Vaccinia-Expressed Human Papillomavirus 16 and 18 E6 and E7 as a Therapeutic Vaccination for Vulval and Vaginal Intraepithelial Neoplasia

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ABSTRACT

Purpose: Anogenital intraepithelial neoplasia is a chronic disorder associated with infection by high-risk human papillomavirus (HPV) types. It is frequently multifocal and recurrence after conventional treatment is high. Boosting HPV-specific cell-mediated immune responses may reduce progression to carcinoma and could lead to disease clearance. We have tested the safety, immunogenicity, and efficacy of a recombinant vaccinia candidate vaccine (TA-HPV) in women with anogenital intraepithelial neoplasia.

Experimental Design: Twelve women, aged 42–54 years with high-grade HPV-positive vulval or vaginal intraepithelial neoplasia of up to 15 years duration, completed a Phase II study of TA-HPV, a live recombinant vaccinia virus, expressing modified versions of the E6 and E7 open reading frames from HPV-16 and HPV-18.

Results: The vaccine was well tolerated. Five of 12 (42%) patients showed at least a 50% reduction in total lesion diameter over 24 weeks with 1 patient showing complete regression of her lesion. Overall, 83% of women showed some improvement with an average decrease in lesion size of 40%. All cases showed an increased IgG titer and T-cell response to the vaccinia virus. An IFN-γ enzyme-linked immunospot assay using pooled 22-mer peptides spanning HPV-16 E6 and E7 showed an increased specific T-cell response after vaccination in 6 of the 10 cases available for testing. There was no increase in specific cytotoxic response to selected individual HLA class I-restricted HPV-16 E6/7 peptides.

Conclusions: The results suggest that the vaccine may have an effect on HPV-positive vulval intraepithelial neoplasia/vaginal intraepithelial neoplasia and that additional studies are warranted to develop an effective therapeutic vaccine.

INTRODUCTION

Squamous intraepithelial neoplasia is common to several anogenital sites and carries a risk of progression to invasive SCC. Both intraepithelial neoplasia and SCC are commonly associated with HR-HPVs. Indeed, for cervical disease, the development of sensitive PCR methodology has demonstrated HR-HPV to be present in almost all cases of SCC (1), and HR-HPV infection is now widely accepted as a necessary cause for cervical cancer (2). Over 85 HPV types have been fully sequenced, and many other potential types have been identified in mucosal and cutaneous tissue (3). Interestingly, the vast majority of cases of cervical neoplasia are attributable to a small subset of HR-HPV types (namely types 16, 18, 31, 33, 45, and 56; Refs. 1, 4). Persistent HR-HPV infection appears to be required for the development of CIN (5) and may increase the risk of disease progression at this site (6). HPV DNA is found in 80–100% of biopsies of high-grade VIN (grade II–III; Refs. 7, 8) and VAIN (8, 9). Classically, both HPV-dependent and -independent SCC of the vulva have been previously described (10), with younger women showing a high prevalence (85%) of HR-HPV infection (11). In common with cervical neoplasia, a limited group of HR-HPVs are associated with vulvar neoplasia, with >90% of cases involving HPV types 16, 18, or 33 (8, 12).

AGIN at sites other than the cervix poses a challenging therapeutic problem for two reasons. Firstly, the disease is increasing in incidence, especially in the vulvar area of premenopausal women (13, 14) in whom lesions are frequently HPV positive. Secondly, the disease is difficult to manage. Many
treatments are in current use for AGIN. Surgical excision or ablation of affected skin is the most successful way of treating the disease, but because AGIN is commonly multifocal and sometimes multicentric, this approach may be extensive and disfiguring and can carry considerable psychosexual morbidity. Recurrence rates are high, with a reported return of high-grade VIN in ~50% of patients (15, 16), usually within the first 4 years after treatment. Furthermore, surgical excision does not eliminate the risk of progression to invasive SCC, which remains of the order of 5% even after treatment (15–17). If untreated, a small study of only eight cases has suggested a much higher progression rate of 88% (17).

