

The relationships between late cutaneous responses and specific antibody responses with outcome of immunotherapy for seasonal allergic rhinitis

William A. Parker, Jr., MD, MAJ, USAF, Bonnie A. Whisman, MS,
Stephen J. Apaliski, MD, MAJ, USAF, and
Michael J. Reid, MD, CAPT, USN Lackland Air Force Base, Texas

Mountain cedar (MC) (Juniperus ashei) causes a significant and isolated seasonal allergic rhinitis in south-central Texas during the winter months. Retrospective studies have indicated that patients segregate into two categories based on skin test reactions: single positive skin test to MC only and multiple positive skin tests. These two populations differed in age, personal and family history of atopy, levels of both IgE and total MC-specific IgE (sIgE), and symptomatology. It has been speculated that the subjects with only a single positive skin test may actually be nonatopic and develop an IgE response to MC because of some peculiarity of the antigen. In a prospective, randomized, controlled trial, we tested the efficacy of immunotherapy (IT) with MC extract in 51 subjects, 12 single positive skin tests and 39 multiple positive skin tests, to determine if these differences indeed exist and if IT is equally effective in both groups. We failed to demonstrate significant differences in age, sex, MC sIgE, total IgE, initial immediate cutaneous response, initial late cutaneous response, or personal or family history of atopy. IT was equally effective in both groups of subjects with no significant differences noted in response to MC sIgE, MC sIgG1, MC sIgG4, or with suppression of the late cutaneous response. In addition, we found that suppression of the late cutaneous response correlated significantly with cumulative dose of MC extract, postseasonal level of MC sIgG1 and MC sIgG4, and improvement of symptomatology. Suppression of the late cutaneous response may be a clinically useful parameter to follow in monitoring patients during IT. Caution is advised because this procedure may result in systemic reactions. (J ALLERGY CLIN IMMUNOL 1989;84:667-77.)

Pollen from the tree, *Juniperus ashei*, commonly called MC, is an important cause of respiratory allergy in central Texas and areas of New Mexico, Oklahoma, and northern Mexico.^{1, 2} In south-central Texas, this

From the Allergy-Immunology Service, Division of Medicine, Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, Texas.

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Reprint requests: Michael J. Reid, MD, Allergy-Immunology Clinic, Naval Hospital Oakland, Oakland, CA 94627-5000.

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Abbreviations used

FBS:	Fetal bovine serum
HSA:	Human serum albumin
MC:	Mountain cedar
sIgE:	Specific IgE
sIgG1:	Specific IgG1
sIgG4:	Specific IgG4
OPD:	o-Phenylenediamine
PBS:	Phosphate-buffered saline
r_s :	Spearman's rank correlation coefficient
IT:	Immunotherapy

tree pollinates heavily during the months of December, January, and February, and is the only pollen present in significant amounts during this time period.³ As such, it provides an excellent model to study respiratory allergy and the response of pollinosis to IT without significant interference from other aeroallergens.

Several investigators have noted the existence of two unique groups of patients sensitive to MC based on skin testing.^{4,5} Both groups have symptoms typical of allergic rhinoconjunctivitis during the MC-pollen season. However, one group has positive skin tests for several allergens, including MC, whereas the second group is skin test positive to MC alone. Although MC allergens have not been studied extensively, Ramirez⁴ suggested that MC pollen has unique allergic properties, because he found a high percentage of patients with single positive skin tests in his clinic population. Thirty-four percent of unselected MC-allergic patients were sensitive to MC alone, and 37% of the patients in a previously reported trial of IT for MC pollinosis had a single positive skin test.⁶ Ramirez⁴ also found that these patients with single positive skin tests were significantly older, required longer periods of exposure to develop sensitivity, and had significantly less total IgE than patients with multiple positive skin tests. Reid et al.,⁵ in another retrospective study, also noted the older age of patients with single positive skin tests and found a significantly lower level of MC sIgE initially and during the peak season in patients with single positive skin tests. The IT-treated patients with single positive skin tests had significantly lower symptom-medication scores than IT-treated patients with multiple positive skin tests, but the response of MC sIgE, MC sIgG1, and MC sIgG4 to IT was not different in the two groups. Subsequently Fling et al.,⁷ in a prospective, randomized, placebo-controlled study, found that in MC-sensitive subjects, IT caused suppression of the late cutaneous response but not the immediate cutaneous response and that this suppression correlated with MC sIgG4. No significant correlation with MC sIgG1 or symptom-medication scores was found. Our study was to attempt to confirm, in a prospective manner, the existence of these two groups and to determine any differences in clinical or immunologic response to IT manifested by symptom-medication scores, late cutaneous response, MC sIgE, MC sIgG1, or MC sIgG4.

