Intralesional Injection of Mumps or Candida Skin Test Antigens

A Novel Immunotherapy for Warts

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**Background:** Warts are common and induce physical and emotional discomfort. Numerous therapies exist, yet none is optimal. Despite theoretical advantages, immunotherapeutic modalities are often neglected as first-line wart therapies.

**Objective:** To compare treatment with intralesional skin test antigen injection of 1 wart vs cryotherapy of all warts.

**Design:** Pilot study.

**Setting:** University dermatology outpatient clinic.

**Patients:** A total of 115 consecutive patients with at least 1 nongenital wart.

**Interventions:** Patients with warts were tested for immunity to mumps and Candida using commercial antigens. Nonresponders received cryotherapy and immune individuals received cryotherapy or intralesional injection of 1 antiserum.

**Results:** Thirty-four (30%) of the 115 patients did not respond to the test injections and 81 (70%) had detectable immunity. Of the immune group, 26 (32%) received cryotherapy, 45 (56%) received intralesional mumps antiserum, and 10 (12%) received intralesional Candida antiserum. Of the anergic patients, 28 (82%) were treated with cryotherapy; 6 (18%) refused cryotherapy. Of the 39 patients who were treated with immunotherapy and completed the protocol, 29 (74%) had complete clearing of the treated wart. Fourteen (78%) of 18 patients with complete resolution of their immunotherapy-treated wart also had resolution of untreated, distant warts.

**Conclusions:** Intralesional injection of mumps or Candida antigens into warts of immune individuals represents effective treatment. Observation of clearing of anatomically distinct and distant warts suggests acquisition of human papillomavirus–directed immunity in some patients. We conclude that this novel approach to immunotherapy may serve as first-line treatment in immune individuals with multiple or large warts and as second-line treatment in immune patients for whom cryotherapy fails.

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HE HUMAN papillomavirus (HPV) causes the common wart or verruca vulgaris. Other cutaneous diseases attributable to HPV include flat warts (verruca plana), genital warts (condyloma acuminate), epidermodysplasia verruciformis, and cutaneous squamous cell carcinoma in immunocompromised patients. A pathogenic role for HPV at noncutaneous sites exists for cervical dysplasia, cervical carcinoma, and laryngeal papilloma. Estimates of the prevalence of HPV infection in the population range as high as 79%, encompassing those with evident clinical disease and those carrying the virus subclinically.1

Warts may arise on any skin surface, but occur most commonly at acral sites. Patients actively seek therapy because of pain and embarrassment. Subclinical infection persists after eradication of the wart itself, yet treatment of evident disease is thought to diminish spread of HPV in the skin of the infected patient and possibly to uninfected contacts.2

Treatments for warts are legion. Cryotherapy remains the first-line treatment despite the use of alternative agents such as keratolytics or cantharidin, laser ablation, surgical excision, and immunologic manipulation. Immunotherapy possesses theoretical advantage in that the immune system modulates proliferation of HPV.3,4 Clear evidence of this fact can be found in (1) the uncontrolled proliferation of warts, both common and genital, in human immunodeficiency virus–infected patients with high viral loads and...
PATIENTS AND METHODS

The study protocol received approval from the institutional review boards of the University of Arkansas for Medical Sciences, the Central Arkansas Veterans Health Care Systems, and the Arkansas Children's Hospital, Little Rock. Figure 1 provides a schematic overview of the study design.

Patients with the clinical diagnosis of 1 or multiple warts provided informed consent and received an intradermal injection of mumps (Connaught, Swiftwater, Pa) and Candida (Bayer, Spokane, Wash) test antigen preparations (0.1 mL each) in the left and right forearms, respectively. The presence or absence and size of the ensuing reactions were noted after 48 to 72 hours. Determination of a positive reaction necessitated erythema and induration of at least 5 mm in diameter. Nonresponders received cryotherapy by standard technique. Responders were randomized by sequential number of entry into the protocol to receive cryotherapy or immunotherapy with the test antigen that induced the greater response, or they received immunotherapy if expressly referred for this technique. Thus, for the purposes of analysis, 2 groups were formed, randomized and nonrandomized. Cryotherapy was administered by liquid nitrogen application such that a 1- to 2-mm zone of frozen tissue was created and maintained around lesional skin for a period of about 30 seconds. The area was allowed to thaw and then treated similarly a second time. Immunotherapy consisted of intraleisional injection of mumps or Candida antiserum in a volume determined by the size of the test reaction as follows: 0.3 mL injected with test site induration of 5 to 20 mm, 0.2 mL injected with test site induration of 21 to 40 mm, and 0.1 mL with test site induration of greater than 40 mm. Only the largest wart, based on surface area, was treated in patients with multiple warts. Both cryotherapy and immunotherapy were repeated every 3 weeks until complete clearing of the treated wart was achieved or for a maximum of 3 treatments. Immunotherapy nonresponders subsequently received cryotherapy as described above if no clinical response was observed after 3 treatments.