The high prevalence of HR-HPV infection in AGIN has lead to the suggestion that therapy for the disease should aim at the immunological eradication of virus-infected cells. Local immunotherapy has already been described with some encouraging results. IFN has been reported to induce short-term disease regression (18) and local immune modulation with imiquimod has also led to disease clearance (19). An alternative approach is to induce a systemic cell-mediated immune response with the aim of effecting local cytotoxicity against the virus-infected cells. This has been attempted in cervical HPV-associated malignant and premalignant disease (20–23) with successful induction of a HPV-specific immune response but little, if any, effect on the disease.

In this Phase II study, the safety and efficacy of a candidate therapeutic vaccine against HPV (TA-HPV; Xenova Research Ltd.) was tested in women with VIN or VAIN. All women had high-grade disease, and the clinical effect was assessed by measurement of the visible areas of abnormal skin or mucosa.

PATIENTS AND METHODS

Subjects

Women between the ages of 18 and 60 with high-grade VIN, VAIN, or AIN were assessed for inclusion in the trial. They were diagnosed by histological interpretation of a biopsy and screened for hematological, biochemical, and immunological abnormalities by blood testing. Women were not considered for inclusion if the use of live vaccinia virus could be expected to be detrimental either to their health or to the health of their close contacts. Thus, women with any degree of immunosuppression (including those receiving therapeutic immunosuppressive agents), a history of severe allergic reaction, active atopic eczema, or other widespread inflammatory or exfoliative skin disease were not considered for the trial. In addition, women were excluded from consideration for vaccination if they were in close contact with children under the age of 5 or with individuals with known or suspected immune suppression or active eczema. Pregnant women or those at risk of pregnancy were also excluded from the trial.

After screening investigations, patients were excluded if they had any hematological evidence of immunosuppression or abnormal renal or liver function. Fourteen women were screened for inclusion in the trial, but 2 were excluded, 1 on the basis of a CD4 lymphocyte count below the normal limit and the other because of abnormal liver function tests requiring additional investigation. During the recruitment period, no women below the age of 42 years were eligible for consideration for inclusion in the trial.

Twelve women entered the trial and all completed the treatment and follow-up. Eleven women had VIN, and one had lower vaginal disease. After vaccination, all subjects were followed for a 6-month period. Trial visits were initially monthly until 3 months, followed by a final review at 6 months. No other treatment for the genital intraepithelial neoplasia was given during the trial or in the month preceding the vaccination.

The study was approved by the local research ethics committee of the hospital, the local genetic manipulation committee, the national Gene Therapy Advisory Committee, the National Health and Safety Executive, and the Medicines Control Agency.

Vaccination

The recombinant vaccinia virus expressing HPV-16 and HPV-18 E6 and E7 (TA-HPV) has been described previously (20, 24, 25). It consists of the fused E6 and E7 open reading frames of HPV-16 and HPV-18, each under the control of a vaccinia promoter within the Wyeth strain of vaccinia virus. The E7 gene in both cases was modified by mutation of the retinoblastoma gene product binding sequence so that there can be no binding of the resultant viral protein with retinoblastoma protein. The fused E6 and modified E7 genes have previously been demonstrated to show no transforming activity. The virus was prepared at a concentration of 1 × 10⁸ plaque-forming units/ml as used previously and known to be able to stimulate an immune response (20, 24, 25).

TA-HPV (in an estimated dose of 2.5 × 10⁵ plaque-forming units) was introduced into the patient by scarification through a 50 μl drop of the virus suspension applied to the skin of the upper arm overlying the deltoid muscle. The area was allowed to dry before covering with a waterproof dressing (Opsite Plus, Smith & Nephew). The dressing was changed twice a week for ~4 weeks until the scab, which formed as a result of the virus-induced inflammation, had separated and the vaccination site was healed. Samples for virological detection were taken from the surface of the dressing every week before its removal for test to the risk of possible contamination of the environment, and on separation, the final scab was tested for the presence of live virus.

