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Letters

Tolerance to poison ivy following vaccine delivery by precipitation

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Previous poison ivy vaccines, comprising urushiol in sterile vegetable oils injected subcutaneously, were withdrawn from the market in 1994 for failure to demonstrate statistical efficacy (J. Slater, personal email communication, March 25, 1999). We had anecdotally found these vaccines to be safe and effective in some patients.

For highly allergic patients for whom avoidance was not practical we offered immunotherapy with home-made vaccines in accordance with Declaration of Helsinki guidelines for ethical use of unproven interventions.[1] Informed consent was obtained from all patients. For simplicity and because ethanol is self-sterilizing, we extracted urushiol and prepared vaccines in ethanol. We injected the vaccines intramuscularly because concentrated ethanol is a tissue irritant and small volumes injected intramuscularly would be rapidly diluted by tissue fluid. We measured urushiol concentration by gas chromatography–mass spectrometry. We quantitated patch test sensitivity by the method of Marks et al[2] with 10-fold increasing concentrations of urushiol and used the number of micrograms of urushiol to produce Mark's grade 3 reactions as our measure of sensitivity. Of our first 4 patients, the most sensitive 2 achieved durable, measurable tolerance,[3] challenging us to figure out how we were achieving more effective tolerance to poison ivy than ever reported in previously sensitized patients and how to increase the percentage responding.

Previous poison ivy vaccines injected subcutaneously in sterile vegetable oils became repositories from which urushiol diffused by the molecule. Vaccines in ethanol injected intramuscularly precipitate solid urushiol particles within the injected muscle as the ethanol is diluted by tissue water and the urushiol becomes insoluble. The more rapid the dilution, the larger the number and smaller the size of the particles. Xiang et al[4] reviewed uptake of vaccines as a function of particle size. They reported efficient uptake of 0.5- to 5-μm particles by naive dendritic cells by macropinocytosis.

A 0.1 mL of a 25-mg/mL solution of urushiol in ethanol contains 2.5 μg of urushiol. If precipitated into 2-μm-diameter particles, it will deposit 0.6 billion such particles around the injection site. Vaccine delivery by precipitation deposits hundreds of millions of antigen particles sized for efficient uptake by naive dendritic cells, which in muscle appear to be of lineages and in a cytokine milieu favorable for tolerance induction.

Table 1 lists the outcomes for our first 35 courses of treatment. Poison ivy vaccines were labeled Pi1 through Pi7. Pi1 and Pi2 were crude 7-day ethanol extract of fresh leaves that contained 1.12 and 1.92 mg/mL of urushiol. Pi3 was a 50-mg/mL concentrate purified by the method of ElSohly et al.[5] Pi4 was 75% by volume Pi2 mixed with 25% Pi3. Pi5 (50 mg/mL) and Pi6 (110 mg/mL) were vacuum-concentrated unpurified crude extracts. Pi7 was 50% Pi6 and 50% Pi2.

The most sensitive 2 of our first 4 cases responded to cumulative urushiol doses of 0.78 to 0.84 mg. Injected ethanol volume is a limiting factor for both safety (increasing volume increases both discomfort and risk of tissue injury because larger volumes will be diluted more slowly) and efficacy (slower dilution of larger injected volumes will yield smaller numbers of larger particles and may miss the 0.5- to 5-μm target range for efficient dendritic cell uptake. Therefore, we made Pi3, a concentrated formulation of urushiol purified by the method of ElSohly et al.[4] Patient 1 (the first author) did not respond to 0.84 mg of urushiol in crude extract vaccine Pi1. He had a 15-fold reduction in sensitivity to 77.2 mg of urushiol in purified concentrate Pi3 but with eosinophilia and dermographism that lasted 3 months. Patient 11 had a 67-fold sensitivity reduction and 9- to 12-month clinical response to 3.61 mg of Pi2 but did not respond to 6.6 mg of Pi3. We concluded that something with adjuvant activity in crude extracts is lost in purification and that mixtures of crude ethanol extracts with concentrates to increase the dose of urushiol per unit volume of ethanol might be an effective way to increase dose without unacceptable increases in injected ethanol volume. We did this in vaccine Pi4 with which patient 11 had a 10-fold sensitivity reduction and a clinical response still present at 36 months following treatment with 2.59 mg and patient 1 had a 1000-fold sensitivity reduction still present at 28 months after treatment with 15.93 mg.

