FAST TRACK

Chimeric Papillomavirus Virus-like Particles Induce a Murine Self-Antigen-Specific Protective and Therapeutic Antitumor Immune Response

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Abstract   The use of chimeric virus-like particles represents a new strategy for delivering tumor antigens to the immune system for the initiation of antitumor immune responses. Immunization of DBA/2 mice with the P1A peptide derived from the P815 tumor-associated antigen P1A induced specific T-cell tolerance, resulting in progression of a regressor P815 cell line in all animals. However, immunization with a human papillomavirus type 16 L1 virus-like particle containing the P1A peptide in the absence of adjuvant induced a protective immune response in mice against a lethal tumor challenge with a progressor P815 tumor cell line. Additionally, we demonstrated that these chimeric virus-like particles could be used therapeutically to suppress the growth of established tumors, resulting in a significant survival advantage for chimeric virus-like particle-treated mice compared with untreated control mice. Chimeric virus-like particles can thus be used as a universal delivery vehicle for both tolerizing and antigenic peptides to induce a strong protective and therapeutic antigen-specific antitumor immune response. J. Cell. Biochem. 73:145–152, 1999. © 1999 Wiley-Liss, Inc.

The goal of immunotherapy is to develop strategies that boost the immune-mediated tumor defense mechanisms and that focus the immune response to the relevant tumor antigens. Immunization with defined cytotoxic T-lymphocyte (CTL) epitopes that are shared between different cancers is perceived as a potential method of generating common cancer vaccines [Pardoll, 1998]. Several different thera-

pies are being examined or applied in tumor therapy settings that induce an antitumor-specific immune response. These include peptide vaccinations, DNA vaccinations, genetic modifications of tumor cells, peptide-pulsed dendritic cells (DCs), and adoptive transfer of tumor-specific T cells. Chimeric virus-like particles (cVLPs) represent a recently developed alternative strategy for delivering antigens to the immune system. The papillomavirus capsid proteins, L1 and L2, are able to self-assemble into virus-like particles (VLPs) composed solely of L1 or containing both L1 and L2 when expressed in eukaryotic expression systems [Kirnbauer et al., 1992, 1993]. Two previous studies using cVLPs that contain either a HPV16 E7 CTL epitope fused to L1 or the whole E7 protein fused to L2 have shown that immunization with these nonreplicative particles can protect mice against the outgrowth of tumors expressing the HPV16 E7 protein [Greenstone et al., 1998; Peng et al., 1998]. The chimeric VLPs were able to deliver the antigenic epitope to the MHC class I processing pathway leading to

induction of class I-restricted CTLs. To extend studies on the use of cVLPs as universal antigen delivery vehicles, the murine P1A tumor-associated antigen was linked to the HPV16 L1 capsid protein, resulting in the synthesis of HPV16 L1-P1A cVLPs. P1A is a self-antigen expressed in various tumors and normal tissues like the testis and placenta [Amar-Costesee et al., 1994]. $P1A_{35-43}$ is the immunodominant, $H-2L^d$-restricted peptide antigen expressed by the mastocytoma tumor cell line P815 [Lethe et al., 1992; Van den Eynde et al., 1994]. Injection of certain peptides has been shown to tolerize mice for tumor cells expressing the peptide resulting in the inability to reject nontumorigenic tumor cells [Toes et al., 1996a,b]. Therefore, we tested whether immunization with the P1A peptide or the HPV16 L1-P1A cVLPs led to induction of specific tolerance for the P1A antigen. We compared immunization with the P1A peptide to immunization with HPV16 L1-P1A cVLPs for induction of P1A-specific CTLs and prevention of tumor development in mice challenged with a lethal dose of a progressor P815 tumor cell line expressing the P1A antigen. The therapeutic potential of the cVLPs was determined by treating mice 5 or 10 days after they had been given a lethal dose of P1A-expressing tumor cells. The data demonstrated that immunization with HPV16 L1-P1A cVLP induced P1A-specific CTLs and prevented tumor growth in mice challenged with progressor P815 tumor cells. This was in contrast to immunization with the P1A peptide which induced in vivo tolerance leading to progression of a regressor P815R tumor cell line. Immunotherapy with HPV16 L1-P1A cVLPs suppressed the growth of established progressor P815 tumors, resulting in a significant survival advantage of treated mice compared with empty VLP-treated control mice. The results presented in this study support the testing of cVLPs for immunotherapy of cancer patients.