HPV Typing

Biopsies from subjects were immediately snap frozen in liquid nitrogen. DNA was subsequently extracted by proteinase K digestion overnight in an extraction mix (comprising 200 μg/ml proteinase K, 0.5% SDS, 10 mM Tris, and 10 mM EDTA at pH 7.5). PCR using primers directed at a 268-base region of the β-globin gene (26) was used to assess the adequacy of the sample DNA for HPV typing. Typing was then performed by PCR with the GP5+/GP6+ primer pair (27) to amplify a region within the L1 gene of genital HPV types. The DNA amplification reaction was performed in 50 μl using 2.5 μl of a hot-start Taq polymerase (HotStarTaq; Qiagen) and the PCR product purified away from the other reaction reagents using a PCR purification kit (Qiagen). The HPV PCR product was sequenced directly and the sequence compared with the L1 open reading frame.
frame of all known genital HPV sequences available in the HPV database (Los Alamos National Laboratory, University of California).  

Antivaccinia ELISA

Whole blood was collected in one 7.5-ml S-Monovette tube (Starstedt 01.1601.001) at days 0, 28, 56, and 84. Serum was separated and stored at −20°C until required for analysis. Vaccinia-specific IgG was measured in patient sera by ELISA using Wyeth strain vaccinia-infected Vero cell lysates as antigen and mock-infected Vero cell lysates as the control (20). The titer at absorbance 0.5 was determined using the linear fit from the graph of the log sample dilution versus sample Wyeth absorbance minus sample Vero absorbance.

ELISPOT Assays

Whole blood was collected in S-Monovette 8.5 ml of citrate phosphate dextrose adenine tubes (Starstedt 01.1610.001), and PBMCs were isolated by density centrifugation on the same day. Cells were cryopreserved in vapor phase nitrogen until required.

Analysis of HPV-16-specific T-Cell Responses with Overlapping E6 and E7 Peptides. IFN-γ ELISPOT assays were performed as described previously (23). Briefly, PBMCs were thawed and seeded at a density of 2 × 10^6 cells/well of a 24-well plate (Costar, Cambridge, MA) in 1 ml of Iscove’s medium (Life Technologies, Inc.) enriched with 10% human antibody serum, in the presence or absence of indicated E6 and E7 peptide pools. As a positive control, PBMCs were cultured in the presence of a memory recall mix, consisting of a mixture of tetanus toxoid (0.75 flocculation units/ml final concentration; National Institute of Public Health and Environment, Bilthoven, the Netherlands), Mycobacterium tuberculosis sonicate (2.5 g/ml; generously donated by Dr. P. Klatser; Royal Tropical Institute, Amsterdam, the Netherlands) and Candida albicans (0.005%; HAL Allergenen Lab, Haarlem, the Netherlands). The peptides used spanned the HPV-16 E6 and E7 protein and consisted of 15 E6 and 9 E7 overlapping 22-mer peptides. Peptides were used in pools of four to five peptides at a concentration of 5 g/ml/peptide. The peptides, as indicated by their first and last amino acid, were used in the following pools: E6-I, 1–22, 11–32, 21–42, and 31–52; E6-II, 41–62, 51–72, 61–82, and 71–92; E6-III, 81–102, 91–112, 101–122, and 111–132; E6-IV, 111–132, 121–142, 131–152, and 137–158; E7-I, 1–22, 11–32, 21–42, and 31–52; and E7-II, 41–62, 51–72, 61–82, 71–92, and 77–98 (23). After 4 days of incubation at 37°C, PBMCs were harvested and seeded in four replicate wells at a density of 10^5 cells/well in 100 μl of Iscove’s medium (Life Technologies, Inc.) enriched with 10% FCS in a multi-screen 96-well plate (Millipore, Eten-Leur, the Netherlands) coated with an IFN-γ-catch antibody (Mabtech AB, Nacha, Sweden). The ELISPOT was otherwise performed according to the instructions of the manufacturer (Mabtech AB). The number of spots was analyzed with a fully automated computer-assisted video imaging analysis system (Carl Zeiss Vision). The background number of spots in the medium controls was 2.3 ± 2 spots/50,000 PBMCs. Specific spots were calculated by subtracting the mean number of spots +2× SD of the medium only control from the mean number of spots in experimental wells. Antigen-specific T-cell frequencies were considered to be increased compared with nonresponders when specific T-cell frequencies were >1/10,000 (23). T-cell frequencies were considered to be boosted by the vaccine when they were at least 3-fold higher than before vaccination. In our experience, the use of these long peptides, which need to be taken up, processed and presented by antigen-presenting cells, together with the long incubation period, highly favors the response of CD4+ cells (23, 28, 29).

