCs that constitutively express high levels of MHC class I and II and costimulatory molecules are efficient inducers of T-cell immunity (17). CTL responses were generated with DCs pulsed with peptides that comprise CTL epitopes (18–20). DCs can also take up and process protein antigen for MHC class II-restricted presentation (21–23). Whereas this processing ability was attributed to immature DCs (21, 23), it was recently also demonstrated for mature DCs (22). In addition, uptake and processing of exogenous protein by DCs have also been shown for MHC class I-restricted presentation (24–26). Obviously, vaccines targeted at presenting antigens through DCs may be a very efficient way to induce cellular immunity.

HPVs represent a heterogeneous group of viruses that infect epithelial tissues. Several types of HPV are associated with lesions that can progress to malignancy (27). HPV16 is one of the types that is closely linked to development of cervical carcinoma and is found in most (60%) cervical cancer patients (28). A role for the immune system in the control of HPV-related malignancies is suggested by the observation that immunosuppressed individuals are more prone to develop cervical carcinomas (29, 30). Expression of the nuclear proteins E6 (binding to p53) and E7 (binding to the retinoblastoma protein) of HPV is required for the malignant phenotype (31). Because E6 and E7 are intracellular proteins, CTLs specific for these proteins are desirable immune effectors in recognizing and destroying HPV-infected cells. Therefore, HPV16 E6 and E7 proteins are logical targets for immunotherapy against cervical cancer.

Various studies in animal models indicate that it is possible to induce protection against HPV-containing tumors (32–34) or to generate E6/E7-specific CTLs (35–40). Different modes of immunizations have been used in these studies: (a) recombinant E6/E7 vaccinia viruses (32, 36, 37, 39, 40); (b) syngeneic cells transfected with E7 (33, 34); and (c) peptides corresponding to a CTL epitope in E7 (35). Also, immunization with proteins in different formulations was shown to mount specific CTL responses, such as HPV16 E7 protein antigen administered s.c. in adjuvant (37) or immunostimulating complexes containing fusion proteins of HPV11 E6 or E7 (38). However, no tumor protection data were presented in the latter reports. Therefore, the role of CTL responses in anti-HPV tumor activity remains to be determined.

A major advantage of protein vaccines over peptide vaccines is their lack of predetermined MHC allele specificity, because host APCs process the protein antigen for presentation by individual MHC alleles of each host. Also, peptide vaccination can sometimes lead to enhanced tumor growth through specific T-cell tolerance induction (41). Safety concerns are involved in the use of recombinant viruses and genetically modified tumor cells. With a protein vaccine, dangerous side effects such as transformation are not anticipated.

Our studies show that HPV16 E7-specific CTLs can be generated by immunization with DCs pulsed with E7 protein, as well as by immunization with E7 protein in IFA, resulting in long-lasting protection against a challenge with HPV16-transformed tumor cells.