MATERIAL AND METHODS

Patient selection

All adult patients (ages 18 to 70 years) with a history consistent with MC-allergen rhinoconjunctivitis and a positive prick skin test to MC extract (1:20 wt/vol), observed in our clinic from January to July 1987, were asked to participate in the study. Subjects younger than 18 years of age, pregnant women, patients receiving β -blocking medications, and patients who had been receiving IT during the previous 5 years were excluded. All patients filled out a questionnaire, received a history and physical examination, and underwent prick skin testing on the back with a routine battery of 40 aeroallergen extracts, including white ash, box elder, cottonwood, American elm, hackberry, mulberry,

mesquite, live oak, pecan, sycamore, Bermuda grass, brome, Johnson grass, timothy grass, perennial ryegrass, burning bush, lamb's-quarter, pigweed, Russian thistle, cocklebur, Franseria, marsh elder, ragweed (giant and short), sagebrush, *Alternaria*, *Cladosporium*, *Helminthosporium*, *Epicoccum*, *Aspergillus*, *Penicillium*, *Fusarium*, *Pullularia*, house dust, *Dermatophagoides farinae*, cat, dog cockroach, diluent, and histamine controls. Skin tests were interpreted as follows: 0, no erythema or wheal; 1+, erythema <20 mm; 2+, erythema >20 mm; 3+, erythema with wheal >3 mm; and 4+, erythema with wheal and pseudopods. Patients who agreed to enter the study signed an informed consent form and underwent titrated prick skin testing with serial tenfold dilutions of MC extract from 1:100 to 1:100,000.

IT

Patients were matched within the two groups (multiple positive skin test and single positive skin test) by end point titration prick skin tests and randomly assigned in a blind fashion to IT, either with MC extract or placebo. MC extract (Hollister-Stier Laboratories, Spokane, Wash., lot No. H87DO44401) was used for all skin testing and IT. This extract was demonstrated according to the method of Lowry et al.⁸ to contain 3.18 mg of protein per milliliter. The actively treated group received conventional high-dose IT with MC extract beginning with 0.1 ml of 1:50,000 wt/vol dilution progressing by 0.05 to 0.1 ml increments until 0.5 ml was reached. A tenfold higher concentration was then administered in the same dosing increments until the highest tolerated dose or 0.5 ml of 1:50 wt/vol was reached. The placebo-treated group received an injection from a vial consisting of 0.5 ml of caramelized glucose, 4.5 ml of 0.03% HSA, and 0.6 mg of histamine phosphate as the final concentrations from which serial dilutions were made. All patients received one to three injections per week until maintenance was reached, at which time they received injections on a weekly basis. All patients achieved maintenance doses of either active or placebo injections before the MC-pollen season, although two actively treated patients had one episode each of generalized urticaria after an injection.

Immediate cutaneous response and late cutaneous response

Before beginning injections, each patient had an intradermal skin test with MC extract, 0.02 ml of 1:1000 wt/vol. The size of the induced wheal was measured at 15 minutes, and the area of tissue swelling was recorded at 6 hours after injection. Both reactions were outlined in pen and transferred with tape onto paper, and the area was measured with a Zidas digital planimeter (Carl Zeiss, Inc., Thornwood, N.Y.). These reactions were elicited and measured in a similar manner again at the end of the study (after season), and all results were recorded in square centimeters.