Analysis of CD8+ T-Cell Responses with HLA Class I-restricted HPV Epitopes. ELISPOT plates (MAIPS410; Millipore) were prepared with the following antigens in triplicate wells: (PHA) 1 μg/ml; synthetic peptides (Immune Systems Ltd.) of HPV-16 E629–38, 59–67, 80–88, and E711–20, 82–90, 86–93 (30, 31) influenza virus M158–66 and EBV BMLF1280–288 (ill at 25 μg/ml or inactivated vaccinia virus and control-uninfected Vero cell lysate 1:2500. Patient PBMCs from each time point were thawed from cryopreserved stocks and resuspended in medium (RPMI (Life Technologies, Inc.), 10% human penicillin, 100 μg/ml streptomycin, 2 mM l-glutamine (all Life Technologies, Inc.) at 5 × 10^5/ml. PBMCs were then added to wells in triplicate, 2.5 × 10^5 to the PHA wells and 5 × 10^5 to each of the remaining wells containing antigen or medium alone. After overnight incubation and washing, biotinylated monoclonal antibody to IFN-γ (7-B6-1-biotin; Mabtech) was added, followed by streptavidin-conjugated horseradish peroxidase (Mabtech). The assay was developed according to the manufacturer’s instructions (Mabtech). A positive response to antigen was defined as >20 spots (IFN-γ-producing cells)/10^6 PBMCs in response to antigen (after subtraction of background). A positive response after vaccination was recorded if the frequency of IFN-γ-producing cells post-vaccination was greater than twice the prevaccination response to an antigen. In our experience, the use of well-defined minimal CTL peptide-epitopes results in strong responses of CD8+ T cells, whereas CD4+ T-cell reactivity is usually weak or not detectable because of the short incubation time (23).

Clinical Response

The extent of the disease was assessed by direct measurement and photographic record of up to two marker lesions in each case. The longest diameter of each marker lesion was recorded serially through the study. Three biopsies of the visible areas were taken at time points: before the vaccination and at 12 and 24 weeks after vaccination. Care was taken to ensure that the biopsies were taken so as not to directly influence the diameter of the lesion being studied. The biopsies were analyzed by two independent pathologists (R. P. M. and N. C.) and the international Society for the Study of Vulvovaginal Disease classification. The presence of HPV detectable by polymerase chain amplification in tissue was sought at each biopsy point. Any symptomatic change was recorded with particular reference to irritation and pain. Assessment was by subjective grading of symptoms into one of four categories: nil; mild; moderate; or severe. After vaccination, subjects were actively questioned to...

3 Internet address: http://hpv-web.lanl.gov/stdgen/virus/hpv/.
ascertain the nature of any systemic symptoms potentially attributable to vaccination, and all additional medications were recorded.

**RESULTS**

**Patients and HPV Type**

Twelve women were recruited for vaccination (Table 1). The age range was 42–54 years with a mean of 46 years. The diagnosis of AGIN had been made between 3 months and 14 years (mean, 3.4 years) before the start of the vaccine trial. The disease was multifocal in 8 women. Eleven women had VIN (grade III), and 1 woman (no. 5) had VAIN (grade II). Previous treatments received by half the patients included excisional surgery, laser ablation, and topical imiquimod cream. All patients were positive for HR-HPV, with 11 of the 12 women infected by HPV-16. HPV-33 was identified in 1 woman (no. 10). Nine of the patients were HLA A2 haplotype.

