Intralesional Immunotherapy of Warts With Mumps, Candida, and Trichophyton Skin Test Antigens

A Single-blinded, Randomized, and Controlled Trial

Background: Warts occur commonly in humans. Destructive modalities are generally the first physician-administered therapy. Other treatment options include immunotherapy. Intralesional immunotherapy using mumps, Candida, or Trichophyton skin test antigens has proved efficacy in the treatment of warts.

Objectives: To determine rates of wart resolution in response to injection of antigen alone, antigen plus interferon alfa-2b, interferon alfa-2b alone, and normal saline; and to compare response according to viral type, major histocompatibility complex antigens, and peripheral blood mononuclear cell proliferation to autologous human papillomavirus antigen before and after injection.

Design: Randomized, single-blinded, placebo-controlled, clinical trial.

Setting: Medical school–based dermatology department.

Patients: Two hundred thirty-three patients clinically diagnosed as having 1 or more warts.

Main Outcome Measure: Clinical resolution of warts in response to intralesional immunotherapy.

Results: Responders were observed in all treatment arms, but were significantly more likely to have received antigen (P<.001). Resolution of distant untreated warts was observed, and was significantly more likely in subjects receiving antigen (P<.001). Interferon did not significantly enhance the response rate (P=.20) and did not differ from normal saline (P=.65). No viral type or major histocompatibility complex antigen correlated with response or lack of response (P>.99 and P=.86, respectively). A positive peripheral blood mononuclear cell proliferation assay result (2 times pretreatment levels) was significantly more likely among responders (P=.002). While there was no significant difference in response based on sex (P=.56), older subjects (>40 years) were less likely to respond (P=.01).

Conclusions: Intralesional immunotherapy using injection of Candida, mumps, or Trichophyton skin test antigens is an effective treatment for warts, as indicated by significantly higher response rates and distant response rates in subjects receiving antigen. Viral type and major histocompatibility complex antigens did not seem to influence treatment response. Response is accompanied by proliferation of peripheral blood mononuclear cells to human papillomavirus antigens, suggesting that a human papillomavirus–directed cell-mediated immune response plays a role in wart resolution.

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WARTS ARE COMMON IN HUMANS AND ACCOUNT FOR 8% OF VISITS TO DERMATOLOGISTS.1 WHILE MANY WARTS RESOLVE SPONTANEOUSLY OVER SEVERAL YEARS, MOST PATIENTS SEEK TREATMENT, BECAUSE THE WARTS ARE UNSIGHTLY AND OFTEN TENDER OR PAINFUL. PRIMARY TREATMENT FOR VERRUCA VULGARIS INCLUDES Destructive Therapies, SUCH AS TOPICAL SALICYLIC ACID, CRYOTHERAPY WITH LIQUID NITROGEN, EXCISION, LASER VAPORIZATION, AND BLEOMYCIN SULFATE INJECTION. BECAUSE WART PROLIFERATION IS CONTROLLED BY THE IMMUNE SYSTEM,2 VARIOUS METHODS HAVE BEEN USED TO STIMULATE THE IMMUNORESPONSE TO THE HUMAN PAPILLOMAVIRUS (HPV). AMONG THESE ARE TOPICALLY APPLIED INORGANIC MOLECULES CAPABLE OF ELICITING CONTACT HYPERSENSITIVITY, IMQUIMOD, AND INTRALESIONAL INTERFERONS.

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Researchers3,4 have shown the effectiveness, in the treatment of common warts, of intralesional injection of antigen preparations of mumps, Candida, or Trichophyton, normally used to assay the status of the cellular immune system via intradermal injection. In an earlier study,3,4 74% of subjects receiving immunotherapy experienced reso-
lution of the treated wart and 78% of subjects with multiple warts experienced resolution of untreated distant warts. Of subjects treated in the cryotherapy arm of the same study, 57% experienced resolution of the treated wart, while no distant wart responses occurred. In a separate report evaluating the effectiveness of this treatment in children, in all of whom 2 treatment modalities had failed, 47% of subjects responded to immunotherapy and 34% cleared untreated distant and anatomically distinct warts.

Herein, we extend these observations on intralesional injection of skin test antigens for common warts and report on a single-blinded, randomized, and controlled trial comparing immunotherapy, immunotherapy plus intralesional interferon alfa-2b, intralesional interferon alfa-2b alone, and normal saline. Data correlating treatment success or failure in individual subjects to HPV type, major histocompatibility complex (MHC) antigens, and degree of pretreatment and posttreatment peripheral blood mononuclear cell (PBMC) proliferation in response to HPV antigens are presented.

