MVA E2 therapeutic vaccine for marked reduction in likelihood of recurrence of respiratory papillomatosis

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Abstract
Background: Recurrent respiratory papillomatosis (RRP) or laryngeal papillomatosis is a disease caused by papillomavirus infection.

Methods: In this phase I/II clinical trial, we evaluated the efficacy of the modified vaccinia Ankara (MVA) E2 virus in the treatment of RRP. Twenty-nine patients (18 female and 11 male) underwent injection of MVA E2 directly into the borders of the vocal cords where lesions were seen and were monitored by direct laryngoscopy. The immune response was assessed by the determination of CD3+, CD4+, and CD8+ lymphocytes counts. The presence of papillomavirus was determined by polymerase chain reaction analysis.

Results: Lesions were completely eliminated in 13 patients (44.8%). In 16 patients (55.2%), lesions recurred between 6 and 18 months after treatment; these patients received a second round of treatment with MVA E2, and they are not seen with new recurrences.

Conclusion: The MVA E2 vaccine has excellent potential for generating complete regression of RRP lesions.

KEYWORDS
human papillomavirus, MVA, papillomatosis, phase I/II clinical trial, Vaccinia virus

1 INTRODUCTION

Recurrent respiratory papillomatosis (RRP) is a benign neoplasia of the larynx. It is usually associated with the presence of human papillomavirus (HPV) types 6 or 11,1 and sometimes with HPV subtypes 16 or 18.2 Although it is generally accepted that HPV is the main causative agent in the development of RRP, other factors may also play vital roles, such as the patient's immune system, the timing and quantity of virus exposure, local traumas, and smoking.3–5 RRP is characterized by the presence of multiple papillomas in the respiratory tract. These papillomas primarily affect the vocal cords, epiglottis, trachea, and bronchi, and sometimes even the lungs. Clinically, RRP can manifest as dysphonia that progresses to aphony; dyspnea and dysphagia can also develop and become progressively worse until the affected person can no longer breathe and dies.6–8 The incidence of RRP has been reported to be 1.9 and 7.0 cases per 100 000 population, depending on the geographical area.6,9 It has been hypothesized that HPV can be transmitted vertically from mother to the neonate during passage through the birth canal.10 This hypothesis is supported by a report showing that when maternal condylomata was present during pregnancy, the risk of RRP increased by 200-fold.11 Furthermore, a recent study showed that a 12% of neonates developed HPV infections via transplacental transmission.12 However, in adults, the most likely route of transmission is oral sex, and the risk of transmission increases with increasing numbers of sexual partners.13,14 The papillomas are most commonly removed using instruments such as microscopic or endoscopic lasers, a microdebrider, or micro-forceps. However, although surgery is effective for removing the
lesions, recurrences of papilloma are still very common. The main reasons for these recurrences are incomplete removal of the lesions and the persistence of the HPV within the tissue adjacent to lesions. Recurrences are currently treated using adjuvant therapies, such as indole-3-carbinol, cidofovir, ribavirin, mumps vaccine, and photodynamic therapy. Unfortunately, recurrences often persist despite these treatments. The recombinant modified vaccinia Ankara (MVA) E2 vaccine containing the bovine papilloma virus E2 protein has been shown to eradicate all HPV as well as the HPV virus by inducing a strong immune response involving the generation of antibodies and a cytotoxic activity against HPV-infected cells. Patients treated with MVA E2 have been reported to show no recurrence of lesions during the 2 years after treatment.

The aim of this study was to evaluate the therapeutic potential of MVA E2 in patients with RRP. In this study, patients who underwent surgical elimination of papilloma lesions followed by injection of MVA E2 directly into the mucosa at the site of the lesions remained free of recurrences thereafter.

2 PATIENTS AND METHODS

2.1 Study design and subjects
All patients with RRP (n = 29) diagnosed at the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán or Instituto Nacional de Rehabilitación, in Mexico City, were enrolled in this phase I/II clinical trial and treated with the MVA E2 recombinant therapeutic vaccine. The study group included 18 female and 11 male patients. Patients were considered eligible to participate in the study if they were positive for RRP, suspected to being infected with HPV, and of any age. A complete physical examination was performed and history taken in all patients. Laboratory investigations, including hematology, blood chemistry, and urinalysis, were also performed in all cases.

