A vaccine candidate against the human papillomavirus: an alternative to treat cervico-uterine tumors

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ABSTRACT

In this study, we obtained a vaccine against tumors generated by the human papillomavirus type 16 (HPV16), a current and highly relevant issue in the field of cancer immunotherapy. In contrast to some vaccines currently being tested in clinical trials against the HPV, of which none of them have been approved, the novelty of this result is the design of an original vaccine, based on a synthetic peptide spanning the minimal sequence of a cytotoxic T lymphocyte (CTL) epitope from the HPV tumor antigen E7, which was adjuvanted with VSSP (Very Small Size Proteoliposomes, Center for Molecular Immunology, Cuba). Its proof-of-concept in the murine HPV16 tumor model demonstrated the induction of CD8+ T cells specific against the E7 epitope in mice treated with this vaccine candidate, as well as tumor regression and a significant increase in survival. This is the first report describing the induction of tumor regression by therapeutic immunization with a minimal CTL epitope and VSSP, and the first example of cancer immunotherapy by combining VSSP with a viral antigen. These results have been patented, presented in several international scientific conferences and published in “Vaccine” (Torres I, Mendoza O, Batte A, Reyes O, Fernandez LE, Mesa C, et al. Immunotherapy with CTL peptide and VSSP eradicated established human papillomavirus (HPV) type 16 E7-expressing tumors. Vaccine 2005;23:5768-74).

Introduction

Cervical cancer is the second cause of death by cancer worldwide; the human papillomavirus (HPV), specifically type 16 (HPV16) being associated to it. More than 99% of cervical cancers and their precursor lesions are diagnosed with HPV DNA. The HPV oncogenic proteins E6 and E7 are relevant for the induction and persistence of cellular transformation, being co-expressed in most of the HPV-containing cervical cancers. Therefore, these oncogenic proteins target antigens for developing vaccines and immunotherapeutic strategies against HPV-associated tumors [1].

In recent years, clinical trials have been carried out with several vaccines containing peptides restricted to the major histocompatibility complex type I (MHC-I) in patients with progressive malignancies associated to HPV and other types of tumors. However, the results have been discouraging because of the induction of a weak T cell response, and therefore, an irrelevant clinical benefit.

Some studies describe different strategies to solve the failure of these peptide vaccines to induce a potent and sustained immunity, although they are still ineffective. The novelty of this result is the design of an original vaccine, based on a synthetic peptide spanning the minimal sequence of a cytotoxic T lymphocyte (CTL) epitope from the HPV tumor antigen E7 that combined with VSSP (Very Small Size Proteoliposomes, Center for Molecular Immunology, Cuba) [2] was able to induce a potent T-cell response against pre-established tumors in the absence of oil-based components. The aim of this study was to demonstrate our hypothesis that this type of peptide-based vaccine increases its immunogenicity and anti-cancer properties when combined with a complete adjuvant. Thus, our findings are highly original, with a novel approach for the development of therapeutic vaccines with the perspective of treating cancer [3]. These results were also patented [4].

This work consisted of several steps: chemical synthesis of the CTL peptide, establishing the TC 1 murine model and the demonstration of the HPV16 E7 protein expression in TC-1 cells and in animal tumors, evaluating protection against tumor challenge in a prophylactic setting of immunized animals, evaluating protection after a second tumor challenge, evaluating the complete regression of pre-established tumors in a therapeutic setting, evaluating survival in the vaccinated animals and assessing the immunogenicity of the vaccine candidate through IFN-γ ELISPOT and IL-10 ELISA assays.

Results

Chemical synthesis of the CTL peptide

The peptide selected for the proof of concept was a H2-Dα-restricted CTL peptide in mice, corresponding to aas. 49-57 in the E7 oncoprotein of HPV16 (RAHYNIVTF), which was called E7(p).

The chemical synthesis of E7(p) was carried out on a solid phase; the E7(p) being further purified by high pressure liquid chromatography procedures at more than 95%. Peptide identity was confirmed by mass spectrometry, using the electro-spray ionization technique.

The peptide was stored at 4 ºC until use. It was dissolved in water for injection at 1 mg/mL just before its administration.

application. Fifty micrograms of the E7(p) peptide was then mixed with 160 µg of VSSP to a volume of 0.2 mL; the E7(p)+VSSP vaccine dose was ready to be administered to the experimental animals.