**Safety**

There was no detectable adverse effect on kidney, liver, or bone marrow function as assessed by blood samples and in no patient was there a significant increase in disease area or progression. All patients remained generally well during and after the vaccination. A local reaction at the site of vaccination at ~7–10 days was common but temporarily limited arm movement in only 2 patients. The swabs taken from the outside of the dressing each week before its removal showed no evidence in any case of escape of the active virus from beneath the dressing to the exterior. A total of 57 swabs from 12 patients was assayed, and no virus particles were detected in any of the samples.

Occlusive dressings were worn over the vaccination site until the scab formed, dried, and then separated from the skin. Gauze samples from under each dressing were tested for the presence of live TA-HPV. Live virus was detected in the under surface of the dressing in all patients after vaccination for a mean of 21 days. In 5 of the 12 patients, live virus was detected in the final sample containing the scab. In 7 patients, no live virus was detected in the final sample. A small area of scarring was left at the vaccination site.

**Clinical Response**

Ten of the 12 treated women (83%) experienced some reduction in the size of the affected area over the 6 months after vaccination with a mean reduction in marker lesion diameter of 40% (Fig. 1). In 1 woman (no. 5), there was complete clearance of the abnormal area with histological normality at 3 months after vaccination. In 9 women, the grade of the marker lesion(s) remained unchanged, but there was a reduction of the extent of the abnormal epithelium. The size of the affected area was assessed by the longest diameter of identifiable lesion(s). In these 9 individuals, the reduction in length ranged from 22 to 65% with 5 of the treated women showing at least 50% reduction in total maximum lesional diameter. Two women (nos. 1 and 10) showed no reduction in the size of the affected area of vulval disease.

In the 11 women in whom VIN was still present at the end of the study, the same HPV was still detectable in the abnormal epithelium. In 1 patient (no. 5) whose VAIN cleared after vaccination, HPV-16 was no longer detectable in the previously abnormal area of the vagina.

The symptoms of VIN experienced by the patients in the study included irritation, soreness, and pain. There was no overall change in symptom score between pre- and postvaccination, with 3 women noticing a slight worsening of symptoms, 3 women having a slight improvement, and 6 women experiencing no change.

**Immune Response**

**Vaccine-Induced Immunity against the Vaccinia Vector.** To monitor the impact of the vaccine on the immune system, the responses to vaccinia were measured. Both antibody

**Table 1** Patients enrolled in study

<table>
<thead>
<tr>
<th>Patient study no.</th>
<th>Age (yr)</th>
<th>Disease</th>
<th>Duration of disease</th>
<th>HPV type</th>
<th>HLA A type</th>
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<tr>
<td>1</td>
<td>48</td>
<td>VIN 3</td>
<td>11 years</td>
<td>16</td>
<td>2, 68</td>
</tr>
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<td>49</td>
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<td>8 years</td>
<td>16</td>
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<td>16</td>
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<td>49</td>
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<td>2.5 years</td>
<td>16</td>
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<td>44</td>
<td>VAIN 2</td>
<td>3 months</td>
<td>16</td>
<td>2, 3</td>
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<td>6</td>
<td>47</td>
<td>VIN 3</td>
<td>6 months</td>
<td>16</td>
<td>1, 2</td>
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<td>7</td>
<td>42</td>
<td>VIN 3</td>
<td>1 year</td>
<td>16</td>
<td>2, 32</td>
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<tr>
<td>8</td>
<td>49</td>
<td>VIN 3</td>
<td>2 years</td>
<td>16</td>
<td>2, 24</td>
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<td>12</td>
<td>53</td>
<td>VIN 3</td>
<td>3 months</td>
<td>16</td>
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**Fig. 1** Percentage reduction in lesion size. Sum of widest diameter(s) at 6 months shown as a percentage reduction compared with the diameter prevaccination.
Fig. 2 Vaccination induced immunity against the vaccinia vector. Pre- and postvaccination antibody and T-cell response above background to the vaccinia vector were detected in patient serum and PBMC samples, respectively, taken before vaccination (day 0) and at days 28, 56, and 84 after vaccination. Peak serum antibody (IgG) responses were measured by ELISA before vaccination (A) and postvaccination (B). Frequency of IFN-γ-producing T cells responding to vaccinia vector was measured by ELISPOT in patient PBMC prevaccination (■) and postvaccination (□). In each case, the maximum response postvaccination is shown.