MATERIALS AND METHODS

Animals. C57BL/6 (B6, H-2b) mice were obtained from IFFA Credo (Paris, France) and maintained in our institute under specific pathogen-free conditions. The mice were used at 7–10 weeks of age.
Cell Lines. MECs, C3, and AR5 (all of C57BL/6 origin, H-2b) cells were maintained in IMDM (Biocrom; Seromed, Berlin, Germany) supplemented with 10% FCS (Hyclone Laboratories, Inc., Logan, UT), 2-ME (2 x 10⁻⁵ M), penicillin (100 IU/ml), and glutamine (292 /µg/ml) at 37°C in humidified air containing 5% CO₂. The C3 cell line was produced by transfecting second-passage cultures of B6 MECs with a plasmid containing HPV16 E + L, pEJ-ras, and pEG-neo plasmids (35). mRNA and protein expression of both E6 and E7 by C3 was demonstrated in reverse transcription-PCR and intra-cellular immunofluorescence. The AR5 cell line expressing AdSE1A and EJ-ras was generated by transfection with pAdSE1A (PstI), pEJ-ras, and pTK-neo (41). The OVA8+ K⁺-specific CTL clone 4G3 was kindly provided by Dr. Y. Sykulev (Massachusetts Institute of Technology, Cambridge, MA). The FaC3 CTL clone was generated in a limiting dilution assay of splenic T cells of a B6 mouse immunized twice with 100 µg of RAHYNNIVTF peptide in IFA, restimulated in vitro once with RAHYNNIVTF-loaded RMA-S cells, and maintained by weekly in vitro restimulation with IFN-γ-treated irradiated C3 cells (35). CTLs were stimulated once every week with the appropriate cell lines, EG7OVA (42) or C3 (35), and 10 units/ml IL-2 in IMDM with 10% FCS, penicillin, glutamine, and 2-ME. EG7OVA cells were maintained in IMDM with 10% FCS, penicillin, glutamine, and 400 µg/ml G418 (Genetec). Peptide Synthesis. Peptides were synthesized on an Applied Biosystems 431A synthesizer with amino and carboxyl ends. All peptides were purified with C18 reversed-phase high-performance liquid chromatography. The sequences of the peptides are OVA8 (257-264):SIINFEKL and HPV16E7 (49-57):RAHYNNIVTF.

Proteins. The genes for HPV16 E6 and E7 were cloned in expression vector pET19b (Novagen) by PCR, using forward primers containing a XhoI site upstream of the stop codons and reverse primers containing a BamHI site annealed downstream of the stop codons. Proteins were expressed as fusion proteins containing 10 histidine residues plus a 13-amino acid linker attached to its amino terminus. For overproduction, the Escherichia coli strain BL21(DE) was used, in which the T7 RNA polymerase gene is under control of the lac promoter (43). Proteins were purified by nickel-chelate affinity resin according to the recommendations of the supplier (Qiagen, Chatsworth, CA). The eluted fraction was dialyzed against 10% acetic acid and lyophilized. The purified proteins were analyzed by SDS-PAGE on 12.5% gels. Purity was assessed at 80–90% for both the E6 and the E7 protein. OVA, grade VI, was purchased from Sigma.

Purification of DCs. SC DCs were isolated by injecting 250 µl of collagenase (100 IU/ml) at multiple sites in murine spleens. These spleens were each cut in four parts that were incubated for 15 min at 37°C in 400 IU/ml collagenase and then passed through a nylon mesh filter. This cell suspension was spun on a discontinuous BSA gradient of 10, 28, and 35% BSA at 4°C for 30 min at 10,000 x g. The low-density cells at the interphase between 10 and 28% BSA were collected and cultured for 90 min at 37°C in glass Petri dishes. Nonadherent cells were discarded, and the adherent cells were cultured in 28% BSA were collected and cultured for 90 min at 37°C in glass Petri dishes. These spleens were each cut in two parts that were incubated for 30 min at 37°C in glass Petri dishes. These spleens were each cut in four parts that were incubated for 15 min at 37°C in 400 IU/ml collagenase and then passed through a nylon mesh filter. This cell suspension was spun on a discontinuous BSA gradient of 10, 28, and 35% BSA at 4°C for 30 min at 10,000 x g. The nonadherent cells were discarded, and the adherent cells were cultured in IMDM, 15% FCS, 2-ME, glutamine, and penicillin for 3 days in the presence of 50 µg/ml protein (OVA or E7) in IMDM supplemented with 10% FCS, 2-ME, penicillin and glutamine at 37°C in humidified air containing 5% CO₂. Live cells were harvested and recultured for 5 x 10⁶ cells per well in 24 well plates in medium with 1% of Rat Factor, i.e., supernatant of mitogen-stimulated rat spleen cells containing 500 units/ml IL-2 and 2500 units/ml IFN-γ. After 5 days, live cells were harvested and restimulated for 3 days with 50 µg/ml protein at 5 x 10⁶ cells per well in medium containing 1% of Rat Factor. The cultures were performed in IMDM supplemented with 10% FCS, 2-ME (2 x 10⁻⁵ M), penicillin (100 IU/ml), and glutamine (292 µg/ml) at 37°C in humidified air containing 5% CO₂. Stimulator cells were prepared as follows: spleen cells were cultured for 3 days in medium containing 30 µg/ml lipopolysaccharide (Bacto Laboratory; DIFCO Laboratories, Detroit, MI). The B-cell
blasts were harvested over a density gradient (lymphocyte M) and infected for 16 h at 37°C with recombinant vaccinia virus containing HPV16 E7 (Ref. 32; kindly provided by Dr. M. Krul, Free University, Amsterdam, the Netherlands) at a multiplicity of infection of 5. The next day, live cells were harvested over lymphocyte M (Cedarlane, Hornby, Canada). Expression of the E7 protein was demonstrated by intracellular immunofluorescence in 60–80% of these cells. After fixation with 1% paraformaldehyde, washing, quenching with 0.2 M glycine, and two more washes, these cells were used for in vitro stimulation. After 5 days of culturing responder spleen cells with stimulator B cells, live cells were harvested by density centrifugation on lympholyte M. These effector cells were used in a cell-mediated lymphocyte cytotoxicity assay.