2.2 Compliance with ethical standards
The study protocol was approved by the ethics and scientific committees of both participating hospitals and was conducted in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Before the administration of MVA E2, a physician reviewed all the data and confirmed that the patient was eligible to undergo the study protocol. Each patient provided written informed consent after receiving an explanation from a physician of all relevant procedures, clinical protocols, treatment plans, and expectations of compliance. If the subject was a child, informed parental consent was obtained. The attending physician maintained an accurate and complete record of each visit, including a nasofibrolaryngoscopic exploration. The principal investigator ensured the confidentiality of all patient information. The patients and the principal investigator could contact each other at any time in the event of the safety concerns. The demographics and clinical characteristics of patients with intraepithelial lesions diagnosed between 2003 and 2013 were reviewed. Twenty-nine patients were considered for inclusion in the study. Most patients who agreed to participate in the study did so because they felt that their dysphonia was very severe or because they were concerned about the possibility of developing cancer.

2.3 Eligibility criteria
Patients were deemed eligible for inclusion in the study if they had diagnosis of RRP, were confirmed clinically and histological analysis or a tissue biopsy result suggesting possible HPV infection, were not pregnant, had normal medullar and kidney reserve, and had normal blood chemistry. The study exclusion criteria were as follows: pregnancy or lactation, presence of cancer cells in the diagnostic biopsy, administration of alternative adjuvant therapies for papillomatosis, a positive human immunodeficiency virus test, presence of disease(s) other than RRP, and previous treatment with a steroid or immune modulator.

2.4 Protocol
Prior to treatment, patients were intubated with consideration given to their age, sex, weight, and height to ensure that there was adequate space for normal functioning of the larynx. Patients were ventilated with a mixture of 60% oxygen and room air, and anesthesia was maintained by intravenous agents. Saline solution (1 mL) was injected into the papilloma lesions, which were then excised using micro-forceps. Next, MVA E2 vaccine was administered. Each patient underwent 4 surgeries, each performed 2 weeks apart. Briefly, a small portion of each papilloma was removed from alternating sides of the vocal cords during these sessions to avoid compromising both sides of the vocal cords simultaneously and to prevent scarring and webbing. During each intervention, MVA E2 recombinant virus was injected directly at the site of the lesions. Each dose consisted of $10^7$ virus particles and was applied locally using a long-fabricated syringe to reach the papillomas in the glottic or subglottic areas.

2.5 Control group
The 29 patients in this study served as their own controls, because it is known that RRP is a highly unpredictable disease with a variable course, such that the progression and interval between recurrences of lesions varies widely even in patients of the same age and sex. The patients in this study developed new lesions within approximately 1-6 months after surgery and some had undergone more than 10 surgical interventions before enrollment in the study. The time course
of recurrences before and after treatment with MVA E2 was determined in each patient.

2.6 Adverse events

All possible adverse events were classified as general, musculoskeletal, gastrointestinal, urogenital, nervous system, skin, or respiratory system, in accordance with the Common Terminology Criteria for Adverse Events of the National Cancer Institute. Using these criteria, the physicians registered any adverse events that could have been related to the administration of the MVA E2 vaccine. Adverse events were considered to be of grade 1 (mild), if clinical intervention was not indicated; grade 2 (moderate), if minimal, local, or noninvasive clinical intervention was required; grade 3 (severe), if symptoms were medically significant but not immediately life-threatening; and grade 4 (life-threatening), if life was compromised and urgent medical intervention was indicated.23

2.7 Virus preparation

The MVA E2 therapeutic vaccine was prepared as previously described.22 Briefly, chicken embryo fibroblasts cells isolated from 11-day-old fertilized eggs were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% horse serum (HBS) (Gibco BRL; Gaithersburg, Maryland), glutamine 20 μM, penicillin 50 units/mL, and streptomycin 50 μg/mL in an atmosphere containing humidified air and 5% CO2 at 37°C. The chicken embryo fibroblasts were attached immediately and grown on microcarriers-cytodex (Amersham, Wisconsin, USA) in a 15-L Bioreactor brand Celligen plus (New Brunswick, New Jersey, USA) for 2 days. The cells were infected with MVA E2 virus and incubated for 48 hours. Microcarriers containing the virus were collected by gravity, and the virus was purified from this cell lysate containing the virus were collected by gravity, and the virus were dissolved on a PBS buffer pH (7.4). The MVA E2 and E2 protein were then diluted in a 0.1 mol/L carbonate buffer (pH 9.5) and 100 μL were added to wells of microtiter plates and incubated overnight at 4°C. Sera dilutions from patients were collected at the beginning (week 0) and at the end of the treatment (week 8) and added to plates and incubated overnight. Plates were rinsed with PBS and then incubated with a Horseradish peroxidase-conjugate Protein A for an hour at room temperature. After washing 5 times, reaction was started by adding the substrate o-phenylene diamine (Sigma Aldrich, St Louis MO, USA). Absorbance was read at 405 nm on a microtiter plate reader.