and T-cell responses against vaccinia were found to be strongly increased after vaccination in almost all patients (Fig. 2). Before vaccination antivaccinia IgG was detected in 9 of 12 patients (nos. 2, 3, 4, 5, 6, 8, 9, 10, and 12). Postvaccination IgG responses in 11 of 12 patients (not no. 8) increased >10-fold. Prevaccination T-cell responses to the Wyeth strain vaccinia virus as measured by ELISPOT were positive in 6 of 12 patients (nos. 2, 4, 5, 6, 9, and 10), with frequencies ranging from 1:13,000 to 50,000. After vaccination, the IFN-γ-producing T-cell frequency to the vaccinia vector increased by 1:13,000 to 50,000. After vaccination, the IFN-γ-producing T-cell frequency to the vaccinia vector increased by 1:13,000 to 50,000.

Prevaccination HPV 16-Specific Immune Responses. Preexisting HPV-16-specific immunity was detected in 6 of the 10 patients (nos. 2, 4, 5, 7, 10, and 12; Table 2), all of whom showed reactivity within the HPV-16 E6 protein. Three subjects (nos. 4, 5, and 7) also demonstrated HPV-16 E7-specific T-cells.

Vaccine-Induced HPV 16-Specific Immune Responses. TA-HPV-induced HPV16 E6 and E7-specific T-cell immunity was analyzed by the stimulation of PBMCs with a set of overlapping peptides covering the complete amino acid sequence of the E6 and E7 proteins. From 10 of 12 patients, enough material was available to study the HPV-16-specific immune response before vaccination as well as 4 and/or 8 weeks after vaccination with TA-HPV (Table 2). Upon vaccination, 4 patients (nos. 1, 4, 6, and 10) did not mount an increased response to the HPV-16 peptide pools. Two of these patients (nos. 1 and 10) also showed no clinical reduction in the size of the lesion(s). Importantly, HPV-16 E6- and E7-specific T cells were specifically enhanced after vaccination in 6 of the 10 patients tested (nos. 2, 3, 5, 7, 11, and 12; Table 2 and Fig. 3). In 1 case (no. 3) the T-cell response was focused to one peptide pool, whereas in the remainder (nos. 2, 5, 7, 11, and 12), T-cell reactivity was detected against two or three different peptide pools. All patients reacted to the positive memory recall mix.

In addition, PBMCs were stimulated with six CTL epitopes identified within HPV-16 E6/E7 and chosen for their ability to bind MHC class I molecules of HLA-A*0201-positive individuals (30, 31). Of the 10 patients typed as HLA-A2 (Table 1), 1 patient (no. 3) responded weakly to peptide HPV-16 E50–88 (T-cell frequency 1/50,000). This response was already present before vaccination and the single injection of TA-HPV failed to enhance this reactivity. Two patients (nos. 7 and 12) showed an enhanced production of IFN-γ in response to the whole HPV-16 E6 and E7 protein as expressed in TA-CIN after vaccination with TA-HPV. No other patient reacted to TA-CIN either pre- or postvaccination.

DISCUSSION

We have analyzed the safety and immunogenicity of the recombinant vaccinia vector expressing HPV-16 and HPV-18 E6 and E7 proteins (TA-HPV) in a Phase II clinical trial. In this study of 12 women with high-grade VIN and VAIN, no adverse effects of the vaccination were found. In general, the patients felt well without definite evidence of pyrexia. The vaccination site was tender and uncomfortable in all women with duration of discomfort ranging between 1 and 13 days, which was maximal ~1 week after vaccination. The vaccination site was dressed for a mean duration of 25 days (range, 15–32 days) and was acceptable to all patients. Vulval symptoms were not significantly improved by vaccination. It may be that an alternative reporting system, e.g., visual analogue scale might prove more sensitive in detecting symptom change.