MATERIALS AND METHODS
Mice

In this study, 6- to 8-week-old DBA/2 ($H-2^d$) male mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were kept under specified pathogen-free conditions.

Cell Lines and Peptides

The P815R regressor was derived by in vitro cloning from the P815 cell line (American Type Culture Collection No.TIB-64) in order to obtain a tumor cell line with different in vivo growth characteristics. P815R showed the phenotype of a regressor tumor and maintained expression of the tumor rejection antigen P1A as confirmed by reverse transcription polymerase chain reaction (RT-PCR) (data not shown). P815 cell lines were cultured in Iscove's modified Dulbecco's medium (Biowhittaker, Walkersville, MD) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin, and 50 mM 2-mercaptoethanol. On the day of tumor challenge, P815 or P815R cells were harvested, washed twice with Hank's balanced salt solution (HBSS) (Sigma, St. Louis, MO), and resuspended in $1\times$ HBSS to the designated concentration for injection. LPS B-cell blasts were made by culturing DBA/2 spleen cells in the presence of 30 mg/ml lipopolysaccharide (Sigma, St Louis, MO) for 5 days, after which they were harvested over a ficoll gradient (Nycomed Pharma AS, Oslo, Norway). The $P1A_{35-43}$ peptide (LPYLGWLVF) was synthesized at Loyola University Chicago using FMOC chemistry on an Applied Biosystems Synergy 432A Peptide Synthesizer (Perkin Elmer, Norwalk, CT). The purity of the peptide was greater than 95% pure as determined by reverse-phase high-performance liquid chromatography (HPLC) and peptide sequencing.

Chimeric Virus-like Particle (cVLP) Construction and Expression

A 90-bp DNA sequence, containing the immunodominant P1A epitope LPYLGWLVF of P815 [Van den Eynde et al., 1994], was amplified by PCR. The PCR primers were designed to reconstitute *Eco*RV restriction enzyme sites at the 5′ and 3′ ends. A *Cla*I site was added to the 3′ end of the construct for orientation purposes. The forward and reverse primers were 5′-ATCCTG-GAAGAAATTCTGTCCTTATC-3′ and 5′-ATC-GATGAACATCTGGAGCGCCAGAAAAC-3′, respectively. PCR amplification was performed using DNA from the P815 cell line as a template with *pfu* DNA polymerase (Stratagene, La Jolla, CA). The PCR product was ligated into the *Eco*RV site of pZErO-1 (Invitrogen, Carlsbad, CA) with T4 DNA ligase overnight at 16°C. Positive clones were sequenced using the SP6

and T7 primers. The P1A sequence was subcloned in frame into the *Eco*RV site of the HPV16L1DC [Müller et al., 1997]. The orientation was checked by *Cla*I-*Hin*dIII digestion. The resulting 16L1-P1A fusion gene was cloned in the baculovirus expression vector pVL1392 (Invitrogen) as a *Xba*I-*Sma*I fragment to generate recombinant baculoviruses as previously described [Müller et al., 1997]. The HPV16 L1-P1A VLPs were made as described previously [Müller et al., 1997].

cVLP and Peptide Immunization, cVLP Therapy, and Tumor Challenge

For protection experiments, mice were immunized 2 weeks before tumor challenge with 25 µg P1A peptide injected subcutaneously (s.c.) suspended in 100 µl of a 50% phosphate-buffered saline (PBS):50% incomplete Freund's adjuvant (IFA) suspension. 10 µg VLPs were injected s.c. suspended in 100 µl PBS. VLP therapy was given as one injection of 10 µg cVLPs on day 5 or day 10 after mice were challenged with P815 tumor cells. Mice were boosted 1 week later with 10 µg of cVLPs on day 12 or day 17 after tumor challenge, respectively. Tumor challenge was performed by injecting 5×10^4 P815 suspended in 100 µl HBSS. Tumor diameter was measured 3 times per week along two axes, and tumor surface area was calculated as (diameter 1 $\times$ diameter 2). Differences in the results of tumor challenge assays were evaluated by the log-rank test. *P*-values of < 0.05 were considered significant.