2.10 Enzyme-linked immunosorbent assay

An optimized enzyme-linked immunosorbent assay was performed to detect the humoral immune response to MVA E2 and E2 protein.22 Briefly, MVA E2 and E2 protein peptide were dissolved on a PBS buffer pH (7.4). The MVA E2 and E2 protein were then diluted in a 0.1 mol/L carbonate buffer (pH 9.5) and 100 μL were added to wells of microtiter plates and incubated overnight at 4°C. Sera dilutions from patients were collected at the beginning (week 0) and at the end of the treatment (week 8) and added to plates and incubated overnight. Plates were rinsed with PBS and then incubated with a Horseradish peroxidase-conjugate Protein A for an hour at room temperature. After washing 5 times, reaction was started by adding the substrate o-phenylene diamine (Sigma Aldrich, St Louis MO, USA). Absorbance was read at 405 nm on a microtiter plate reader.

2.11 Detection of HPV DNA

The presence of HPV DNA was determined by polymerase chain reaction (PCR) analysis. Tissue samples from papilloma lesions were analyzed by PCR, first with PCR MY09/11 standard primers and then with specific primers for each different type of HPV. PCR was performed in 2.5 mM MgCl2, 250 μM of each deoxynucleotide, 0.5 μM of sense and anti-sense primers, 5 μL template, and 1 unit of Taq DNA polymerase (PE Applied Biosystems, Roche, Foster City, California) with 40 cycles of denaturation at 94°C for 1 minute, annealing at 56°C for 1 min and extension at 72°C for 1 min. The amplified DNA fragments were identified using electrophoresis in 1.5% agarose gels stained with ethidium bromide. The PCR-amplified DNA fragments were also purified and sequenced by using the BigDye Terminator v3.2 Cycle Sequencing Kit (PE Applied Biosystems). The sequences were compared with those reported sequences in the GenBank server (www.ncbi.nlm.nih.gov/blast).

2.12 Lymphocyte counts

The CD3+, CD4+, and CD8+ lymphocytes counts were determined in each patient before and after MVA E2 treatment by flow cytometry. Peripheral blood was collected in
tubes containing EDTA before and 3 months after MVA E2 treatment. Mature T-lymphocytes (CD3⁺) and subsets (CD4⁺ and CD8⁺) were identified in the whole blood, via specific antibodies. T-lymphocyte counts were determined using a FACSCount system (Becton-Dickinson, Mexico City, Mexico) in accordance with the manufacturer’s instructions. The results are reported as the absolute count (total number of cells per milliliter of blood).

2.13 | Statistical analysis
The efficacy of treatment was analyzed by comparing the recurrence rates in each patient after administration of conventional treatment or adjuvant MVA E2 recombinant vaccine in the 2-5 years after treatment. A recurrence index (designated “X”, ie, the number of patients with recurrences during an 18-month period) was determined before and after treatment with the MVA E2 vaccine. The Wilcoxon signed-rank test was used to evaluate the difference in the recurrence index between patients treated and not treated with MVA E2. Differences were considered statistically significant if the P-value was ≤.05.

3 | RESULTS

3.1 | Demographic and clinical characteristics of patients with RRP
All patients enrolled in the study were Latin American mestizos (Hispanic), ranged in age from 18 months to 65 years, and had undergone from 1 to 16 surgeries to remove papillomas (Table 1). The average rate of recurrence of lesions in patients before treatment with MVA E2 varied widely from 1 to 8 months (Table 1). Approximately 27.6% of the patients experienced recurrences in the 3 months after the surgery and most (62%) experienced recurrences at 4-6 months after surgery (Table 1). Despite successful removal of their papilloma lesions, all patients developed new lesions in less than 6 months.

We assessed the presence of HPV DNA before treatment in all patients by PCR analysis. Four patients were positive for HPV type 11, 2 were positive for HPV 6, and 1 was positive for HPV 16 (Table 1). However, definitive detection was difficult in the other patients.

3.2 | MVA E2 recombinant virus decreased recurrences of respiratory papillomatosis
Four weeks after completion of treatment, all patients were free of lesions as determined by clinical and histological examinations. (Table 2). Thirteen (44.8%) of the 29 patients were free of lesions with no recurrences after treatment with MVA E2 (Table 2, Figure 1). However, 11 patients (37.9%) experienced recurrences of papillomas in the first year after treatment, 4 experienced recurrences between 12 and 18 months after the treatment, and 1 experienced an initial recurrence 37 months after the treatment (Table 2). Noting that the lesions recurred within a short time in 16 patients, we performed a second series of 3 injections of MVA E2 in these patients after the papillomas were diagnosed. After this second round of MVA E2 injections, the time to recurrence in these patients was increased by a few months up to 3-5 years when compared with that after the first application of MVA E2 (Table 3).