## METHODS

The study protocol received approval from the institutional investigational review boards of the University of Arkansas for Medical Sciences, the Central Arkansas Veterans Healthcare System, Arkansas Children's Hospital, and the General Clinical Research Center of the University of Arkansas for Medical Sciences.

### PATIENTS AND IMMUNOTHERAPY PROCEDURES

Patients (or their guardians) clinically diagnosed as having 1 or multiple warts provided informed consent and were randomized after obtaining a positive intradermal pretest result to 1 of 4 groups: (1) intralesional injection with mumps (Connaught, Swifwater, Pa), Candida (Bayer, Spokane, Wash), or Trichophyton (Alk-Abello, Round Rock, Tex) skin test antigen preparation (mumps antigen is not currently commercially available); (2) intralesional injection with mumps, Candida, or Trichophyton skin test antigen preparation plus interferon alfa-2b, 1 × 10⁶ IU; (3) intralesional injection with interferon alfa-2b, 1 × 10⁶ IU; or (4) intralesional injection with saline. Mumps skin test antigen is a concentrated product derived from virus killed with formaldehyde; solution and formulated to contain a minimum of 40 colony-forming units per milliliter. Candida and Trichophyton skin test antigens represent allergenic extracts in which crude allergen is added to extracting solution (sodium chloride, sodium bicarbonate, glycerin, and phenol or thimerosal) in specific ratios of allergen to solution, and expressed as protein nitrogen units or weight per volume. We used products of 1000 protein nitrogen units per milliliter and 1.500 wt/vol, respectively. Randomization was computer generated, and the sequence was provided to the investigators in sealed envelopes. All subjects were tested for existing immunity to each antigen preparation by placing 0.1 mL intradermally into the skin of the forearm (pretest). Determination of a positive reaction necessitated induration of at least 5 mm in diameter. The test antigen that induced the greatest response was then used for intralesional injection. If equal reactions occurred, then an arbitrary assignment to Candida first, then Trichophyton, and then mumps was used. If all skin test results were nonreactive, the patient was excluded from the study.

Intralesional injection was performed using the following volumes per group: (1) 0.3 mL of antigen preparation, (2) 0.3 mL of antigen preparation plus 0.08 mL of interferon alfa-2b, (3) 0.08 mL of interferon alfa-2b, and (4) 0.3 mL of saline. The effectiveness of this amount of antigen preparation was previously established. Only the largest wart, based on surface area, was treated in patients with multiple warts. All subjects received injections every 3 weeks into the same wart until complete clearing of the treated wart was achieved or for a maximum of 5 treatments.

Patients were examined at study initiation and at each episode of treatment with notation as to the number and surface area of warts. Surface area was estimated by measurement of diameter using a ruler. At the follow-up visits, 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 and normal skin markings returned. 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%. For analytic purposes, only those subjects with greater than 75% improvement in the injected or distal- responding warts were defined as responders.

Exclusion criteria consisted of prior allergic response to any of the antigen preparations, pregnancy, lactation, and evidence of human immunodeficiency virus type 1, clinical evidence of epidermodysplasia verruciformis, iatrogenic immunosuppression, primary immunosuppression, or any generalized dermatitis.

Only subjects with multiple warts were chosen for viral typing and assay of PBMC proliferation because these procedures required biopsy of 1 wart. Fresh tissue was used for viral typing, as described later. Protein extracted from a portion of the tissue was used in the PBMC stimulation assay, as described later.