**Establish of TC-1 murine tumoral model. Demonstration of expression of HPV E7 protein in TC-1 cells and animal tumors**

We used the TC-1 murine tumor model, consisting of immunocompetent C57BL/6 mice which were challenged with TC-1 tumor cells. The TC-1 tumor cell line, expressing the HPV16 E7 protein, derives from a primary culture of C57BL/6 mice lung cells immortalized and transformed with HPV16 E6 and E7 genes and a human activated c-Hras gene [5]. This tumor cell line was kindly provided by Dr. TC Wu (The Johns Hopkins Medical Institute, Baltimore, Md., USA). It has been widely used in the proof-of-concept of several vaccine preparations developed in laboratories throughout the world.

The actions for its establishment in our laboratory were: (i) to make the tumor cell line syngeneic with C57BL/6 mice purchased from the National Center for the Production of Laboratory Animals (CENPALAB, Cuba); (ii) the demonstration of HPV16 E7 protein expression by reverse transcription and polymerase chain reaction and Western blot procedures in cells and tumors isolated from these mice; and (iii) repeated experiments of tumor inoculation, followed by the analysis of tumor implantation to obtain homogeneous and repetitive results.

**Prophylactic evaluation of protection against tumor challenge of animals immunized with the vaccine candidate**

In this first assay, C57BL/6 mice were subcutaneously immunized in the right flank with two doses given 14 days apart. Seven days after the second immunization, the animals were challenged with 5x10^4 TC-1 tumor cells administrated subcutaneously in the right hind limb. Three additional control groups were included in the study, receiving: VSSP, E7(p) + incomplete Freund’s adjuvant (IFA) and phosphate buffered saline (PBS), respectively. The results indicated that mice immunized and transformed with HPV16 E6 and E7 genes and a human activated c-Hras gene [5]. This tumor cell line was kindly provided by Dr. TC Wu (The Johns Hopkins Medical Institute, Baltimore, Md., USA). It has been widely used in the proof-of-concept of several vaccine preparations developed in laboratories throughout the world.

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**Evaluation of survival in animals treated with the vaccine candidate**

Mice were observed for 100 days after treatment to determine the effects of the E7(p) + VSSP therapy on survival. The Kaplan-Meier plot (Figure 1b) shows survival results of 80% for the group treated with E7(p) + VSSP (8/10), a result that is statistically significant compared to the control group (5/10) and the group treated with VSSP (3/10). The results indicate that the E7(p) + VSSP vaccine preparation is effective in prolonging survival in mice challenged with TC-1 tumors.

**Table 1. Results of tumor implantation in immunized mice**

<table>
<thead>
<tr>
<th>Group</th>
<th>Incidence of tumor / Total</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10/10</td>
<td>50 µg E7(p) + IFA</td>
</tr>
<tr>
<td>II</td>
<td>2/10</td>
<td>50 µg E7(p) + VSSP</td>
</tr>
<tr>
<td>III</td>
<td>10/10</td>
<td>VSSP</td>
</tr>
<tr>
<td>IV</td>
<td>10/10</td>
<td>Placebo (PBS)</td>
</tr>
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**Figure 1.** The therapeutic immunization with the E7(p) + VSSP induces TC-1 tumor regression and long-term survival. (a) C57BL/6 mice (10 per group) were inoculated with 2 x 10^5 TC-1 cells in a hind limb. Ten and 24 days after tumor graft, mice were immunized with PBS (□), VSSP (●), E7(p)+IFA (△), or E7(p)+VSSP (○). Tumor volume was measured for 50 days. (b) Mice were observed and survival recorded for 100 days.
cally highly significant when compared to the groups treated with E7(p) + IFA, VSSP or PBS, respectively (P < 0.0001).

**Immunogenicity of the vaccine candidate as assessed by IFN-γ ELISPOT and ELISA assays**

As an initial step to identify the effector mechanisms involved in tumor eradication, the immune response of cytokine secretion induced by immunizing with E7(p) + VSSP was characterized by an ELISA assay. An anti-IFN-γ ELISPOT assay was carried out to compare the immune response mediated by anti-E7 specific CTLs developed in the different groups of vaccinated mice. Splenocytes from mice immunized with the E7(p) + VSSP secreted high levels of IFN-γ in response to the stimulation with the peptide, and low levels of IL-10. These were indicators of a T helper lymphocyte type 1 response. The highest number of IFN-γ-secreting cells was induced in mice immunized with E7(p) + VSSP, suggesting the induction of a cytotoxic CD8+ T cell response (Figure 2).

![Figure 2](image_url)