Most of the treated patients were followed up after the intervention. Thirteen patients (44.8%) had no recurrences during an average follow-up duration of 5 years (Table 2), and the 16 patients who received a second treatment with MVA E2 have had no recurrences up to the present time (Table 3).

To investigate the efficacy of the treatment with MVA E2 further, the recurrence index (X) was calculated for the
13 patients who received 1 round of MVA E2 injections and did not experience a recurrence for 3-5 years (Table 2) and for the 16 patients who receive a second round of MVA E2 injections after experiencing a recurrence. In the first group, X = 13 patients/18 months (0.72 patients/month) before treatment with MVA E2; in the same group, X = 0 patients/month after 1 round of MVA E2 injections (Table 2). In the second group, X = 16 patients/18 months (0.88 patients/month) before treatment with MVA E2; in the same group, X = 15 patients/18 months (0.83 patients/month) after 1 series of MVA E2 injections (Table 2), suggesting that a single round of recombinant vaccine injections was ineffective. However, after receiving a second round of MVA E2 injections, none of the patients in that group have experienced any lesions in the subsequent months or years (X = 0) (Table 3). The Wilcoxon signed-rank test was used to analyze the data from all 29 patients before and after the first MVA E2 treatment. Our null hypothesis was that there would be no difference in the recurrence index before and after treatment with MVA E2. With a level of significance of $\alpha = 0.005$, the critical $z$ value from the statistical Wilcoxon tables is 2.576, which differs significantly from the $z$ value of 8.7416 derived from our data. Our results indicate that adjuvant treatment with MVA E2 efficiently stimulated regression of intraepithelial lesions and eliminated recurrences. Furthermore, treatment with MVA E2 is capable of maintaining patients free of lesions.

3.3 | Humoral immune responses to HPV E2 protein and the MVA E2 therapeutic vaccine

Specific antibodies against the MVA E2 virus were detected in all treated patients. Serum titers increased during treatment with MVA E2 and were between 1/500 and 1/1000 dilutions. Antibodies against the BPV E2 protein were also detected in all treated patients and serum titers ranged from 1/128 to 1/256. In contrast, no antibodies were detected in untreated patients. Similar results were obtained in our previous phase I and II trials.18–20

3.4 | CD3+, CD4+, and CD8+ cells counts

Three months after treatment with MVA E2, increases in particular cell populations were detected in some patients while decreases were detected in other patients (Figure 2). Increases in the CD8+, CD4+, and CD3+ cells counts were observed in 3 patients; in 1 patient, there were increases in CD3+ and CD8+ cells but a decrease in CD4+ cells. In another patient, there was an increase in CD4+ cells but decreases in CD3+ and CD8+ cells. There were neither significant correlations between regression of lesions and lymphocyte subset counts nor any significant associations between lymphocytes subset counts and the number of

### TABLE 2 Clinical characteristics of 29 patients with recurrent respiratory papillomatosis, treated with MVA E2

| Patient number | Sex | Average onset of recurrence (months)a | Age at the start of MVA E2 treatment years | Rate of recurrence (months)b | Rate of recurrence (months)c | No recurrence (months) |
|---------------|-----|---------------------------------------|---------------------------------------------|-----------------------------|-----------------------------|-----------------------|
| 1             | Female | 2 | 5 | 3 | ... | ... |
| 2             | Female | 4 | 14 | 5 | ... | ... |
| 3             | Female | 5 | 28 | ... | ... | 62 |
| 4             | Female | 4 | 20 | 7 | ... | ... |
| 5             | Female | 5 | 19 | ... | ... | 42 |
| 6             | Female | 4 | 20 | ... | ... | 96 |
| 7             | Female | 1 | 20 | ... | 14 | ... |
| 8             | Female | 4 | 24 | 6 | ... | ... |
| 9             | Female | 3 | 30 | ... | ... | 41 |
| 10            | Female | 4 | 43 | ... | ... | 72 |
| 11            | Female | 5 | 58 | ... | 13 | ... |
| 12            | Male | 6 | 3 | ... | ... | 66 |
| 13            | Male | 5 | 10 | 6 | ... | ... |
| 14            | Male | 8 | 32 | ... | 18 | ... |
| 15            | Male | 5 | 28 | ... | ... | 67 |
| 16            | Male | 4 | 32 | 5 | ... | ... |
| 17            | Male | 2 | 35 | 2 | ... | ... |
| 18            | Male | 1 | 39 | ... | 37 | ... |
| 19            | Male | 4 | 48 | ... | ... | 14 |
| 20            | Male | 4 | 42 | ... | ... | 14 |
| 21            | Male | 3 | 44 | 12 | ... | ... |
| 22            | Male | 6 | 46 | 12 | ... | ... |
| 23            | Male | 2 | 48 | ... | ... | 48 |
| 24            | Male | 1 | 51 | 2 | ... | ... |
| 25            | Male | 3 | 52 | ... | 18 | ... |
| 26            | Male | 6 | 62 | ... | ... | 14 |
| 27            | Male | 6 | 62 | ... | ... | 12 |
| 28            | Male | 4 | 73 | ... | ... | 10 |
| 29            | Male | 5 | 66 | 3 | ... | ... |