**Cell-mediated Lymphocyte Cytotoxicity.** Cell-mediated cytotoxicity was measured in a europium (Eu³⁺) release assay. In short, varying numbers of effector cells were added to 1000 Eu³⁺-labeled target cells in 150 μl of culture medium in 96-well U-bottomed plates. After 4 h at 37°C, supernatants were collected and mixed with Enhancer solution (Wallac, Turku, Finland). Measurement of the samples took place in a 1234 Delfia fluorometer (Wallac). The mean percentage of specific lysis of triplicate wells was calculated as follows: % specific lysis = (cpm experimental release - cpm spontaneous release)/(cpm maximum, 2% Triton X-100, release - cpm spontaneous release) x 100. The SE of triplicate cultures was less than 5% specific release.

Alternatively, a ⁵¹Cr release assay was performed when vaccinia-infected B cells were used as targets. E6 and E7 protein expression was confirmed by intracellular immunofluorescence. Varying numbers of effector cells were added to 5000 Na₂⁵¹CrO₄ (⁵¹Cr)-labeled target cells and incubated for 5 h at 37°C. Target cells were labeled for 1.5 h with 100 μCi of ⁵¹Cr per 1 X 10⁶ cells. The percentage of specific ⁵¹Cr release was calculated as described above.

**RESULTS**

**Complete and Long-Lasting Protection against a Challenge with HPV16-induced Tumor Cells.** DCs are prominent candidates for immunotherapeutic intervention (45, 51, 52). These cells trigger vigorous T-cell responses not only when loaded with peptide antigen but also when pulsed with peptide antigen (53, 54). We were interested at first in investigating the properties of DCs pulsed with the HPV16 E7 protein in eliciting protection against a HPV16-containing syngeneic tumor cell line. When immunized with IFA only, most C57BL/6 mice develop a tumor within 3–4 weeks after challenge with the HPV16-containing C3 tumor cells, as demonstrated in the experiment shown in Fig. 1. The E7 CTL epitope RAHYNIVTF, administered in IFA s.c., induces a clearly protective effect against the C3 tumor (Fig. 1b), as has been demonstrated before (35). Immunizations with “naked” DCs, i.e., DCs not pulsed with HPV protein, provide a minor but not significant protective effect against tumor development (Fig. 1c), as previously observed. This is probably due to the presentation of FCS-derived peptides by DCs inducing CTL responses that may recognize C3 cells, which were cultured in the presence of FCS before in vivo use. In this experiment, some of the DCs were pulsed with HPV16 E6 protein, which was produced similarly to E7. Although immunogenic peptides from the HPV16 E6 sequence have been described previously (55), naturally processed E6-derived CTL epitopes for H-2b mice have not been demonstrated. We failed to induce E6-specific CTL responses in C57BL/6 mice by a protocol similar to that used to induce E7-specific CTLs (see the next paragraph). In contrast to immunizations with DCs pulsed with HPV16 E6 protein (Fig. 1d), immunizations with E7-pulsed DCs provide complete protection against tumor growth, which lasted 75 days, and even longer than 9 months after challenge (Fig. 1e; data not shown). We also immunized mice s.c. with the HPV proteins in IFA. We observed that 4 of 10 mice immunized with E6 protein in IFA developed a tumor (Fig. 1f), which is not significantly different (P = 0.115) from the tumor take in the control group (Fig. 1a). Interestingly, we found that immunizations with E7 protein in IFA completely protected B6 mice against a C3 challenge (Fig. 1g), whereas no protection was observed by the same E7/IFA immunization in a syngeneic adenovirus-induced tumor model, AR5 (Fig. 1h). Therefore, specific tumor protection is generated by immunization with HPV16 E7 in IFA or loaded on DCs.