Cytotoxic T-Lymphocyte Assay

DBA/2 mice were immunized s.c. with 10 µg of HPV-16 L1-P1A cVLPs in PBS. Control mice were immunized with HPV-16 L1 VLPs. The cytotoxic T-lymphocyte (CTL) response assay was performed according to standard protocols. In short, 5×10^6 nylon wool passed spleen cells from individual mice were added to 1×10^6 irradiated P815 cells and cultured for 5 days in the presence of 1% T-STIM Culture Supplement (Collaborative Biomedical Products, Bedford, MA). The responder T cells were isolated over a ficoll gradient, washed, and used in a cytotoxicity assay at the indicated effector target (E/T) ratio. Target cells used were P815 (H2^d) or DBA/2 LPS blasts. The percentage of specific lysis was calculated according to the following formula:

$$\text{Specific lysis} = (\text{experimental release}$$
$$- \text{spontaneous release})/(\text{maximum release}$$
$$- \text{spontaneous release}) \times 100.$$

Maximum release was obtained by adding 100 µl of 1% Triton X-100 to 50 µl of target cells, and spontaneous release was obtained from 50 µl of target cells incubated with 100 µl of medium. All assays were performed in triplicate wells.

RESULTS
Immunization With the P1A Peptide, But Not L1-P1A cVLPs, Induces Tolerance

Previous studies have demonstrated that immunization with CTL epitope peptides derived from certain tumor antigens tolerize mice for tumor cells expressing these antigens [Toes et al., 1996a,b]. We compared the in vivo presentation of the P1A antigen administered as a peptide and the P1A antigen administered as a cVLP. It was hypothesized that immunization with P1A-containing cVLPs may lead to activation instead of tolerance for the peptide because of the way the antigen is presented to the immune system. DBA/2 mice were immunized with the P1A peptide in incomplete Freund's adjuvant (IFA) or with HPV16 L1-P1A cVLPs in PBS. After 2 weeks, the mice were challenged with a regressor P815 tumor cell line derived from P815, designated P815R. In immunocompetent naive mice, the regressor P815R cell line initially formed tumors; these tumors regressed in all mice within 4 weeks (Fig. 1). When mice were immunized with the P1A peptide, the regressor P815R cells formed tumors but, in contrast to PBS-treated mice, these tumors continued to grow, causing the death of all animals as a result of a massive tumor load. However, when the P1A peptide was delivered in a cVLP, small tumors arose but, like control mice, they regressed within 3 weeks after challenge (Fig. 1). When 100% of mice had tumors, the mean tumor surface area in naive mice was >30 mm^2, while the mean tumor surface area in HPV16 L1-P1A cVLP immunized mice was <3mm^2 (data not shown). These data indicate that in vivo immunization with the P1A peptide alone induced tolerance such that the mice were susceptible to the outgrowth of a normally immunogenic regressor P815R tumor. By contrast, administration of P1A peptide in a cVLP did not induce tolerance, but in fact enhanced the antitumor immune response, since tumors
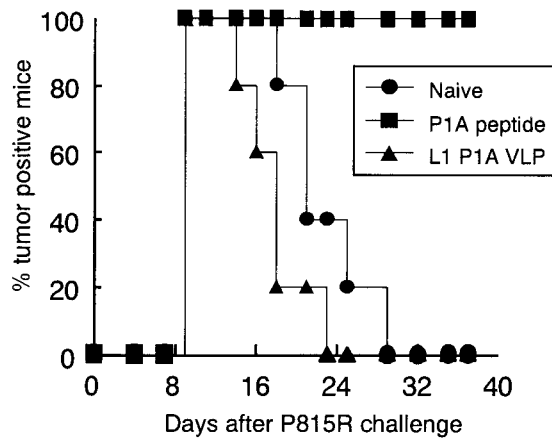
Fig. 1. Immunization with the P1A peptide induces tolerance for a regressor cell line expressing the P1A antigen. DBA/2 mice (5 per group) were immunized subcutaneously (s.c) with either PBS (●), 25 µg P1A peptide in IFA (■), or 10 µg HPV16 L1-P1A cVLPs in PBS (▲). After 2 weeks, mice were challenged s.c. with 5×10^4 regressor P815R cells and tumor occurrence was assessed 3 times per week. Results are representative of three individual experiments.

regressed earlier and were smaller in HPV16 L1-P1A cVLP-immunized mice than in control mice.