Patients were examined at study initiation and at each episode of treatment with notation as to the number and surface area of warts. At the follow-up visit, presence or absence of response to treatment and approximate decrease in size of warts in responders were recorded. Complete resolution was judged to have occurred when the thickening, hyperkeratosis, and dilated vasculature of the treated wart were no longer evident. No response was judged to have occurred if there was less than 25% decrease in surface area of the treated wart. Partial responses were estimated as follows: 25% to 50%, 51% to 75%, and greater than 75% but less than 100%.

Exclusion criteria consisted of prior allergic response to mumps or Candida antiserum, pregnancy, infection with human immunodeficiency virus type 1, iatrogenic immunosuppression, primary immunosuppression, or any generalized dermatitis.

RESULTS

PATIENT CHARACTERISTICS

A total of 115 patients (49 women, 66 men) were treated in the protocol (Table 1). The age range was 5 to 72

Figure 1. Protocol flowchart. DTH indicates delayed-type hypersensitivity; VV, verruca vulgaris.

low T-lymphocyte cell counts, (2) the profusion of warts and squamous cell carcinomas in solid organ transplant recipients, and (3) the innumerable flat warts found in patients with epidermodysplasia verruciformis.5

Traditionally, immunotherapy has relied on application of inorganic chemicals in a sensitization phase followed, when successful, by application of the sensitizer to the surface of the wart. Dinitrochlorobenzene, a popular substance for such use, was found to be mutagenic in the Ames assay and thus is rarely used.4 Diphencyprone and squaric acid are other chemicals sometimes used in the treatment of warts today. More recently, imiquimod, an agent capable of cytokine up-regulation, has gained use for external anogenital warts. Of 109 patients treated with 5% imiquimod cream, 50% experienced clinical resolution. Of these responders, only 13% experienced a recurrence. The utility of imiquimod for thick hyperkeratotic verruca vulgaris is unclear.6

We have developed a novel approach to immunotherapy, taking advantage of the high prevalence of immunity to mumps and Candida in the general population. Our hypotheses are (1) injection of skin test antigens into the warts of immune individuals will result in clearance of the treated wart, (2) injection of skin test antigens into 1 wart in immune individuals will result in systemic HPV-directed immunity that will provide for clearing of untreated warts.

Briefly, immune individuals receive a titrated volume of mumps or Candida antigen, routinely used to detect immunity to these organisms, injected directly into a wart. Herein, we report our experience in 115 patients with common warts who received either cryotherapy or immunotherapy utilizing intraleional injection of mumps or Candida antigens.
years (average age, 32.2 years). The average age for cryotherapy-treated patients was 34.8 years, 31.3 years for mumps immunotherapy–treated patients, and 22.1 years for Candida immunotherapy–treated patients. All patients had periungual or palmoplantar warts except for 1 patient with flat warts.

TEST SITE RESULTS

Thirty-four patients (30%) did not have reactions to either antigen. Thirty-eight (33%) had reactions only to mumps, 7 (6%) had reactions only to Candida, and 36 (31%) had reactions to both test antigens. Of the responders, the test site diameter was 5 to 10 mm in 29 patients, 11 to 20 mm in 30, 21 to 30 mm in 12, 31 to 40 mm in 6, and greater than 40 mm in 4. Of the 34 nonresponders, 6 refused cryotherapy and were not treated in the protocol. These 6 patients are not included in Table 1 and are considered as discontinued from treatment in the row of skin test–negative patients treated with cryotherapy in Table 2.