If an essential adjuvant was lost in purification, we thought vacuum concentration without purification might be a simple way to retain it in vaccines Pi5 and Pi6. Because only 2 of 5 patients treated with these 2 vaccines had the 10-fold or greater sensitivity reduction that correlates with durable clinical response, we decided to mix our most potent concentrate (Pi6) with an equal volume of fresh crude extract as vaccine Pi7, to which 13 of 14 treated patients aquired tolerance.

Our observational series did not have preselected end points. The parameters we identified as most relevant are termed *reduct-fold*, the factor by which patch test sensitivity was reduced by treatment, and *clinical response* (yes or no as reported by the patient). When available, we also tracked the intervals at which tolerance was still present (by tolerance to patch test and/or reported natural exposure) and lost. Cases 16, 18, and 32 with "NT" in the reduct-fold column did not return for posttreatment testing, but gave convincing reports of clinical tolerance to exposures previously followed by severe exacerbations.

Eighteen of 20 courses in 18 patients treated with vaccines containing at least 50% by volume of unmodified crude ethanol extract spiked with purified or unpurified concentrated urushiol (to increase urushiol dose) produced convincing clinical and/or patch test tolerance at urushiol doses from 3.36 to 22.4 mg.

Disclosures: The authors hold US and EU patents for the technology reported in this article.

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Table 1
Clinical Experience With Intramuscular Poison Ivy Urushiol Vaccine in Ethanol, 2009-2017

Case No.	Patient No.	Patient age, y/sex	Tx complete	Pre-Tx grade 3 patch test dose, μg	Tx dose, mg	Ethanol volume injected, mL	No. of Tx steps	Pre-Tx grade 3 patch test dose	Post-Tx grade 3 patch test dose, μg	Time tested post-Tx	Reduct-fold	Vaccine	Clinical response	Response maintained	Lost protection by	Adverse effect no.[a]
1	1a	70/M	06/24/09	0.5085	0.84	0.86	6	1652	1.13	1 mo	2	Pi1	N	NA	NA	
2	2	31/M	08/06/09	0.0226	0.78	0.96	7	34425	2.26	9 mo > 3 wk	100	Pi1	Y	9 mo	14 mo	
3	3	57/F	09/08/09	0.113	0.84	0.96	7	7442	0.113	2 mo	1	Pi1	N	NA	NA	
4	4	14/F	12/26/09	0.005085	0.84	0.52	8	165,388	0.113	3 mo = 3 wk	22	Pi1	Y	≥3 mo	?	
5	8a	66/F	07/26/10	0.00384	1.43	0.96	7	371,875	19.2	8mo > 1 mo	5000	Pi2	Y	≥40 mo	63 mo	
6	10	12/M	09/21/10	0.0576	1.43	0.99	8	24,809	0.0576	1 mo	1	Pi2	N	NA	NA	
7	11a	65/F	07/15/11	0.0576	3.61	1.71	10	62,674	3.84	2 mo	67	Pi2	Y	9 mo	12 mo	
8	1b	73/M	04/23/12	0.5	77.20	2.49	14	154,400	7.5	1 mo = 3 mo	15	Pi3	No exposure	>3 to <16 mo	16 mo	1
9	11b	66/F	12/27/12	0.05	6.60	0.71	7	132,100	0.05	3 mo	1	Pi3	N	NA	NA	
10	11c	67/F	10/10/13	0.05	2.59	0.44	2	51,720	0.5	3 wk	10	Pi4	Y	≥36 mo		2
11	1c	74/M	11/14/13	0.5	15.93	1.23	8	31,860	500	6 mo = 14 mo	1000	Pi4	No exposure	≥28 mo		3
12	19	55/F	04/08/14	0.015	7.56	0.44	5	504,000	1	6 wk	67	Pi5	Y	≥6 mo		
13[b]	14	57/M	07/31/14	22.5	17.56	0.54	6	780	22.5	0 d	1	Pi5	Y			
14[c]	20	53/M	08/14/14	0.225	17.56	0.64	6	78,044	0.5	4 mo	2	Pi5	Y			
15	21	65/F	08/21/14	0.015	17.56	0.64	6	1,170,667	0.5	4 mo	33	Pi5	Y	≥16 mo clinical response, 16 mo patch		
16	23	23/F	06/29/15	5	17.37	1.24	7	3474	Did not return for post-Tx testing		NT	Pi4	Y: farmer reported full tolerance to same exposure.			
17	24a	25/M	06/22/15	0.035	17.37	1.24	7	496,286	5	2 mo	143	Pi4	Y	≥3 mo, 4 mo partial		
18	24b		09/30/15	Tree trimmer with partial loss at 4 mo, full restore after 11.2-mg booster								Pi4	Y	≥14 mo		
19	28	29/M	08/06/15	5	18.2	1.3	2	3640	Did not return for post-Tx testing		NT	Pi4	Y: tree-trimmer reported full tolerance			4
20	26	16/M	10/05/15	0.0225	17.37	1.24	7	772,000	0.015	1 mo	1	Pi4	No exposure			
21	31a	65/M	10/21/15	150	17.92	0.32	2	119	1500		10	Pi7	Y (VG not E)	≥3 mo		
22	31b		03/24/16	VG but not 100% initial protection, 100% for ≥9 mo after 16.8-mg booster								Pi7	Y	≥9 mo		
23	8b	72/F	02/04/16	0.5	8.68	0.36	6	17,360	1000	3 mo	2000	Pi7	Y	≥6 mo		5
24	37a	47/F	02/08/16	0.5	18.7	0.17	2	37,400	0.33	3 mo	1	Pi6	Unsure (Definite response to Pi7 on next line)			
25	37b	47/F	05/09/16	0.35	17.9	0.32	1	51,143	5	6 wk	14	Pi7	Y	≥11 mo		
26	35	46/F	03/31/16	0.023	17.9	0.32	1	778,261	25	3 mo	1087	Pi7	Y	≥6 mo		6
27	36	15/M	03/31/16	0.05	17.9	0.32	1	358,000	1.5	3 mo	30	Pi7	Y	≥6 mo		
28	38	44/F	05/09/16	0.225	17.9	0.32	2	79,556	2.25	7 mo	10	Pi7	Y	≥6 mo		
29	42	53/M	07/25/16	0.15	22.4	0.4	1	149,333	3.5	6 mo	23	Pi7	Y	≥6 mo		7
30	43	24/M	08/04/16	0.05	21.3	0.38	2	426,000	22.5	3 mo	450	Pi7	Y	≥7 mo		
31	39	47/F	07/21/16	0.0035	3.36	0.06	1	960,000	0.225	6 mo	64	Pi7	Y	≥7 mo		8
32	46	39/M	08/29/16	0.005	22.4	0.4	1	4,480,000	Did not return for post-Tx testing		NT	Pi7	Y: 99% tolerant to previously hi-morbidity exposures			
33	47	59/F	09/22/16	0.011	5.04	0.18	2	458,182	7.7	3 mo	700	Pi7	Y	≥7 mo		
34	48	71/F	10/12/16	0.035	0.56	0.1	1	16,000	Patient chose to stop after first dose		NT	Pi7	(N)	9		9
35	49	64/F	02/06/17	0.5	5.04	0.18	2	10,080	0.5 6 wk		1	Pi7	?	10		10

