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A human papillomavirus type 16 vaccine by oral delivery of L1 protein

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Abstract

To establish an edible HPV16 vaccine, we constructed a recombinant HPV16 L1-expressing *Schizosaccharomyces pombe* yeast strain (HPV16L1 yeast). A preliminary study revealed that freeze-dried yeast cells could be delivered safely, and were digested in the mouse intestine. The freeze-dried HPV16 L1 yeast was administered orally as an edible vaccine, with or without the mucosal adjuvant heat-labile toxin LT (R192G), to 18 female BALB/c mice. After the third immunization, none of the mice that received the edible HPV16 vaccine showed specific antibody responses, whereas all of the positive controls that were administered intranasally with 5 μg of HPV16-virus-like particles (VLP) had serum IgG, and genital IgA and IgG that reacted with HPV16-VLP in enzyme-linked immunosorbent assays (ELISAs). When a suboptimal dose (1 μg) of HPV16-VLP was administered to all the mice, including the negative control mice, 50% of the mice that were pre-immunized with the edible HPV16 vaccine showed positive serum IgG responses, while none of the negative controls showed any response. Vaginal IgG and IgA antibodies were also elicited in 33 and 39%, respectively, of the mice that were given with the edible HPV16 vaccine and the intranasal boost. All of the antibodies reacted more strongly to intact HPV16-VLP than to denatured HPV16-L1 protein suggesting that the edible vaccine primes for antibody responses against conformation-dependent epitopes. The inclusion of adjuvant in the vaccine formulation marginally increased the genital IgA response ($P = 0.06$). HPV16-L1 protein in the yeast might induce tolerance in the vaccinated animals that could be recovered by intranasal boosting with a suboptimal dose of HPV-VLP. This freeze-dried yeast system may be useful as an oral delivery of HPV 16 L1 protein.

Keywords: Fission yeast; Mucosal immunity; Iga; Igg; Neutrolization

1. Introduction

Although mortality due to cervical cancer has recently decreased in developed countries, this type of cancer frequently occurs as a secondary cancer, and it is the fifth leading cause of death in women worldwide (Parkin et al., 2001). Certain types of the human papillomavirus (HPV), which is sexually transmitted, represent the most important risk factors for cervical cancer (zur Hausen, 1991). Recent reports show that 30–50% of young women who have recently had sexual intercourse for the first time are infected with HPV in their cervices (Ho et al., 1998; Franco et al., 1999; Molano et al., 2003). Surprisingly, most cervical HPV infections involve high-risk types that are likely to induce cancer. HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68,73 and 82, and possibly some other types, are considered to be high-risk types (Munoz et al., 2003). We have identified HPV types 16, 18, 31, 33, 35, 45, 51, 52, 56, 58, and 67 as single infecting types in cervical cancer and precursor lesions in Japan

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(Sasagawa et al., 2001). Our recent report showed that more than 60% of women in their late teens, who visited outpatient obstetrics and gynecology clinics for various reasons, were infected with HPV, and that 50% of these women were infected with high-risk HPV types (Sasagawa et al., in press). This alarmingly high prevalence of HPV infection in young women suggests that education and social health programs aimed at preventing HPV infections may not be sufficiently effective in combating cervical cancer, especially in poor resource settings, in which cervical cancer prevention should be a priority (Yang et al., 2004). HPV testing and cytological screening may not be the best choices in these areas, due to the costs of screening tests and treatments. Thus, nationwide use of a prophylactic vaccine against the high-risk HPV types may prove advantageous in the prevention of cervical cancer.

The development of HPV vaccines has been hampered for many years due to a lack of animal systems for HPV propagation and of a methodology for the synthesis of HPV virions. Rose et al. (1993) showed that high-level production of HPV-11 L1 protein led to the assembly of virus-like particles (VLPs) in an insect cell system. Kirnbauer et al. (1993) succeeded in synthesizing HPV16-VLPs in this insect cell system. Subsequently, we succeeded in producing HPV6- and HPV16-derived VLPs in the yeast *Schizosaccharomyces pombe* (Sasagawa et al., 1995). Although the yield of VLPs from yeast is less than that obtained from the insect cell system, the yeast system confers advantages in terms of large-scale VLP production and safety of use in humans. Regardless of the method used for their production, HPV-VLPs are good candidates for a human vaccine, since VLPs have been shown to have the same antigenic properties as the native virions, and they do not carry any potentially oncogenic viral genes. Koutsky et al. (2002) were the first to demonstrate that parenteral immunization with a HPV16-VLP vaccine conferred 100% protection against HPV16 infection in women. It has also been reported that a HPV vaccine that targets HPV16 and HPV18 may reduce cervical cancer incidence by more than 50% (Kulasingam and Myers, 2003; Goldie et al., 2003).