Of the 81 patients with a positive skin test result, 26 received cryotherapy and 55 received immunotherapy (45 with mumps antigen and 10 with Candida antigen). Of 54 cryotherapy-treated patients, 28 were anergic and 26 were reactive to the skin test antigens.

RESPONSE TO TREATMENT IN PATIENTS COMPLETING THE PROTOCOL

Response data are shown in Table 2. A flowchart summarizing the results is given in Figure 2.

Thirty patients did not complete the protocol: 14 in the cryotherapy arm and 16 in the immunotherapy arm. Of the 4 patients who are considered as discontinued from treatment in the mumps immunotherapy group, 3 discontinued because of adverse effects as described later and 1 discontinued because she was trying to become pregnant.

RESPONSE OF ANATOMICALLY DISTINCT UNTREATED WARTS

In the patients treated with immunotherapy who had clearing of the treated wart, 18 had more than 1 wart. Fourteen of 18 patients (78%; exact 95% confidence interval: 52%-94%) experienced clearing of all anatomically distinct warts despite receiving antigen injections into only the largest wart. All warts regressed at any anatomic location, regardless of where the treated wart was located. For instance, warts on the hands cleared when a wart on the foot was injected. Of these 14 patients, 10 received mumps immunotherapy and 4 received Candida immunotherapy. One other patient treated with mumps immunotherapy experienced resolution of distant warts without resolution of the treated wart. Complete resolution of large warts was repeatedly observed despite injection of the antigen into only a small portion of the wart. Patients in the cryotherapy arm had all of their warts treated. Therefore, a statement about distant wart response in these patients cannot be made based on the results of this study.

PARTIAL RESPONDERS IN THE IMMUNOTHERAPY ARM

Five patients who received mumps antigen injections experienced partial resolution (decrease in surface area of 50% to 75%) of warts. Two were lost to follow-up during the protocol and 3 completed the protocol with only partial resolution.

ADVERSE EFFECTS

Six patients treated with immunotherapy developed a flu-like illness less than 12 hours after the first treatment with antigen injection into a wart. The symptoms lasted no more than 24 hours and improved with the administration of nonsteroidal anti-inflammatory agents. Of these patients, 3 discontinued the protocol, 1 experienced complete resolution of the treated wart after the first injection, and 2 continued the protocol with complete clearing of the treated wart. These latter patients were pretreated with acetaminophen without additional symptoms.
Common complaints in the immunotherapy arm included the immediate pain of intralesional injection and pruritus in the injected wart during the ensuing 24 hours. There were no reports of persistent pain beyond the immediate time of injection. Common complaints in the cryotherapy arm included the immediate pain of treatment that persisted beyond the actual time of treatment. Cryotherapy-treated patients also reported local skin reactions and discomforts related to cryotherapy. Although the variable of discomfort was not assessed in our study, patients treated with immunotherapy generally stated that they preferred the injections to cryotherapy.

**FOLLOW-UP**

All patients were contacted by telephone every 4 months for up to 12 months after completion of the protocol. The average time of follow-up to date is 12 months. Three relapses occurred: 2 in the cryotherapy arm and 1 in the mumps antigen treatment arm.

**STATISTICAL CONSIDERATIONS**

Using an intent-to-treat analysis, there was no statistical difference (P = .7, Fisher exact test) between cryotherapy and immunotherapy in terms of clearing of treated warts among the 115 patients in the protocol. This analysis includes the 6 patients who refused cryotherapy as nonresponders to cryotherapy. The 2 groups were again not statistically different when considering complete and partial responses among only the randomized patients (P = .24). No statistical differences in terms of sex or age were observed between responders and nonresponders in the cryotherapy or immunotherapy treatment arms.

**COMMENT**

The HPV causes significant morbidity. The large number of therapies for the common wart and related conditions attests to the fact that treatment options are less than optimal. Immunotherapy for warts, the manipulation of the immune system to achieve an HPV-targeted immune reaction, offers a theoretical advantage in that effective control of viral proliferation should be sustained and widespread in the individual. Specific factors accounting for the variability of innate responsiveness to HPV are incompletely understood.