Immunization With L1-P1A cVLP Can Protect Against a Lethal Tumor Challenge

From the above experiments, we determined that the P1A peptide-containing cVLP did not tolerize mice to regressor P815 tumors. To determine whether P1A peptide incorporated into VLPs was able to induce a protective immune response against a lethal tumor challenge, DBA/2 mice were immunized with the HPV16 L1-P1A cVLPs and challenged 2 weeks later with a lethal dose of the P1A-expressing progressor P815 cell line. If the cVLPs induced a P1A-specific immune response in immunized mice, tumor growth should be controlled and mice should have a survival advantage compared with control-treated mice. Indeed, HPV16 L1-P1A cVLP-immunized mice were able to confine the growth of the tumor over an extended period (Fig. 2A). By contrast, lethal tumors quickly arose in P1A peptide-immunized mice. Mice immunized with control HPV16 L1 VLPs alone also developed tumors after progressor P815 challenge similar to peptide-immunized mice, indicating that the protective effect was mediated through the delivery of the P1A peptide by the cVLP, and not by nonspecific stimulation of the immune system by the particulate
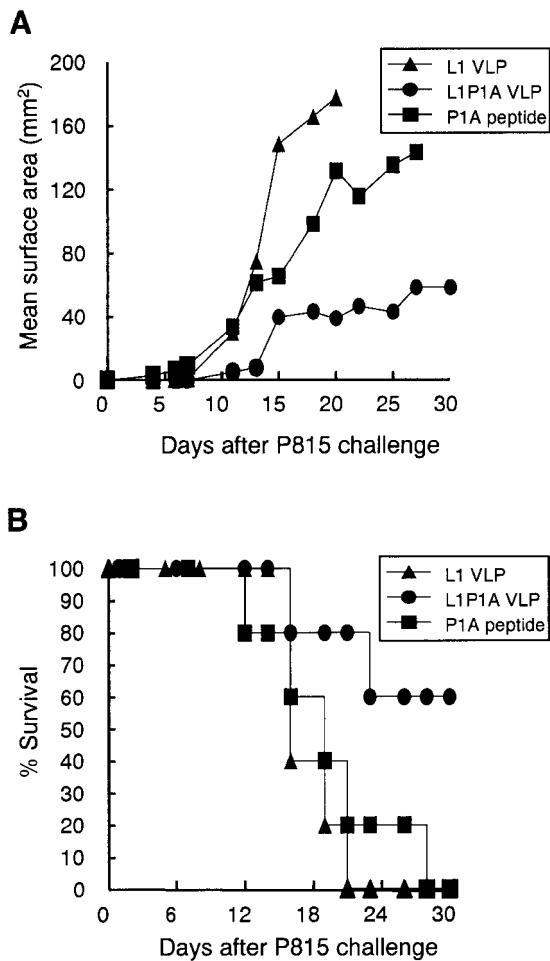

Fig. 2. Immunization with HPV16 L1-P1A cVLPs protects mice from progressor P815 tumor outgrowth. DBA/2 mice (5 per group) were immunized s.c. with either 10 µg HPV16 L1 VLPs in phosphate-buffered saline (PBS) (▲), 25 µg P1A peptide in incomplete Freund's adjuvant (IFA) (■), or 10 µg HPV16 L1-P1A cVLPs in PBS (●). After 2 weeks, mice were challenged with 5×10^4 progressor P815 cells and tumor occurrence were assessed 3 times per week. Shown are the mean tumor surface areas **(A)** and survival curve **(B)**. Results are representative of three individual experiments.

antigen (Fig. 2A). Survival curves indicated that 60% of the HPV16 L1-P1A cVLP-immunized mice stayed tumor free, resulting in a significant overall survival advantage over control mice (Fig. 2B). Taken together, these data show that incorporation of the self-peptide P1A into a cVLP did not tolerize mice for the antigen, but instead immunized and protected against the outgrowth of a progressively growing P1A-expressing tumor.