Abbreviation: MVA, modified vaccinia Ankara.

a Before MVA E2 treatment.

b Within 1 year after MVA E2 treatment.

c Within 12 and 18 months after MVA E2 treatment.

**FIGURE 1** Laryngoscopy of papilloma lesions from patients treated with modified vaccinia Ankara (MVA) E2 therapeutic vaccine. Photographs of the larynx in representative patients (7 and 10). The patients were free of lesions after MVA E2 treatment.
MVA E2 treatment applications. In a previous study that included both tumor-bearing animals and patients with papilloma lesions, we found that a therapeutic vaccination with MVA E2 was able to induce the generation of cytotoxic cells specifically directed against tumor cells. However, in this study, it was not possible to conduct an HPV-specific cytotoxicity assay, because of the limited number of the biopsy samples taken from the patients.

3.5 | MVA E2 recombinant virus therapy did not induce adverse events

All possible adverse events were classified as body in general, musculoskeletal, gastrointestinal, urogenital, nervous system, skin, and respiratory system, in accordance with the Common Terminology Criteria for Adverse Events of the National Cancer Institute. No adverse events were detected by the physicians in this study.

4 | DISCUSSION

RRP is a virus-induced disease affecting the upper aerodigestive tract. Although papillomas can be found anywhere in the body, the larynx is the most common site. At present, there is no satisfactory treatment for RRP and most patients are seen with recurrences within a short period of time. Clearly, new therapeutic strategies are urgently required to control RRP in HPV-infected patients. We have previously demonstrated that MVA E2 recombinant virus is capable of eliminating cervical Intraepithelial lesions grade 1, 2 and 3 in a therapeutic vaccination protocol. In this study, we found that MVA E2 vaccine was able to eliminate RRP in patients infected with HPV. This is the first report to demonstrate that MVA E2 can eliminate HPV lesions in patients with RRP. Furthermore, no recurrences were observed in the

| TABLE 3 | Clinical characteristics of 16 patients with recurrent respiratory papillomatosis, treated twice with the MVA E2 |
| Patient number | Sex | Rate of recurrence (months)<sup>a</sup> | No recurrence (months)<sup>b</sup> |
|---|---|---|---|
| 1 | Female | 3 | 26 |
| 2 | Female | 5 | 9 |
| 4 | Female | 7 | 17 |
| 7 | Female | 14 | 36 |
| 8 | Female | 6 | 6 |
| 11 | Female | 13 | 40 |
| 13 | Male | 6 | 11 |
| 14 | Male | 18 | 14 |
| 16 | Male | 5 | 16 |
| 17 | Male | 2 | 9 |
| 18 | Male | 37 | 3 |
| 21 | Male | 12 | 60 |
| 22 | Male | 12 | 14 |
| 24 | Male | 2 | 8 |
| 25 | Male | 18 | 36 |
| 29 | Male | 3 | 7 |

Abbreviation: MVA, modified vaccinia Ankara.

* Recurrence after first MVA E2 treatment.

* No recurrence after second MVA E2 treatment.