We document the effectiveness of intralesional injection of mumps or *Candida* antigens into common warts in patients with known reactive intradermal skin tests to these antigens. Seventy-four percent of warts treated in this manner resolved, compared with 55% of lesions treated with cryotherapy in similarly immune individuals and 58% of warts treated with cryotherapy in those nonreactive to mumps and *Candida* antigen intradermal testing. Seventy-eight percent of patients with multiple warts treated with immunotherapy experienced resolution of anatomically distinct, untreated warts. These data indicate that immunotherapy and cryotherapy are statistically comparable regimens (P = .7). The advantage of immunotherapy resides in the acquisition of HPV-directed immunity and the potential for resolution of untreated warts at anatomically distant sites. Based on these observations, we propose that this approach to immunotherapy should serve as first-line treatment for immune individuals with numerous (>5) or large (>1 cm) warts. Furthermore, this modality should serve as an effective second-line treatment in immune individuals who are judged to have failed cryotherapy.

Our study was designed to compare immunotherapy with cryotherapy to determine the potential clinical utility of the former. It is likely that injection with the diluent of the antigen preparations will result in some clinical responses. Using *Candida* antigen injection, other investigators have reported up to 25% clearing using vehicle control. The trauma and subsequent inflammatory reaction of the intralesional injection itself may result in an HPV-directed immunologic reaction leading to clinical response. Regardless of the mechanism, our data argue strongly for the induction of HPV-targeted immunity given the repeated observation of clearing of untreated lesions.

The mechanism by which lack of immune responsiveness to HPV is overcome is unclear. The high reported rates of recurrence (39% for cryotherapy) may be due to a lack of immune response. Several explanations exist for the apparent lack of immunity. First, patients with warts may lack a memory T-cell population able to target HPV. Second, patients may possess HPV-specific T cells, but these lymphocytes fail to expand clonally with appropriate stimulation. Third, patients possess sufficient circulating HPV-specific T cells, but these lymphocytes are unable to traffic to the sites of HPV infection. Fourth, HPV-specific lymphocytes are weak effectors in terms of elaboration of necessary cytokines and/or recruitment of additional effector cells. And finally, HPV may elude host recognition. In the immunotherapy arm of the present study, 1 patient experienced recurrence of the treated wart and 2 patients in the cryotherapy arm experienced recurrence with average follow-up of 12 months. Acquisition of HPV-directed immunity will, it is hoped, provide improved HPV surveillance and fewer relapses than with other forms of therapy, but follow-up to date is too limited to prove this hypothesis.

Six patients undergoing immunotherapy experienced a flulike illness within 12 hours of intraleisional antigen injection, lasting up to 24 hours. Symptoms consisted of fever, malaise, and myalgia and were controlled by administration of nonsteroidal anti-inflammatory medications. No other side effects were identified. In this group, 3 patients had subsequent resolution of the
treated wart. Possible explanations for the flulike illness include injection of some of the immunogen into the circulation with subsequent immunologic response and/or a brisk elaboration of cytokines in response to local injection with subsequent systemic effect. Intramuscular administration of various interferons is known to produce a similar constellation of symptoms.8

The 115 patients enrolled in the protocol were not entirely randomized. Soon after initiation of the study, it became apparent that immunotherapy was effective. It was especially useful in patients with large or numerous warts who were recalcitrant to other therapies. This led community dermatologists to refer difficult and persistent cases to the University of Arkansas for immunotherapy. The enrollment of these cases in the intraleisional mump/Candida injection arm of the study introduced a bias toward the treatment of recalcitrant cases with the skin test antigens instead of cryotherapy. It is possible that our response rates would be higher than reported if a less selected patient population had been enrolled.

The novel approach to immunotherapy described herein should find clinical utility as noted previously. The likely induction of HPV-directed immunity through the establishment of a delayed-type hypersensitivity reaction intraleisonally to an otherwise irrelevant immunogen suggests other applications for this technique, namely, in condyloma, laryngeal papilloma, and cervical dysplasia and carcinoma. Immunogens other than mumps and Candida antigens will likely be as, if not more, effective and the addition of other antigens to the pretreatment panel may allow more patients to receive this effective treatment for a common disorder.

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