Abbreviations: E, excellent; NA, not applicable; NT, no posttreatment testing; Pi, poison ivy [vaccine]; Tx, treatment; VG, very good clinical response.

[a]The following adverse effects were found: (1) dermographism lasting approximately 3 months with eosinophilia (to 1200/mm^3) after high-dose (total of 77 mg) urushiol (eosinophil count of 300/mm^3 at 4 months); (2) slowly resolving (weeks) tender local lumps to 3 cm on abbreviated (rapid build-up) dosing schedule (did not give prednisone); (3) eosinophilia (eosinophil counts to 800/mm^3) without dermographism 2 days after a cumulative dose of 16 mg (eosinophil count of 100/mm^3 at 7 months); (4) painful local swelling required 4 days' prednisone at 40 mg/d for each of 2 doses; (5) painful local induration to 6 cm by 6 days at step 4 of 5-dose schedule, responded to 4 days of 40 mg/d of prednisone then tapered to 20 mg/d every other day, 10 mg/d every other day, and discontinued, and good clinical and patch test responses with the fifth dose; (6) dermographism with eosinophil counts up from baseline to 210 to 440/mm^3; (7) transient tender lumps at 1 step injection sites, local poison ivy at 1 of 4 sites, in all patients quantity was not sufficient and they wanted treatment, by 12 days there was mild poison ivy partially overlapping sites with recent grade 6 and 7 patch test reactions (eosinophil count of 300/mm^3 and baseline count of 200/mm^3); (8) tender swelling lasting 2 days at each of the first 1.68 mg injections (planned 2-step schedule), with second treatment stopped with good clinical control and response confirmed by posttreatment patch; (9) vesicular reaction at first dose shot sites, triamcinolone not sufficient to control, needed 7-day prednisone taper, and did not achieve protection but decided not to continue treatments; and (10) mild local and first set shots, uncomfortable local lumps and overlapping poison ivy dermatitis lasting 3 weeks despite a second dose of topical triamcinolone (third dose deferred).