FIGURE 2 | CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> lymphocyte counts in patients before and after modified vaccinia Ankara E2 treatment. Patients 6, 7, 11, 25, and 27 are representative [Color figure can be viewed at wileyonlinelibrary.com]
3-8 years after treatment. However, these patients are being followed up to assess the ability of the MVA E2 vaccine to protect against recurrence in the longer term. The present data strongly suggest that adjuvant treatment with MVA E2 stimulates regression of lesions and is capable of maintaining patients free of lesions and preventing recurrences for several years in most patients (Tables 2 and 3; Figure 1). However, the effectiveness of MVA E2 for prevention of recurrences was variable. In some patients, one series of 4 injections of MVA E2 was sufficient to completely eliminate the recurrence, whereas other patients required a second round of 3 MVA E2 injections because they experienced recurrences within 18 months of the first round of injections. Moreover, the duration of follow-up in this study was variable because not all patients were enrolled at the same time. At present, all the patients who participated in this study remain free of recurrences. Our results suggest that the immune response in the individual could be important for the elimination and prevention of recurrences. All patients treated with MVA E2 in our study developed antibodies against the MVA E2 virus and the BPV E2 protein. The presence of antibodies against MVA E2 suggests that the immune system recognized HPV-transformed cells. There was no significant difference in antibody titers between patients who received 4 doses of MVA E2 vaccine and those who received 7 doses, suggesting that a cellular immune response was probably responsible for eliminating the lesions and decreasing the likelihood of recurrence. We did not detect any significant differences in the CD3+, CD4+, and CD8+ lymphocyte counts in our patients before and after treatment. Concordant with these observations, previous studies using the bacillus Calmette-Guerin vaccine as an immunomodulator have shown no differences in the absolute counts or percentages of T-cells, B-cells, or natural killer cells in patients with RRP before and after treatment. Moreover, in a clinical trial using a linoleic acid conjugate to treat laryngeal papillomatosis, there was no significant change in the CD3+, or CD4+, cell count before and after treatment. It was clear that a cellular immune response was probably responsible for the elimination or reduction of lesions in most patients. Notably, in previously reported clinical studies, MVA E2 was shown to induce strong cytotoxic activity against HPV-transformed cells, and the mechanisms of the elimination of the lesions induced by MVA E2 were comprehensively described. In addition, several studies have shown that T-cell responses can be generated by several vaccines designed to treat HPV in patients with anogenital HPV infection. Studies using cidofovir (an analogue of cytosine) have reported a reduction in the recurrence of RRP lesions. Although the mechanism of action is not well understood, cidofovir is known to induce apoptosis and stimulate the immune system against virus-infected cells. In addition, interferon-α has also been used as an immune-based therapy in patients with RRP, but recurrences occurred within a short time. An interesting question is whether development of immunity to the vector may interfere with the efficacy to the vaccine in patients who undergo a second round of MVA E2 injections. We know from this study and previous reports that patients vaccinated with MVA E2 generated antibodies against the MVA vector. In our study, a second round of MVA E2 injections prevented recurrence very efficiently, suggesting that the presence of antibodies against the vector did not compromise the effectiveness of subsequent MVA E2 injections. Furthermore, it has been demonstrated that high levels of expression of the antigens encoded by MVA are produced even in the presence of immunity against the vector.

Of note, in this study, the 29 patients who received MVA E2 served as their own controls. The reason for this is that RRP has a highly unpredictable course, and progression of the disease varies widely from patient to patient. Therefore, a meaningful comparison between 2 parallel groups of patients, that is, one treated with MVA E2 and the other treated with surgery alone, would be unrealistic.

PCR was used to identify HPV DNA in the patients with RRP and detected low-risk HPV 6 and HPV 11 in 7 cases. This finding is concordant with previous reports of low-risk HPV 6 and 11 being the types of HPV most frequently detected in the larynx. Notably, HPV DNA was not detected in the other patients, probably because of the low levels of viral DNA present and the small amount of tissue collected at the time of each surgical procedure. Because all patients were free of lesions after the initial treatment with MVA E2, biopsy samples were not taken for PCR analysis after the first round of injections. The elimination of lesions and the reduction in recurrences were likely attributable to the elimination of HPV and the generation of a strong immune responses against HPV-transformed cells. These findings are in line with our earlier work demonstrating that the MVA E2 therapeutic vaccine can eliminate papillomavirus and generated a strong immune responses against HPV-transformed cells in patients with intraepithelial lesions. Conventional surgical treatments can efficiently eliminate lesions, but the removal of the damaged tissue does not necessarily guarantee the elimination of viral DNA. HPV-infected mucosal cells are likely to remain but be clinically undetectable. It is clear that recurrences occur in patients with RRP because of the persistence of HPV DNA. A deficient host-immune response against HPV-
infected cells is another factor that can affect the elimination of these lesions.

Novel strategies for the prevention of HPV infections have recently been reported, including the tetravalent vaccine Gardasil (Merck) which is designed to protect against HPV 6, 11, 16, and 18 infections. In a recent clinical trial, there was a decrease in recurrences of RRP in patients vaccinated with Gardasil, but recurrences did occur within in a short time. In this study, the MVA E2 vaccine proved to be a relatively safe treatment, in that none of the patients experienced any serious adverse events. This finding is consistent with previous reports of no serious adverse events when MVA E2 was used to treat HPV lesions. As already established in many clinical trials, administration of the MVA virus is vector of choice for novel HPV therapeutic vaccines.