### VIRAL TYPING

Sample specimens of wart obtained from consenting patients were received in the laboratory and processed for HPV typing. A section of wart tissue, approximately 2 mm³, was placed in a sterile microtube and mixed in 200 µL of HPV digestion solution, consisting of a fresh mixture of 1 mL of 1M Tris buffer, pH 8.0; 1 mL of 1M Tris buffer, pH 9.0; 80 µL of 0.5M EDTA, pH 8.0; 200 µL of Tween 20; and 37.1 mL of sterile water and endopeptidase K in a ratio of 197.1 µL of HPV digestion solution to 3 mL of endopeptidase K. The microtubes were incubated overnight in a 65°C water bath, followed by heating at 95°C to inactivate the endopeptidase K. The microtubes were then centrifuged at 6000 rpm in a microcentrifuge (Eppendorf) for 5 minutes. The resulting digests were decanted and stored on ice or at –80°C. Viral phenotyping was then performed on the wart digests via polymerase chain reaction (PCR) using sets of PCR primers specific for the HPV genotypes. Template (watt digest or negative control), 2 µL, was added to a PCR master mix consisting of the following components: 45 µL of Supermix (Gibco BRL, Gaithersburg, Md), 2 µL of primer A, and 2 µL of primer B on ice (primers A and B represent primer pairs). The PCR cycling program was run under the following conditions: step 1, 2 minutes at 95°C for the initial melt; step 2, 1 minute at 95°C; step 3, 90 seconds at 50°C; step 4, 2 minutes at 72°C; step 5, repeat steps 2 through 4 a total of 29 times; step 6, 5 minutes at 72°C, and step 7, store at 4°C. The PCR products were then run on a 1.2% agarose gel and identified as to HPV genotype by (1) presence or absence of a specific PCR product and (2) generation of a PCR product of the appropriate band size.

### PBMC PROLIFERATION ASSAY

Venous blood was collected in heparinized test tubes for mononuclear cell isolation before treatment and, on average, 4.5 weeks...
A wart extract from the tissue was prepared by transferring the tissue to a homogenizer (Dounce) containing 1.3 mL of sterile saline or phosphate-buffered saline (PBS). The tissue was homogenized and filtered through a 45-µm filter attached to a 3-mL syringe into sterile cryovials and stored at −20°C.

Venous blood, 15 mL, was transferred to a 50-mL centrifuge tube, diluted to a total volume of 30 mL with saline or PBS, underlaid with a density gradient (Ficoll/Lite-Lymphol1; Atlanta Biologicals, Norcross, Ga), and centrifuged for 20 minutes at 2100 rpm (model 5804R centrifuge; Eppendorf). Interface cells were collected and washed twice with saline or PBS, centrifuged, and resuspended in saline or PBS. Cell counts were performed using a particle counter (Z1; Beckman Coulter, Fullerton, Calif), and the cells were resuspended in freezing media (RPMI/20% human AB serum) and stored at −70°C. Prespecimen and postspecimen PBMCs from each patient were stored for proliferation assays.

Peripheral blood mononuclear cells were thawed, washed twice in saline or PBS, and resuspended at $5 \times 10^8$ cells per milliliter in RPMI/10% human AB serum. Cells were plated at 200 µL per well, and wart extract (5, 10, and 15 µL in quadruplicate) was added to desired wells. Concanavalin A, 5 µg/mL, was added as a positive control. Plates were incubated at 37°C, 5% carbon dioxide, for 5 days and then pulsed with 1 µCi (3.7 MBq) was added as a positive control. Plates were incubated at 37°C, 5% carbon dioxide, for 5 days and then pulsed with 1 µCi (3.7 × 10^4 Bq) tritium-thymidine for an additional 6 hours. Cells were harvested and lysed with an automated cell harvester (Filtermate 196; Packard, Meriden, Conn), the filter was air dried, and cells were counted in an automated beta plate counter (Matrix 9600 Direct Beta Counter; Packard). Results were calculated by averaging the groups and dividing by treated wells with media alone (nonspecific incorporation) to obtain a stimulation index. A positive stimulation index was determined if a subject had a 2-fold increase comparing before and after blood draws.

**MHC Determination**

HLA-A, HLA-B, and HLA-DRB typing was performed by a PCR, followed by a sequence-specific oligonucleotide probes method.6 This method uses locus-specific primers to amplify the polymorphic exons of the gene of interest (exon 2 for HLA-DRB and exons 2 and 3 for HLA-A and HLA-B). Amplified DNA was spotted onto replicate nylon membranes and probed with 30 to 60 different horseradish peroxidase–labeled oligonucleotide probes. After a stringency wash, the positive reactions were detected with a chromogenic substrate (tetramethylbenzidine).