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5 M. L. H. De Bruijn and M. Toes, unpublished observations.
Induction of HPV16 E7-specific Proliferative Responses. Because exogenous protein antigen often stimulate CD4+ proliferative responses, we tested whether immunization with HPV16 E7 protein in IFA induced E7-specific proliferative responses. Two weeks after immunization, CD4+ T cell lines were generated. Splenic T cells from mice immunized with IFA only or OVA in IFA were included in this experiment. We could detect protein-specific proliferative responses induced by immunization with E7 in IFA, as observed for OVA (Fig. 2).

Induction of HPV16 E7-specific CTL Responses. We were interested in investigating whether E7-specific CTL responses were detectable in the protected mice from the experiment shown in Fig. 1. The spleens of selected mice were collected and restimulated in vitro with B-cell blasts infected with rVV-E7. The CTL bulk cultures were harvested after 5 days and tested in a cytotoxicity assay for recognition of syngeneic target cells loaded with the HPV16 E7 CTL epitope RAHYNIVTF and the tumor cell line C3. A control E7-specific CTL clone was included, demonstrating efficient presentation by the target cells of the synthetic peptide RAHYNIVTF and the processed epitope on C3 (Fig. 3a). The CTL bulk culture of a mouse immunized with only IFA did not show reactivity toward either target cell (Fig. 3b). However, CTL bulk cultures obtained from individual mice immunized with either RAHYNIVTF in IFA, E7 in IFA, SC DCs pulsed with E7, or BM DCs pulsed with E7 all showed specific recognition of the peptide-loaded target and the C3 tumor cell (Fig. 3, c-f). In a separate experiment, mice were immunized twice with IFA or HPV16 E7 protein in IFA, and CTL bulk cultures were generated according to the same protocol as in the previous experiment. B-cell blasts were used as target cells in the cytotoxicity assay. They were infected with the vaccinia vector only (rVV—), with vaccinia containing the HPV16 E6 gene (rVV E6), or with vaccinia containing the HPV16 E7 gene (rVV E7). The E7-specific CTL clone recognizes only the rVV E7-infected target cells (Fig. 4a). Whereas the CTL bulk culture from a B6 mouse that was treated with only IFA showed no reactivity toward any of the three targets (Fig. 4b), the CTL bulk culture of a mouse immunized with E7 in IFA clearly lysed the rVV E7-infected target cells only (Fig. 4c). These data demonstrate the presence of E7-specific CTL responses in mice immunized with E7-pulsed DCs or immunized with E7 in IFA. We were
Fig. 4. HPV16 E7-specific CTL responses induced by immunization with E7 protein in IFA. C57BL/6 mice were immunized twice with IFA or 2 nmol of HPV16 E7 in IFA. Two weeks after the boost, the spleens were collected and restimulated in vitro with fixed C57BL/6 B-cell blasts infected with rVV-E7. After 5 days, the effector cells were collected and tested for HPV16 E7-specific CTL responses in a cytotoxicity assay with C57BL/6 B-cell blasts infected with rVV (vector control), rVV-E6, or rVV-E7. The HPV16 E7-specific CTL clone FaC3 recognized the rVV-E7 target only (a). The CTL bulk culture obtained from a mouse immunized with only IFA was negative (b), but CTLs from a mouse immunized with HPV16 E7 responded against the rVV-E7 target (c).