L1-P1A cVLPs Induce P1A-Specific CTLs

As CD8^{+} T cells have been shown to be the most important effector cells in cVLP-induced

protection against other tumors [Greenstone et al., 1998; Peng et al.,1998], the induction of a P1A-specific CTL response is likely to be critical for clearance of the P815 tumor. To determine whether a P1A-specific CTL response was induced by immunization with HPV16 L1-P1A cVLPs, a cytotoxicity assay with splenocytes from HPV16 L1-P1A cVLP-immunized mice was performed. In vitro stimulated T cells from HPV16 L1-P1A cVLP-immunized mice were able to specifically lyse P815 cells but not P1A-negative autologous target cells (Fig. 3, left). T cells from HPV16 L1 VLP-immunized mice did not demonstrate any specific lysis of P815 or DBA/2 autologous targets (Fig. 3, right). The data indicate that immunization with P1A cVLPs induced P1A-specific CTLs and that the protective effect induced by HPV16 L1-P1A VLPs was most likely mediated by P1A antigen-specific CTLs.

Immunotherapy With cVLPs

Although it is of interest to know whether a particular vaccine can be used prophylactically, one of the major goals of tumor immunology is to develop effective strategies for active immunization of cancer-bearing hosts. In order to establish whether cVLPs can be used in a tu-
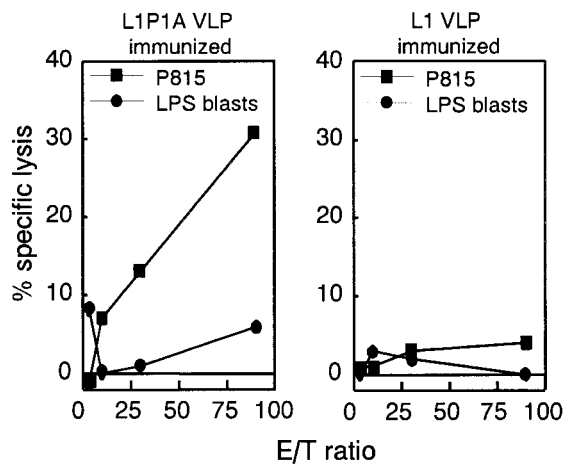
mor therapy setting, the ability of HPV16 L1-P1A cVLPs to eradicate or suppress the growth of established P815 tumors was tested. DBA/2 mice were injected subcutaneously with progressor P815 tumor cells, and the tumors were allowed to grow. On day 5 or day 10 after injection, mice were treated with either HPV16 L1-P1A cVLPs or HPV16 L1 VLPs. These injections were repeated 7 days later. The first tumors were palpable 5 days after injection of tumor cells, and by day 10, all mice had a measurable tumor. Thirty percent of mice treated with HPV16 L1-P1A cVLPs starting on day 5 after tumor inoculation survived past day 50 whereas no control HPV16 L1 VLP-treated mice survived ($p = 0.0001$) (Fig. 4). Within the 30% of mice that had survived, 3 of 30 mice had tumors that regressed and mice became tumor-free (data not shown). Mice receiving therapy starting at day 10 had a lower chance of survival compared with those that received cVLPs at day 5. Nevertheless, they still had a significant survival advantage over HPV16 L1-VLP-treated mice ($p = 0.02$). The data demonstrate that immunotherapy with HPV16 L1-P1A cVLPs suppressed the growth of established progressor P815 tumors and cured 10% of mice, resulting in significant survival advantages for
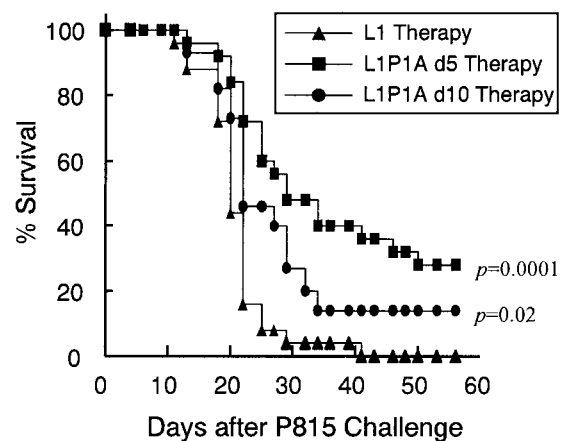

Fig. 3. Immunization with HPV16 L1-P1A cVLPs induces a tumor protective cytotoxic T-lymphocyte (CTL) response. DBA/2 mice were immunized with either 10 µg HPV16 L1-P1A cVLPs in phosphate-buffered saline (PBS) (left) or 10 µg HPV16 L1 VLPs in PBS (right). After 2 weeks mice were sacrificed and spleens were taken. Spleen cells were passed over nylon wool columns and incubated with irradiated P815 stimulator cells. After 5 days, lymphocytes were harvested and tested in a CTL assay for their ability to kill ^{51}Cr labeled P815 cells (■), or P1A-negative DBA/2 LPS blasts (●). Results are shown for one mouse and are representative of five mice tested.