**Statistical Analysis**

**Power/Sample Size**

The study was originally designed to enroll 100 patients in each of 4 study arms. Assuming 40% clearance of the injected warts in the placebo arm, no effect of granulocyte–macrophage colony-stimulating factor (GM-CSF) (later amended to interferon alfa-2b), and an increase to 60% clearance in each immunotherapy arm, the study had 93% power to detect a difference among treatment arms in the primary comparison of response rates, using $\chi^2$. If GM-CSF or interferon alfa-2b had no effect over placebo but boosted the clearance to 70% when combined with immunotherapy, the power to detect an overall difference among the 4 study arms was 99%. After enrollment of 233 patients, however, an unplanned interim analysis was conducted, for administrative reasons (the supply of mumps antigen was no longer assured), with the intention of stopping the trial if significant treatment effects were evident. By using Gordon Lan–DeMets' boundaries, it was determined that the stopping boundary for a significant result at this point in the trial should be $P \leq .005$ (observed). The observed (uncorrected) $P$ value for the primary overall comparison of the 4 groups was less than .001, and enrollment in the trial was stopped. All patients already enrolled in the trial at that point were followed up to planned completion of treatment. Once the decision was made to stop the trial using the Gordon Lan–DeMets criteria, further analyses were reported in terms of nominal (uncorrected) $P$ values.

**Randomization**

Randomization was conducted by means of sealed envelopes prepared by the Department of Biostatistics at the University of Arkansas for Medical Sciences, using randomized blocks of random block sizes. This ensured approximately equal numbers of patients in each treatment arm at any point in the study. No stratification factors were used for randomization. After a patient who had agreed to participate and had signed informed consent was determined to be reactive to at least 1 of the study antigens, the next sealed envelope was opened in sequence, revealing the assigned study arm. The study was single blind in that the patients, but not the study investigators, were blinded to an individual's treatment assignment.

**Data Analysis**

Cross-classified categorical data were analyzed using $\chi^2$ tests. Characteristics measured on a continuous scale (ie, age) were compared between responders and nonresponders using $t$ tests or Wilcoxon rank sum tests and between treatment groups using analysis of variance or Kruskal-Wallis tests. The simultaneous effects of treatment and age on the probability of response were modeled using logistic regression.

**Results**

Two hundred thirty-three subjects enrolled in the study. The initial design included treatment arms using GM-CSF instead of interferon alfa-2b. Because of serious adverse events experienced by subjects receiving GM-CSF, these arms were discontinued and the trial proceeded using interferon alfa-2b in place of GM-CSF. Twenty-three subjects received GM-CSF and are discussed separately, but excluded from analysis, except where expressly noted. Most subjects underwent treatment before enrolling in the study: 49 of 58 in the antigen alone group, 38 of 43 in the antigen plus interferon alfa-2b group, 36 of 48 in the interferon alfa-2b alone group, and 49 of 61 in the saline group. An additional 9 subjects were randomized, but underwent no injections or failed to return for even 1 follow-up visit. Thus, 201 subjects form the main group for analysis. Data on subject age and sex are provided in Table 1. There were no significant differences among the 4 study arms in terms of age ($P = .20$) or sex ($P = .32$). Data regarding positive intradermal skin test results (defined as a ≥5-mm induration) before study initiation for all 233 enrolled subjects are as follows. Positive results to Candida, mumps, and Trichophyton were found in 19 subjects, Candida alone, 53 subjects; mumps alone, 40 subjects; Trichophyton alone, 21 subjects; Candida and mumps, 67 subjects; Candida and Trichophyton, 20 subjects; and mumps and Trichophyton, 13 subjects. Fifty-two of 285 subjects (18.2%) were nonreactive to all antigens.
significant older (39 vs 34 years) than those who did respond antigens. There were no differences in response among the individual sex. The same result was obtained when analyzing the 4 groups separately as when analyzing the 2 groups: (1) antigen with or without interferon alfa-2b against (2) interferon alfa-2b alone and saline alone. Response to interferon alfa-2b injection alone was similar to that to saline (P=.65). Adding interferon alfa-2b did not improve response compared with injection of antigen alone (P=.20). There were no differences in response among the individual antigens (Candida, 59%; mumps, 51%; Trichophyton, 62%; P=.48). Fifty-seven subjects injected with antigen were judged to have 100% resolution of warts at study conclusion, 21 of whom had more than 1 wart and experienced 100% study resolution of all distant warts. In the interferon alfa-2b and saline groups, these numbers were 25 and 11, respectively. There was no difference in response based on sex (P=.56), but subjects who did not respond were significantly older (39 vs 34 years) than those who did respond (P=.01, t test; P=.04, Wilcoxon rank sum test). A multivariate logistic regression analysis indicated that age younger than 40 years was positively associated with the probability of response, after controlling for antigen injection (P=.01). Across responders in all groups, the average number of injections to complete response was 4.6. The average number of treatments in responding subjects receiving antigen was 5.8.