No adverse events have been reported in any of the patients included in the clinical trials using MVA E2 therapeutic vaccine in the past 15 years. Collectively, the evidence suggests that the MVA E2 therapeutic vaccine is a promising adjuvant treatment for RRP.

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CONFLICT OF INTEREST
The authors declare that they have no conflicts of interest with the contents of this article.

REFERENCES
1. Gissmann L, Diehl V, Schultz-Coulon HJ, zur Hausen H. Molecular cloning and characterization of human papilloma virus DNA derived from a laryngeal papilloma. J Virol. 1982;44:393-400.
2. Donne AJ, Hampson L, Homer JJ, Hampson IN. The role of HPV type in recurrent respiratory papillomatosis. Int J Pediatr Otorhinolaryngol. 2010;74:7-14.
3. Larson DA, Derkay CS. Epidemiology of recurrent respiratory papillomatosis. APMA. 2010;118:450-454.
4. Rosenthal DW, DeVoti JA, Steinberg BM, Abramson AL, Bonagura VR. T (H2-like chemokine patterns correlate with disease severity in patients with recurrent respiratory papillomatosis. Mol Med. 2012;18:1338-1345.
5. Shah KV, Stern WF, Shah FK, Bishai D, Kashima HK. Risk factors for juvenile onset recurrent respiratory papillomatosis. Pediatr Infect Dis J. 1998;17:372-376.
6. Derkay CS. Task force on recurrent respiratory papillomas. A preliminary report. Arch Otolaryngol Head Neck Surg. 1995;121:1386-1391.
7. Reeves WC, Ruparelia SS, Swanson KL, Derkay CS, Marcus A, Unger ER. National registry for juvenile-onset recurrent respiratory papillomatosis. Arch Otolaryngol Head Neck Surg. 2003;129:976-982.
8. Sittel C. Pathologies of the larynx and trachea in childhood. Laryngorhinootologie. 2014;93(suppl 1):S70-S83.
9. Derkay CS, Wiatrak B. Recurrent respiratory papillomatosis: a review. Laryngoscope. 2008;118:1236-1247.
10. Venkatesan NN, Pine HS, Underbrink MP. Recurrent respiratory papillomatosis. Otolaryngol Clin North Am. 2012;45:671-694. viii–ix.
11. Silverberg MJ, Thorsen P, Lindeberg H, Grant LA, Shah KV. Condyloma in pregnancy is strongly predictive of juvenile-onset recurrent respiratory papillomatosis. Obstet Gynecol. 2003;101:645-652.
12. Rombaldi RL, Serafini EP, Mandelli J, Zimmermann E, Losquiao KP. Transplacental transmission of human papillomavirus. Virol J. 2008;5:106.
13. Heck JE, Berthiller J, Vaccarella S, et al. Sexual behaviours and the risk of head and neck cancers: a pooled analysis in the international head and neck cancer epidemiology (INHANCE) consortium. Int J Epidemiol. 2010;39:166-181.
14. Smith EM, Ritchie JM, Summersgill KF, et al. Age, sexual behavior and human papillomavirus infection in oral cavity and oropharyngeal cancers. Int J Cancer. 2004;108:766-772.
15. Goon P, Sonnex C, Janni P, Stanley M, Sudhoff H. Recurrent respiratory papillomatosis: an overview of current thinking and treatment. Eur Arch Otorhinolaryngol. 2008;265:147-151.
16. Piguetari S, Smith EM, Gray SD, Shive C, Turek LP. Detection of human papillomavirus infection in diseased and nondiseased sites of the respiratory tract in recurrent respiratory papillomas patients by DNA hybridization. Ann Otol Rhinol Laryngol. 1992;101:408-412.
17. Spiegel JH, Andrus JG, Stefanato CM, Heeren T. Histopathologic effects of cidofovir on cartilage. Otolaryngol Head Neck Surg. 2005;133:666-671.
18. Jumaran YCA, de la Garza A, Cruz Quiroz BJ, et al. MVA E2 recombinant vaccine in the treatment of human papillomavirus infection in men presenting intrarectal flat condyloma: a phase III study. BioDrugs. 2007;21:47-59.
19. Corona Gutierrez CM, Tinoco A, Navarro T, et al. Therapeutic vaccination with MVA E2 can eliminate precancerous lesions (CIN 1, CIN 2, and CIN 3) associated with infection by oncogenic human papillomavirus. Hum Gene Ther. 2004;15:421-431.
20. Garcia-Hernandez E, Gonzalez-Sanchez JL, Andrade-Manzano A, et al. Regression of papilloma high-grade lesions (CIN 2 and CIN 3) is stimulated by therapeutic vaccination with MVA E2 recombinant vaccine. Cancer Gene Ther. 2006;13:592-597.
21. Rosales C, Graham VV, Rosas GA, et al. A recombinant vaccinia virus containing the papilloma E2 protein promotes tumor regression by stimulating macrophage antibody-dependent cytotoxicity. Cancer Immunol Immunother. 2000;49:347-360.
22. Rosales R, Lopez-Contreras M, Rosales C, et al. Regression of human papillomavirus intraepithelial lesions is induced by MVA E2 therapeutic vaccine. Hum Gene Ther. 2014;25:1035-1049.
23. National Cancer Institute. Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. http://evs.nci.nih.gov/CTCAE. Accessed June 14, 2010 QuickReference SX7pdfAccessed:2011 2010.
24. Gibbons JD, Chakraborti S. Nonparametric Statistical Inference. Alabama: Taylor and Francis Group; 5th ed. 1999.
25. Tasca RA, Clarke RW. Recurrent respiratory papillomatosis. Arch Dis Child. 2006;91:689-691.
26. Louw E. Effects of conjugated linoleic acid and high oleic acid safflower oil in the treatment of children with HPV-induced laryngeal papillomatosis: a randomized, double-blinded and crossover preliminary study. Lipids Health Dis. 2012;11:16.
27. Lescaille G, Pitoiset F, Macedo R, et al. Efficacy of DNA vaccines forming e7 recombinant retroviral virus-like particles for the treatment of human papillomavirus-induced cancers. Hum Gene Ther. 2013;24:533-544.
28. van der Burg SH, Ressing ME, Kwappenberg KM, et al. Natural T-helper immunity against human papillomavirus type 16 (HPV16) E7-derived peptide epitopes in patients with HPV16-positive cervical lesions: identification of 3 human leukocyte antigen class II-restricted epitopes. Int J Cancer. 2013;130:612-618.
29. Mandell DL, Arjmand EM, Kay DJ, Casselbrant ML, Rosen CA. Intratissular cidofovir for pediatric recurrent respiratory papillomatosis. Arch Otolaryng Head Neck Surg. 2004;130:1319-1323.
30. Shehab N, Sweet BV, Hogikyan ND. Cidofovir for the treatment of recurrent respiratory papillomatosis: a review of the literature. Pharmacotherapy. 2005;25:977-989.
31. Greene V, Schmandt S, Babkina N, Barysik N, Coerdt W. Human papilloma virus (HPV)-associated gynecological alteration in mothers of children with recurrent respiratory papillomatosis during long-term observation. Cancer Detect Prev. 2007;31:276-281.
32. Ramírez JC, Gherardi MM, Rodríguez D, Esteban M. Attenuated modified vaccinia virus Ankara can be used as an immunizing agent under conditions of preexisting immunity to the vector. J Virol. 2000;74:7651-7655.
33. Major T, Szarka K, Sziklai I, Gergely L, Czegledy J. The characteristics of human papillomavirus DNA in head and neck cancers and papillomas. *J Clin Pathol.* 2005;58:51-55.