Unfortunately, the parenteral HPV16-VLP vaccine is expensive, since it requires advanced techniques and special facilities for production and storage. In addition, repeated injections of the parenteral vaccine, which are usually required for efficacy, are impractical in poor resource settings, with limited numbers of trained clinical staff. Furthermore, it has been reported that parenteral immunization with VLPs is a poor inducer of secretory IgA, which plays a major role in mucosal immunity (Hagensee et al., 1995). Immunization of the mucosa-associated lymphoid tissue (MALT), which is located in the respiratory and digestive tracts, is important for the induction of effective mucosal responses against many viruses. Balmelli et al. (1998) have demonstrated that intranasal administration of HPV16-VLP elicits mucosal antibodies that neutralize HPV16. However, intranasal vaccination also requires the preparation of relatively large amounts of purified HPV-VLP. The gut-associated lymphoid tissue (GALT) constitutes an alternative site for immunization to induce strong mucosal immunity. Recently, two groups have produced edible HPV vaccines from tobacco and potato plants that express the HPV-11 (Warzecha et al., 2003) and HPV-16 (Biemelt et al., 2003) L1 genes, respectively, and they have tested them in animal model systems. Although edible vaccines alone do not induce HPV-specific antibodies, systemic or local booster immunizations with purified VLP elicit HPV-specific serum IgG.

In this study, we used a freeze-dried preparation of a yeast strain that expresses HPV16 L1 protein as an edible vaccine in a mouse model system. Yeasts are good candidate edible vaccine vectors, since they are readily adaptable to large-scale production, may be administered safely to animals and humans, and they incorporate HPV16-VLPs into the nucleus. Indeed, all of the mice willingly ate this freeze-dried yeast; this represents an advantage, in terms of administration, over intranasally administered VLP vaccines, which require anesthesia. Our preliminary study demonstrated that freeze-dried yeast strains could be delivered safely, and that they were digested in the intestine, where GALT is located. In the present study, we investigated the efficacy of this edible yeast vaccine, and evaluated the optimal conditions for immunization, in terms of the dose of yeast and the addition of the *Escherichia coli*-derived mucosal adjuvant LT (R192G) (Freytag and Clements, 1999).

2. Materials and methods

2.1. Construction of a recombinant S. pombe strain that expresses the HPV16 L1 gene

We have previously reported that a strain of the fission yeast *S. pombe*, which expresses the HPV16 L1 gene (B27; wild-type HPV16) under the control of a thiamine-repressive promoter, was able to synthesize virus-like particles (VLPs) (Sasagawa et al., 1995). B27L1 has two amino acid changes at histidine 202 to aspartate and threonine 266 to alanine, and this HPV16 L1 gene could produce 68 times more virus-like particles than the prototype HPV16 L1 (Pushko et al., 1994). In order to increase L1 gene expression in yeast, we introduced B27L1 into a new vector, pTL2M, and expressed the HPV16 L1 protein (Tohda et al., 1994). This vector contains a cytomegalovirus promoter that expresses, constitutively, the introduced foreign gene. We confirmed by Western blot analysis that this recombinant yeast (pTL2-HPV16 L1) expressed the 55-kDa HPV16-L1 protein at the level of about 5% of total protein. The appropriate assembly of the purified HPV16 L1 protein was confirmed by the reactivity of this protein to two HPV16-monoclonal antibodies, Camvir-5 and Camvir-6, which recognized conformation-dependent epitopes in an enzyme-linked immunosorbent assay (ELISA) (kindly provided from Margaret Stanley, Cambridge University, Cambridge, UK). After culturing in YPD medium, the recombinant yeast was collected by centrifugation at $2000 \times g$ at