### IMMUNOTHERAPY WITH ANTIGEN AND DISTANT RESPONSES

Distant response data in each arm of the study are shown in Table 2. One hundred fifty-three subjects had more than 1 wart. Compared with injection with interferon alfa-2b or saline alone, subjects receiving antigen, with or without concomitant interferon injection, were statistically more likely to respond (P<.001). The same result was obtained when analyzing the 4 groups separately as when analyzing the 2 groups, as previously described. The addition of interferon alfa-2b to antigen did not significantly improve response (P=.24). There was no significant difference in response between interferon alfa-2b and saline (P=.23).

### IMMUNOTHERAPY WITH ANTIGEN VS INTERFERON ALFA-2B OR NORMAL SALINE ALONE

Response data in each arm of the study are shown in Table 1. These are from the 201 subjects with at least 1 follow-up. Compared with injection with interferon alfa-2b or saline alone, subjects receiving antigen, with or without concomitant interferon injection, were statistically more likely to respond (P<.001). The same result was obtained when analyzing the 4 groups separately as when analyzing the 2 groups: (1) antigen with or without interferon alfa-2b against (2) interferon alfa-2b alone and saline alone. Response to interferon alfa-2b injection alone was similar to that to saline (P=.65). Adding interferon alfa-2b did not improve response compared with injection of antigen alone (P=.20). There were no differences in response among the individual antigens (Candida, 59%; mumps, 51%; Trichophyton, 62%; P=.48). Fifty-seven subjects injected with antigen were judged to have 100% resolution of warts at study conclusion, 21 of whom had more than 1 wart and experienced 100% study resolution of all distant warts. In the interferon alfa-2b and saline groups, these numbers were 25 and 11, respectively. There was no difference in response based on sex (P=.56), but subjects who did not respond were significantly older (39 vs 34 years) than those who did respond (P=.01, t test; P=.04, Wilcoxon rank sum test). A multivariate logistic regression analysis indicated that age younger than 40 years was positively associated with the probability of response, after controlling for antigen injection (P=.01). Across responders in all groups, the average number of injections to complete response was 4.6. The average number of treatments in responding subjects receiving antigen was 5.8.

### ADVERSE EVENTS

In 1027 episodes of treatment, 47 subjects reported fever and myalgias (4.6% of treatment episodes) and 46 subjects reported edema and erythema at the injection site of acral lesions (4.5% of treatment episodes). Of the 47 subjects experiencing fever and myalgias, 9 received interferon alfa-2b alone, 30 received interferon alfa-2b plus antigen, 7 received mumps antigen alone, and 1 received saline. The 46 subjects experiencing edema and erythema were scattered among all treatment groups. In no case were these adverse events a cause of study discontinuation. Oral antipyretic medications were used to lower temperature. Cool compresses and limb elevation were used to lessen edema and erythema. Signs and symptoms resolved within 24 hours in all subjects.

### GM-CSF AND SERIOUS ADVERSE EFFECTS

Of the 23 subjects receiving GM-CSF, 2 developed hypotension, necessitating medical intervention, within 4 hours of intralesional injection. For this reason, the study arms using GM-CSF were halted, with substitution of interferon alfa-2b for GM-CSF, using the same randomization protocol. Of 10 subjects receiving GM-CSF alone, 2 responded, while 10 of 13 receiving GM-CSF plus antigen responded. These data are excluded from the remainder of the analysis, except as indicated.

### RESPONDERS VS NONRESPONDERS FOR POSITIVE PBMC PROLIFERATION ASSAY RESULTS

Thirty-five subjects consented to the PBMC proliferation assay. Of the 19 who responded to treatment, 17 had significant PBMC proliferation after intralesional injection and 2 did not. Of the 16 who did not respond to treatment, 6 had significant PBMC proliferation after intralesional injection and 10 did not. Because only 35 subjects were studied, the data include subjects receiving GM-CSF and interferon alfa-2b. Nineteen subjects received antigen or antigen plus interferon alfa-2b (or GM-CSF), and 16 received interferon alfa-2b (or GM-CSF) or saline. Responders were more likely to have a positive PBMC proliferation assay result, as previously defined, than nonresponders (P=.002). There was no significant association between use of antigen or antigen plus interferon alfa-2b (or GM-CSF) vs saline or interferon alfa-2b (or GM-CSF) alone and PBMC proliferation assay response.