The inability of the CTLs to recognize E6 protein-pulsed DCs or E6 in IFA implies that the E6 protein is not processed efficiently by APCs. This is consistent with the absence of E6-specific CTL responses in C57BL/6 mice (data not shown). However, immunizations with E6 protein-pulsed DCs or E6 in IFA induce strong E7-specific CTL responses in C57BL/6 mice (data not shown).

Protection against HPV16-induced Tumor Cells after Immunization with HPV16 E7-pulsed DCs or with HPV16 E7 Protein in IFA Requires CD8+ but not CD4+ T Cells. Because we observed the presence of E7-specific Th and CTL responses in mice immunized with E7-pulsed DCs or E7 in IFA, we investigated the role of these T-cell subsets in the protection mediated by these two types of immunizations. To this end, we treated the mice during the priming and effector phase with CD4 and/or CD8 Ab. The scheme of Ab treatment efficiently eliminates CD4+ and CD8+ T cells. In the control group, 90% tumor take was established (Fig. 5a). Protection was provided by immunizations with E7-pulsed DCs (Fig. 5b). The Ab treatments clearly demonstrate that the protection was mediated by the CD8+ cells (Fig. 5, c-e). Similarly, protection provided by E7 in IFA injections (Fig. 5f) was lost by CD8 Ab treatment (Fig. 5g). CD4+ cells did not play a significant role in the protection mediated by E7 in IFA immunizations (Fig. 5, h and i). Thus, we demonstrate that CD8+ cells play a major role in the tumor-protective effect by immunizations with E7-pulsed DCs and E7 in IFA. With regard to the presence of E7-specific CTL responses by these two types of immunizations, we conclude that E7-specific CTLs induced by immunization with E7-pulsed DCs or E7 in IFA are required for the protection against the C3 tumor.

In Vitro and in Vivo Uptake of E7 Protein Antigen by DCs for MHC Class I-restricted Presentation. We evaluated whether in vitro and in vivo loading of DCs with E7 protein could lead to the subsequent surface display of E7 peptide-loaded MHC class I molecules. For the in vitro experiment, we isolated DCs from two different sources: (a) from spleen according to low density and transient adherence; and (b) from bone marrow by culturing in the presence of IL-4 and GM-CSF. SC DCs are considered mature cells, whereas BM DCs are considered more immature. SC DCs and BM DCs were pulsed overnight with 10 μM OVA or HPV16 E7 protein and washed three times before being used as target cells in a CTL recognition assay. Fig. 6A shows specific recognition by the CTL clones of these in vitro protein-pulsed DCs. These data clearly demonstrate that uptake and processing of OVA and HPV16 E7 protein for MHC class I-restricted presentation can occur by both SC DCs and BM DCs. Because E7 in IFA immunizations allows the induction of E7-specific CTL responses, uptake of E7 by APCs in vivo for MHC class I-restricted presentation is a prerequisite. DCs are the most efficient APCs for priming T cells, which urged us to investigate the in vivo uptake of E7 by DCs. Although SC DCs isolated 4 or 24 h after immunization with E7 protein in IFA were not recognized by the E7-specific CTL clone (data not shown), SC DCs isolated 2 weeks after immunization were able to elicit a response (Fig. 6B). Because s.c. administration of antigen in IFA causes slow release of antigen, apparently a 2-week period allowed a sufficient amount of E7 to be taken up and processed for class I-restricted presentation. Thus, E7 protein is taken up not only in vitro but also in vivo by DCs for MHC class I-restricted presentation, and this is likely to play a role in E7-specific CTL response induction.