No progression of disease was observed in any of the 12 women during the 6 months after vaccination. In 11 women, no change in disease grade occurred, but the reduction in size of the measured regions of AGIN seen in 10 of the 12 women was promising. In 5 women, the affected areas were reduced by >50% of the original longest diameter, and in 1 patient, the lesion cleared completely. An effective treatment that would compare favorably with the most common form of treatment, excisional surgery, would be one in which complete clearance occurred and in which recurrence was rare. The responses seen in this small group of women with diverse duration of disease and prior treatment do not achieve this ideal, although as yet, it
is impossible to know if the longer term course of the disease has been influenced by the stimulation of anti-HPV immunity. Interestingly, the patient who showed a complete response (no. 5) also showed resolution of concurrent grade I CIN. Although spontaneous resolution of low-grade cervical disease is common (32), persistence of her clinical response to 24 months after vaccination is extremely encouraging, especially given her prolonged (>10 year) pretrial history of relapsing cervical and cervical disease. A reduction in lesion size also enabled several subjects to undergo relatively minor excisional treatment at the conclusion of the study, compared with the radical approach that might have been used to deal with their disease at enrolment.

It has earlier been reported that women with HPV-16 cervical disease may have T-cell responses to HPV-16 E7 peptides (23, 33, 34). In our patients, we found measurable immune responses to at least one HPV-16 E6 or E7 peptide pool in 6 of the analyzed women (60%) prevacination (Table 2). Immunoreactivity was found against all HPV-16 E6 and E7 peptide pools but with slightly greater frequency or strength to peptides covering the central E6 region from amino acid 41-132. Others have suggested that T-cell responses to HPV-16 E7 peptides may be reduced in women with invasive cancer (23, 35) but relatively increased in persistent and especially high-grade cervical dysplasia (35). The low level of HPV-16 E7-stimulated IFN-γ production by T cells from this cohort of patients with VIN 3 could reflect an apparent suppression of immunity in chronic HPV disease or tolerance to 16 E7. Previously reported preponderance of immunoreactivity to COOH-terminal HPV-16 E7 peptides in patients with CIN (34, 35) or central E7 peptides in high-grade CIN or cervical cancer (23) was not seen in these patients with VIN/VAIN.

A systemic immune response upon vaccination was detected by ELISPOT assays to pooled peptides in 6 of the 10 patient samples available for testing (Table 2 and Fig. 3). All these 6 subjects showed a clinical response. In 4 women (40%), there was no significant increase in the number of lymphocytes producing IFN-γ after exposure to viral peptides after vaccination compared with the prevaccination responses. Two of these 4 women had no clinical response either, but the remaining 2 patients showed a significant (>50%) reduction in marker lesion diameter. There was no strong correlation between the clinical change and the observed in vitro T-cell response. It is possible, however, that the detection of systemic immunity to HPV-16 E6 and E7 peptides after vaccination may not truly reflect the intensity of the immune response at the site of the disease. Nevertheless, the observed T-cell responses in 6 of 8 clinical responders remain remarkable.