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**Table 1. Immunotherapy Response Data in 201 Subjects**

<table>
<thead>
<tr>
<th>Immunotherapy Arm</th>
<th>Total Subjects</th>
<th>Average Age, y</th>
<th>Female-Male Ratio</th>
<th>Responder*</th>
<th>Nonresponder*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen alone</td>
<td>54</td>
<td>37</td>
<td>31:23</td>
<td>29 (54)</td>
<td>25 (46)</td>
</tr>
<tr>
<td>Antigen plus interferon alfa-2b</td>
<td>41</td>
<td>38</td>
<td>29:12</td>
<td>28 (68)</td>
<td>13 (32)</td>
</tr>
<tr>
<td>Interferon alfa-2b alone</td>
<td>46</td>
<td>40</td>
<td>25:21</td>
<td>12 (26)</td>
<td>34 (74)</td>
</tr>
<tr>
<td>Normal saline</td>
<td>60</td>
<td>34</td>
<td>32:28</td>
<td>13 (22)</td>
<td>47 (78)</td>
</tr>
</tbody>
</table>

*Data are given as number (percentage) of each group.
RESPONSE TO IMMUNOTHERAPY AND VIRAL TYPE OR MHC ANTIGEN

Viral type was determined in warts from 146 subjects: type 2a, 27, or 57 were found in 120 subjects, type 1a in 13 subjects, type 3 or 10 in 5 subjects, type 32 or 42 in 3 subjects, and another type in 5 subjects. More than 1 type was not identified in any subject. There was no significant association between viral type and response to any injection ($P > .99$). This finding was also true among those receiving antigen ($P = .86$). Sixty-five subjects underwent MHC determination. There was no association between response and any of the MHC antigens found in this population.

Manipulating the immune system to achieve a therapeutic or protective response against diseases caused by HPV is an active field of investigation. Interferon alfa-2b is Food and Drug Administration approved for the treatment of condyloma, requiring twice-weekly injection for 3 weeks for optimal results. Topical imiquimod is also reported to have utility in the treatment of nongenital warts and has Food and Drug Administration approval for the treatment of condyloma. Dinichlorobenzene, squaric acid dibutyl ester, diphenylcyclopropenone, and Toxicodendron extract have all been successfully used in topical immunotherapy against common warts. The inorganic chemicals require an elicitation phase of several weeks. Use of Toxicodendron extract is arguably a viable option only in those persons known to be sensitive to plants in this genus, because the induction of sensitivity would be undesirable in terms of eliciting allergic contact dermatitis on exposure in nature.

Few prospective controlled trials of wart therapies exist. We document the clinical efficacy of intralesional immunotherapy using Candida, mumps, or Trichophyton skin test antigens. The response of warts injected with antigen alone was lower in this study than in an earlier publication (60% [combined response data from Table 1, antigen alone and antigen plus interferon alfa-2b groups] vs 74%). Most subjects described herein were referred for the protocol, whereas in the earlier work, subjects were mainly recruited from a general clinic population. We speculate that a selection bias toward more recalcitrant disease exists in the patients constituting the present study group. Still, injection of antigen, with or without interferon alfa-2b, resulted in a meaningful clinical response when compared with injection of saline or interferon alfa-2b alone (56% vs 23%; $P < .001$). Similarly, resolution of anatomically distinct untreated warts was more likely when antigen was injected compared with injection of saline or interferon alfa-2b alone (49% vs 16%; $P < .001$). That older subjects (>40 years) were less likely to respond to immunotherapy than younger subjects agrees with other data documenting less robust immunologic responses with increasing age. We do not have long-term follow-up results of these subjects to be able to report on relapse rate.

In mice vaccinated with viruslike particles to the major capsid protein L1, humoral and cellular responses could be enhanced through coinjection of DNA encoding the costimulatory molecule, B7-2. We sought to enhance local immunity through concomitant injection of cytokines. Injection of interferon alfa-2b did not afford a significant clinical benefit, either alone or in combination with antigen. It is possible that a regimen of more frequent injection and a higher dose might enhance outcome, but more frequent visits and larger volumes of injection are inconvenient and uncomfortable for patients. Because we hypothesize that immunologic events in the wart itself contribute to enhanced recognition of epitopes on the HPV, with subsequent expansion of lymphocyte clones targeting the virus, we initially thought to coinject GM-CSF to affect Langerhans cells and trafficking lymphocytes. While the addition of GM-CSF to antigen injection or even injection of GM-CSF alone might provide a therapeutic response and while patients in these arms of the study were observed to respond, the occurrence of hypotension in 2 subjects necessitated discontinuation of GM-CSF in the protocol.