DISCUSSION

A subunit protein vaccine that induces antitumor T-cell immunity constitutes a safe and effective immunotherapeutic agent. Two examples from the literature show the feasibility of such a vaccine in other tumor models (12, 13). We now show the potential of such a vaccine for HPV-related tumors. E7 but not E6 protein-pulsed DCs induce strong protection in a HPV16 mouse tumor model (Fig. 1), showing the efficient T-cell stimulatory capacity of DCs. Interestingly, however, we observed equally strong protection by HPV16 E7 protein in IFA immunization (Fig. 1). The specificity of these immunizations is indicated by the lack of protection with HPV16 E7 in IFA against a syngeneic adenovirus-induced tumor (Fig. 1a). Although E7-specific proliferative responses are generated by E7 in IFA immunization (Fig. 2), the presence of E7-specific CTLs (Figs. 3 and 4) and the requirement for CD8+ but not CD4+ cells (Fig. 5) demonstrate that CD8+ CTL responses are responsible for the tumor protection. DCs, either derived from bone marrow by IL-4 and GM-CSF culture or from spleen, can take up and process protein antigen (OVA and HPV16 E7 in vitro for MHC class I-restricted presentation (Fig. 6A). We assume that DCs play a role in the in vivo activation of protective CTLs by protein immunization, because the E7 epitope is displayed on these cells isolated from immunized mice (Fig. 6B).

The CTL epitope in HPV16 E7, represented by the amino acid sequence RAHYNIVTF (35), is dominant for H-2b mice, because immunization with either peptide or protein in IFA induces CTLs that strongly recognize the peptide-loaded target and the tumor cell line endogenously expressing this epitope (Fig. 3). Although immunogenic peptides were described for HPV16 E6 in H-2b mice by use of a panel of synthetic peptides (35), it has not yet been demonstrated that these represent naturally processed peptides presented by HPV16 E6-infected/transfected cells. In fact, we were unable to generate any HPV16 E6-specific CTL responses in C57Bl/6 mice (data not shown). Also, immunizations with E6 protein-pulsed DCs or E6
Fig. 5. CD8+ T cells but not CD4+ T cells are required for C3 tumor protection obtained by immunization with HPV16 E7-pulsed DCs or with HPV16 E7 protein in IFA. CD4+ and/or CD8+ T cells were depleted in vivo by Ab treatment according to the schedule described in the "Materials and Methods." Immunizations with IFA only (a), 2 × 10⁶ HPV16 E7 protein-pulsed BM DCs (b–e), or 2 nmol of HPV16 E7 in IFA (f–i) were performed, and tumor growth was followed for 4 weeks. Fisher's exact test showed that data in b, c, f, and g were significantly different from the data in a (P = 0.003, 0.001, 0.001, and 0.001, respectively), whereas the data in d, e, h, and i were not (P = 0.500, 0.500, 0.291, and 0.291, respectively).

protein in IFA did not significantly protect B6 mice against a challenge with HPV16 C3 cells (Fig. 1, d and f). The E6 and E7 protein are of equal quality. Therefore, E6 immunizations can be considered as controls for the immunizations performed with HPV16 E7.

It is generally accepted that different T-cell subsets recognize epitopes from protein antigens derived from mutually exclusive sources: Th helper cells are activated by APCs presenting exogenous Ag; and CTLs are activated by APCs presenting endogenous Ag. However, this belief has been challenged by data on CTL response induction by exogenous protein Ag in various formulations. Our data also point out that segregation of MHC pathways cannot be strictly maintained. The fact that we now demonstrate not only that exogenous antigen activates CTL responses in vivo but that it also induces tumor protection, which is mediated by CD8+ T cells alone, offers opportunities for the design of recombinant protein antigens as vaccines that selectively and specifically stimulate antitumor class I-restricted CTL responses.

Details have to be defined for the mechanism of uptake and processing of protein antigens and MHC class I/peptide complex formation. Processes leading to class I-restricted presentation of exogenous protein Ag are probably much less efficient than for class II presentation, although this has not yet been scrutinized for DCs. Even if low numbers of MHC/peptide complexes are presented by DCs, this can
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