### Table 2 TA-HPV enhanced HPV-16 E6- and E7-specific immune responses

<table>
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<th>Patient</th>
<th>Date (weeks)</th>
<th>E6 Pool 1</th>
<th>E6 Pool 2</th>
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Other studies of vaccination as therapy for HR-HPV-associated anogenital disease have been reported recently. The use of DNA (35), peptides (21–23, 36, 37), protein (38), recombinant virus (20), dendritic cells (39), and virus-like particles (40) have all been shown to induce an HPV-specific immune response. The development of a cytotoxic T-cell response probably in combination with a T-helper cell response is likely to produce the best immune attack on virally infected neoplastic cells. HLA class I-restricted HPV-16 E7 peptides most likely to produce a cytotoxic T-cell response have been defined (30, 31), but in our patients, there was no stimulation of T-cell response to the peptides after vaccination. However, the use of a restricted set of peptides to analyze CTL reactivity may not permit the detection of all CTL responses. Other studies of vaccination in humans using these selected peptides have suggested that patients with CIN, cervical cancer, and vaginal cancer are able to mount a CD8+ T-cell response to the peptides (22, 36), although in one study, the lack of a peptide-specific cytotoxic T-cell response was similar to the results presented here (37). In contrast, the responses to class II-restricted epitopes of HPV-16 (23) was stimulated after vaccination in 6 of 10 women tested. These responses have not been tested in other vaccination trials in humans.

The effect of vaccination on HPV infection and HPV-associated neoplastic disease is less clear. Only small numbers of patients with HR-HPV dysplasia or carcinoma have received vaccination as therapy, and as yet, dramatic clinical responses have not occurred. Borysiewicz et al. (20) reported the use of TA-HPV in 8 women with therapy-unresponsive cervical cancer, 1 of whose disease remitted. Although the vaccine has been used as adjuvant therapy in two small trials of women with CIN 3 before laser therapy and in women with early-stage cervical cancer before surgery, the possible effect on the disease was not evaluated (41). HPV peptides have been used as therapeutic vaccines in several small studies. In one of these, 11 women with cervical cancer and 1 woman with vaginal cancer, all of whom were of the HLA-A2 genotype, received immunotherapy with the HPV-16 E786–93 peptide (36). None of these patients showed a clinical response to vaccination, although 2 individuals demonstrated induction of specific and persistent CTL responses. Another HLA-A2-restricted peptide E712–20 has been tested as a therapeutic vaccine in 18 women with HPV-16-positive high-grade intraepithelial neoplasia of the cervix (n = 16) or vulva (n = 2; Ref. 22). Seven of these women also received the same HPV-16 E7 peptide, E786–93 used by Steller et al. (36). There was observed improvement of disease in terms of partial (6 of 18) or complete (3 of 18) clearance of affected areas with improvement only occurring in women with CIN. Ten of 16 women tested had induction of E7 immunoreactivity as assessed by IFN-γ release and chromium release assays. The combination of these two HPV-16 E7 peptides, E711–20 and HPV-E786–93, has also been tested as therapy in 19 women with late-stage HPV-16-positive cervical cancer who were HLA-A*0201-positive (21). The vaccination was well tolerated but produced no clear effect on disease course. A third type of vaccine tested in human disease has recently been reported (35). Plasmid DNA encoding the amino acids 83–95 of HPV-16 E7 was used as a vaccine in patients with AIN. Three of 19 treated...
Therapeutic HPV-16 E6/E7 Vaccination for VIN/VAIN

individuals were found to have changed from high- to low-grade AIN, whereas in the remainder, the disease did not change. Ideally, therapeutic vaccination for HPV-associated AGIN would have an efficacy that induces complete disease regression without recurrence. To produce such an effect, the immune response should be readily induced and maintained and must be able to produce a potent cytotoxic or delayed type hypersensitivity response against epithelial cells harboring the viral DNA. The development of a therapeutic vaccine to combat this virally driven disease is still some way from producing these aims. However, this trial and others suggest that stimulation of the immune response in HR-HPV-infected individuals may be able to produce at least a partial effect on neoplastic disease. Improvements in vaccine design and delivery as well as techniques to optimize immune responses within the diseased epithelium may ensure that therapeutic vaccination becomes an effective treatment choice for this group of conditions.

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REFERENCES


Vaccinia-Expressed Human Papillomavirus 16 and 18 E6 and E7 as a Therapeutic Vaccination for Vulval and Vaginal Intraepithelial Neoplasia

Peter J. Baldwin, Sjoerd H. van der Burg, Christopher M. Boswell, et al.


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