Our repeated observation that untreated warts resolve after injection of only 1 wart prompts the speculation that intralesional immunotherapy induces HPV-directed immunity. Indeed, we have observed resolution of hundreds of flat warts in individual patients after injection of only 1 lesion. We found that increased proliferation of PBMCs in response to coinjection GM-CSF to affect Langerhans cells and trafficking lymphocytes. It is possible that local and distant responses of warts in subjects who received saline or interferon alfa-2b alone develop by the same mechanism as when antigen is injected and that many triggers of an immune response to HPV exist. While injection of saline is an appropriate control, it is not a true placebo. That noted, local and systemic responses to saline injection in this study were far less likely than when antigen was used. While we found enhanced proliferation of PBMCs, we do not know the subtypes of the responding cells or the responsible effector populations leading to clinical resolution of common warts. In this study, we were unable to identify a correlation between wart response and viral type or any MHC antigen. What factors determine response or resistance to therapy is unclear.

### Table 2. Distant Response Data*

<table>
<thead>
<tr>
<th>Study Arm</th>
<th>Responder†</th>
<th>Nonresponder†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antigen alone (n = 37)</td>
<td>15 (41)</td>
<td>22 (59)</td>
</tr>
<tr>
<td>Antigen plus interferon alfa-2b (n = 35)</td>
<td>20 (57)</td>
<td>15 (43)</td>
</tr>
<tr>
<td>Interferon alfa-2b alone (n = 34)</td>
<td>3 (9)</td>
<td>31 (91)</td>
</tr>
<tr>
<td>Normal saline (n = 47)</td>
<td>9 (19)</td>
<td>38 (81)</td>
</tr>
</tbody>
</table>

*Response was defined as 100% resolution of at least 1 anatomically distinct wart.
†Data are given as number (percentage) of each group.

($P > .99$). Proliferative responses to Candida, mumps, or Trichophyton antigen were not assayed to conserve PBMCs for study of HPV response.

COMMENT

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While little work has been performed with cutaneous wart types in terms of understanding the interplay between viral infection and host immunity, the interest in developing effective vaccines against HPV types causing cervical dysplasia and carcinoma has led to significant knowledge in this area. T lymphocytes responding to HPV-16 can be identified in vivo in infected humans. Lymphocyte proliferative responses are documented in humans in response to vaccination with HPV-18 E6 and E4 fusion proteins. Human papillomavirus 16 L1 viruslike particle vaccine of humans has been shown to result in proliferation of CD4+ and CD8+ T lymphocytes. In the same study, high neutralizing antibody titers after initial vaccination correlated with strong cytokine responses after a second vaccination 6 months later, suggesting that the cellular and humoral immune responses are linked. Peripheral blood mononuclear cells from patients with cervical cancer may be stimulated by autologous leukocytes pulsed with HPV-16 E7 to generate cytolytic T lymphocytes with appropriate specificity. Rhesus macaques vaccinated with viruslike particles, chimeric viruslike particles, and plasmid DNA against HPV-16 L1 vary in the nature of immune response (humoral vs cellular) and the degree of immune response according to type of vaccination. Intrale- sional immunotherapy is not a direct vaccination strategy, but may induce an immune response to HPV, perhaps based on mechanisms similar to those documented in the literature. We have mostly treated subjects with common warts but have observed complete responses using intraleional immunotherapy for genital warts as well (unpublished observation, 2004).

Intraleional immunotherapy for common warts is effective and safe. It is unique in affording many patients a therapeutic response in untreated warts and may, through stimulation of HPV-directed immunity, provide fewer recurrent warts. While useful in any patient with warts, intraleional immunotherapy may be particularly useful in patients with numerous lesions or lesions covering large surface areas.

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Financial Disclosure: Drs Horn and Johnson have obtained US patent 6,350,451 and have significant ownership interest in a start-up company formed through the University of Arkansas for Medical Sciences Biomedical Biotechnology Center. This company was created to market a product based on use of the antigens described in this article. Neither author has received payment to perform the work, and the company has not supported any portion of the research described in this article.

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