[b]This patient was tested on the same day treatment was completed because the patient was moving out of the area.

[c]This patient was taking adalimumab for psoriasis from December 2013.

We found a 39,000-fold variation in pretreatment patch test reactivity in patients reporting similar symptoms. Reduct-fold of 10 or higher had an excellent correlation with clinical response. Within this dose range for combination vaccines, pretreatment sensitivity did not.

We found unpurified urushiol concentrates less stable than purified concentrates. Going forward, we plan to apply for formal clinical trials with a combination vaccine (approximately Pi4). We do not believe it is practical to attempt to identify the adjuvant in crude ethanol extracts without an animal model with a tight dose-response curve, which to our knowledge does not exist.

Treatment with approximately 0.5, 2, and 20 mg of urushiol at 2- to 6-week intervals was safe and effective for most patients. Some needed short-term prednisone and/or topical triamcinolone for injection site reactions, usually patients requesting treatment in fewer steps to reduce travel. This treatment did not appear to affect outcomes.

The standard for disclosure in scientific reporting is increasingly becoming that of sufficient detail to enable a reader to completely reproduce the reported observations. However, we encourage readers of this article not to try to reproduce our work without a way to measure the urushiol content of their vaccines. The reason is a greater than 10-fold variation in the urushiol content of similarly prepared crude ethanol extracts, coupled with incomplete knowledge of the factors that affect that yield. Without knowing content, it is impossible to extrapolate our experience for safety or efficacy.

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Association of exhaled nitric oxide with ethnicity and sex in rural Georgia youth

Nitric oxide is produced by the human bronchial epithelium[1] and is associated with airway eosinophilic inflammation seen in patients with asthma and other airway diseases.[2] Airway inflammation is a characteristic of asthma, along with airway hyperresponsiveness and airway obstruction. Inhaled corticosteroids to treat the inflammation are a mainstay of asthma treatment. Measurement of exhaled nitric oxide (eNO) as a biomarker of eosinophilic inflammation is noninvasive, safe, reproducible, and easy to perform; however, the clinical value of measurements has been limited by imprecise reference ranges. Established reference ranges must account for many variables known to affect eNO values. Some have suggested that a threshold value may be more useful than reference ranges.[3]

Studies have evaluated the association of eNO and ethnicity and/or race but this information is limited. A 2017 study investigated factors related to eNO in 716 children between the ages of 6 and 19 years from the 2007-2012 National Health and Nutrition Examination Survey (NHANES).[4] High levels of eNO (defined by American Thoracic Society guidelines as >35 ppb for the ages of 6-11 years and >50 ppb for the ages of 12-19 years) were more frequently found in non-Hispanic black and Hispanic than non-Hispanic white individuals.[4] Recently, a systematic review[5] comprising 12 studies of ethnicity and eNO was published. Ten of these studies presented ethnicity as a significant factor in eNO levels. A different study, including 4718 patients from 2007-2010 NHANES, found that eNO was significantly lower in non-Hispanic whites compared with non-Hispanic blacks and Hispanics between the ages of 6 and 19 years. In individuals older than 19 years, there was no difference in eNO according to ethnicity.[6]

Some studies have evaluated sex as well; however, few investigated both ethnicity and sex groups simultaneously in association with eNO levels. In addition, one plausible variable that may affect the association of eNO with ethnicity and sex is exposure to air pollutants, which are typically greater in urban areas. Previous studies have almost exclusively examined urban populations, making our rural study population unique. Further clarification of fixed influential variables could possibly improve the clinical utility of eNO measurements. The purpose of this study was to examine the associations of race and sex with eNO in a representative sample of rural teenage youth with asthma symptoms.

The initial data were collected as part of the Puff City Georgia randomized controlled trial (ClinicalTrials.gov Identifier: NCT01170676), which tested the effectiveness of a computer-based intervention to improve asthma self-management.[7] The study surveyed high school students in the single public high school in each of 4 rural counties of Southeastern Georgia. The students reported asthma and asthma symptoms using the Lung Health Survey, a questionnaire composed of questions used in the International Study of Asthma and Allergies in Childhood questionnaire. Collected information includes physician diagnosis of asthma, respiratory symptoms, sex, and race. Exhaled NO was measured per manufacturer's protocol using the NIOX MINO eNO instrument by trained research study staff. The study was approved by the Human Assurance Committee of Augusta University, and permission was provided by the respective school superintendent and principal in each county in Georgia.[7]

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