Fig. 4. Treatment of established progressor P815 tumors with HPV16 L1-P1A cVLPs. DBA/2 mice were injected with 5×10^4 progressor P815 tumor cells. Mice were treated with 10 µg of empty HPV16 L1 VLPs (▲) (n = 25) or HPV16 L1-P1A cVLPs (■) (n = 30) 5 days later. Another group of mice (n = 15) was treated with HPV16 L1-P1A cVLPs (●) 10 days after tumor inoculation. Injections were repeated with the same VLPs 7 days later. Tumor occurrence and diameter was assessed 3 times per week. Statistical comparisons of survival (as compared with empty L1 VLPs) were made on the basis of log-rank P-values. P-values of <0.05 are considered statistically significant.

cVLP-treated mice compared with empty VLP-treated control mice.

DISCUSSION

Several studies have reported successful immunization with synthetic peptides derived from tumor antigens [Velders et al., 1998a]. However, one justified concern with the use of peptide-based cancer vaccines for immunization is the induction of specific T-cell tolerance for the tumor antigen causing enhanced tumor outgrowth [Toes et al., 1996a,b; this study]. The success of peptide-based vaccines may therefore depend on the individual pharmacokinetic characteristics of the peptide. This study compared the use of a peptide-based vaccine and chimeric virus-like particles containing the peptide to induce tumor antigen-specific antitumor immunity against a self-antigen. We showed that injection of the P1A peptide in adjuvant induced tolerance in vivo and rendered mice susceptible to the outgrowth of a regressor P1A-expressing tumor. One strategy shown to prevent tolerance induction is to target an antigen or peptide to professional antigen presenting cells (APCs). Peptide-pulsed DCs have been used successfully to activate the cellular arm of the immune response [Gilboa et al., 1998]. A recent report has shown that immunization with a tolerizing peptide loaded onto DCs prevented tolerance induction and resulted in an effective antitumor response, whereas other modifications of the peptide such as lipidation or inclusion of T-helper epitopes did not diminish the capacity of the peptide to tolerize CTLs [Toes et al., 1998]. Although DC-based vaccines are highly effective, they have the disadvantage of requiring ex vivo manipulation of the DCs and reinfusion back into recipients, a laborious, patient-restricted, and more expensive process than using a universal delivery vehicle such as a cVLP. In the case of the P1A self-antigen, previous studies had shown that immunization with the P1A peptide loaded onto DCs, the ultimate APCs, induced a long-lived state of P1A-specific functional unresponsiveness [Grohmann et al., 1997]. Interestingly, when we delivered the P1A peptide to the immune system incorporated into a cVLP, mice were immunized and rejected P1A-expressing tumor cells, indicating that the cVLP delivered the self-peptide to the immune system in a way that prevented tolerance induction and promoted active immunization.

This is the first study to demonstrate that cVLPs can be used to deliver a nonviral self-antigen-derived CTL epitope to the immune system, indicating that VLPs can be used as universal antigen carriers. We chose to fuse the P1A peptide to the C-terminus of the L1 protein, a region that is not required for proper capsid assembly [Müller et al., 1997]. cVLPs composed solely of the L1-P1A fusion protein contain 360 L1 monomers and therefore contain 360 copies of the P1A peptide in each cVLP [Hagensee et al., 1994]. For these cVLPs, the upper size limit for incorporating a foreign epitope is 60 amino acids, although capsid yields are generally lower as the size of the insert is increased most likely because of impediments to VLP assembly [Müller et al., 1997]. cVLPs composed of L1 and L2 capsid proteins can accommodate larger size inserts of up to at least 400 amino acids if the foreign epitope or protein is linked to L2 [Greenstone et al., 1998]. Fusion to L2 allows larger inserts because L2 is not required for capsid assembly and is present at a ratio of one L2 molecule to 30 L1 molecules [Kirnbauer et al., 1992, 1993]. In this case, a given cVLP would contain only 12 monomers of the L2 fusion protein and therefore 12 copies of the insert of interest. For human vaccination, the advantage of using a larger insert is that an entire protein, part of a protein, or multiple epitopes can be incorporated into the particle. cVLPs containing the entire coding sequence of a protein can serve as universal vaccine, as all possible CTL and T-helper epitopes in this protein can be used depending on the HLA type and individual T-cell repertoire of the patient. HPV16 L1/L2 cVLPs incorporating the P1A peptide fused to the L2 protein also effectively immunized mice against P1A-expressing tumors and slowed the growth of P815 tumors when applied in a therapeutic setting (our unpublished results). This finding indicates that both L1 and L1/L2 cVLPs can be used as universal antigen carriers for eliciting protective and therapeutic immune responses to tumor antigens.