34. Zhou C, Sun B, Wang F, et al. Coblation plus photodynamic therapy (PDT) for the treatment of juvenile onset laryngeal papillomatosis: case reports. *World J Surg Oncol.* 2014;12:275.

35. Young DL, Moore MM, Halstead LA. The use of the quadrivalent human papillomavirus vaccine (gardasil) as adjuvant therapy in the treatment of recurrent respiratory papilloma. *J Voice.* 2015;29:223-229.

36. Cavenaugh JS, Awi D, Mendy M, Hill AV, Whittle H, McConkey SJ. Partially randomized, non-blinded trial of DNA and MVA therapeutic vaccines based on hepatitis B virus surface protein for chronic HBV infection. *PLoS One.* 2011;6:e14626.

37. Cosma A, Nagaraj R, Buhler S, et al. Therapeutic vaccination with MVA-HIV-1 nef elicits Nef-specific T-helper cell responses in chronically HIV-1 infected individuals. *Vaccine.* 2003;22:21-29.

38. Jaoko W, Nakwagala FN, Anzala O, et al. Safety and immunogenicity of recombinant low-dosage HIV-1 a vaccine candidates vectored by plasmid pTHr DNA or modified vaccinia virus Ankara (MVA) in humans in East Africa. *Vaccine.* 2008;26:2788-2795.

39. Rosales R, Rosales C. Immune therapy for human papillomaviruses-related cancers. *World J Clin Oncol.* 2014;5:1002-1019.

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