Presentation of tumor antigens by professional APCs may be crucial for the induction of an antigen-specific CTL response [Grabbe et al., 1995]. It is well known that $CD8^+$ cytotoxic T cells are the main effectors involved in antitumor immunity. Although specific $CD8^+$ T cells can be generated following immunization with MHC class I-restricted epitopes in the absence

of CD4$^+$ T-cell help, the additional source of helper function has been shown to increase the immunizing capacity of class I-reactive antigens and to induce long-term CTL memory responses [Armstrong et al., 1998]. In addition to stimulating a strong T-helper cell response characterized by a Th1 phenotype [Loman et al., 1998], cVLPs are unique exogenous antigens that are capable of stimulating potent MHC class I-restricted T-cell responses both to the capsid protein L1 [Dupuy et al., 1997; De Bruijn et al., 1998; M.P. Rudolf, J.D. Nieland, D.M. Da Silva, M.P. Velders, M. Müller, H.L. Greenstone, J.T. Schiller, and W.M. Kast, unpublished communication] and to the nonstructural epitope fused to the capsid protein [Greenstone et al., 1998; Peng et al., 1998; this study]. This is a property that may be necessary for the generation of an effective cytotoxic T-cell response against a tumor-associated antigen. The particulate nature of the VLP probably helps increase the immunostimulatory effects of the vaccine by facilitating uptake and processing for class I and class II presentation by professional APCs [Bachmann et al., 1996; Rock et al., 1996; Schirmbeck et al., 1996]. The precise mechanism whereby VLPs are able to prime CTL precursors is unknown; however, the potential of VLPs to mimic an authentic virus and escape endocytic vesicles to gain access to the cytosol after entry is a likely possibility. Escape from intracellular vesicles would allow conventional endogenous processing of cVLPs to occur, leading to cytosolic degradation of viral and tumor antigens and peptide loading of newly synthesized class I molecules in the endoplasmic reticulum.

The HPV16 L1-P1A cVLP therapy did slow down the growth of tumors and significantly increased the survival of tumor bearing mice. Even when therapy was started 10 days after tumor inoculation, the survival of mice treated with cVLPs was significantly better than that of mice treated with empty VLPs. Only few examples of efficient therapy on 10 day established tumors in mice have been reported previously, indicating that the treatment of aggressively growing tumors in mice is extremely difficult. Active immunization in tumor bearing hosts is a general problem that has not yet been solved [Velders et al., 1998b]. The observation that tumor bearing T-cell receptor transgenic mice could reject a skingraft, but not tumors bearing the same rejection antigen, indicates that neither systemic T cell exhaustion nor systemic T-cell anergy is the reason for failure to eliminate the tumor cells [Wick et al., 1997]. Delivery of tumor antigens or peptides in the form of cVLPs is able to overcome the failure to eliminate the tumor cells and cure or significantly improve survival of tumor-bearing mice. In our model treatment of tumors 5 days after injection was more effective than treatment of 10-day established tumors, as indicated by the total tumor eradication in some animals. That tumor burden of these fast-growing murine tumors at a certain point prevents total tumor eradication is generally accepted. This point of no return will be dependent on the murine model being studied.

Chimeric VLPs elicit a cellular response against the protein or peptide that is packaged into the VLP, even in tumor-bearing hosts. Additionally, HPV VLPs elicit neutralizing antibodies against HPV, a property that makes VLPs a promising prophylactic vaccine. Thus HPV cVLPs have the potential of being a combined prophylactic and therapeutic vaccine, preventing new virus infection by inducing anti-HPV antibodies and targeting the CTL response to tumor rejection antigens. The results presented here open the possibility to use cVLPs as a universal antigen carrier for tumor-specific or tumor-associated antigens when vaccinating cancer patients.

ACKNOWLEDGMENTS

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