Symptom-medication scores

All patients recorded a symptom and medication score sheet on a daily basis during the MC-pollen season. Patients recorded both nasal symptoms (itchy nose, congestion, sneezing, nose blowing, and itchy eyes or throat) and chest

symptoms (wheezing, shortness of breath, chest tightness, or cough). All symptoms were graded on a scale of 1 to 5, depending on severity. Medications were scored as 1 point per standard dose. All scores during the peak MC-pollen season (January 11 to 20) were added to reach a total symptom-medication score for the peak season.

Pollen counts

Pollen counts were performed at 2- to 5-day intervals during the MC-pollen season. Counts were done on a Rotorod (Ted Brown Associates, Los Altos, Calif.) located on the roof of Wilford Hall Medical Center, located in southwest San Antonio.

MC sIgE, MC sIgG1, and MC sIgG4

Blood was obtained from subjects before institution of IT, after maintenance (after IT), and at the end of the MC season (late February). The antigen used was extracted from MC pollen generously supplied by Peter Hauck, Center Laboratories, Port Washington, N.Y. Pollen was extracted in 0.125 mol/L of NH_4HCO_3 at 1/10 wt/vol at 4° C for 16 hours. The extract was then centrifuged. The supernatant was filtered through 8, 3, and 1.2 micron pore-size filters and then filtered through 0.8 and 0.45 micron filters. The filtrate was then dialyzed in 3500 dalton cut-off dialysis tubing against distilled water. The extract was then lyophilized and stored at 4° C until it was used.

MC sIgE antibodies. The MC sIgE assay was performed by a monoclonal antibody-based ELISA transfer method as previously reported.⁹ Briefly, the assay was performed without the use of antigen-free control wells because of the low background of the monoclonal system. Wells were coated with 150 µg/ml of extract in PBS, pH 7.2, incubated for 1 hour at 37° C, aspirated, and incubated with 200 µl of 0.1% HSA in PBS for 1 hour at 37° C. The HSA was aspirated, and the wells were washed three times with PBS-Tween and dried.

One hundred microliters of unknown serum diluted 1:5 in 10% FBS in PBS was added to each well and incubated overnight at 4° C. After being transferred to a second plate with wells coated with MC antigen and incubated at 4° C, the wells were aspirated, washed three times, and dried in the same manner. Sera were transferred to a third plate late in the afternoon. This procedure was repeated until a total of four transfers with five plates were completed. After the plates resulting from the last transfer were washed, 100 µl of a 2.5 µg/ml dilution of mouse monoclonal antihuman IgE antibody (Boehringer Mannheim, Indianapolis, Ind.) in 10% FBS in PBS was added to each well and incubated at room temperature for 3 hours.

All the wells were then aspirated, washed three times, and dried; 150 µl of 0.5 mg OPD per milliliter and citrate buffer was added to each well. The wells were incubated for 30 minutes at room temperature and read at 492 nm.

The MC sIgE concentration was expressed in arbitrary units per milliliter by interpolation from a reference curve analyzed in parallel with the unknowns. The reference curve was developed from a pool of high-titer sera whose content of MC sIgE was arbitrarily assigned.

MC sIgG subclass antibodies 1 and 4. Determination of MC sIgG1 and MC sIgG4 were performed as described

above for IgE antibodies with the following exceptions: (1) Serum samples were diluted 1:10 in 10% FBS in PBS. (2) Murine monoclonal antihuman IgG1 and IgG4 were used. These were all IgG1 isotypes and demonstrated to be human immunoglobulin subclass-specific by direct binding and competitive inhibition assays, as previously described.¹⁰ The anti-IgG1 was catalog No. 411450, clone HPG001, Calbiochem, San Diego, Calif., and the anti-IgG4 was catalog No. 63-063-1, clone sk-44, Miles Scientific, Naperville, Ill. Monoclonal antihuman IgG subclass antibodies were added after incubation for 2 hours at room temperature, aspiration of serum samples, and washing. The monoclonal antibodies were incubated overnight at room temperature at equivalent antigen-binding dilutions. For anti-IgG1, the dilution used was 1:10,000, and for anti-IgG4, 1:20,000 in 10% FBS in PBS. (3) After plates were washed and dried, 100 µl per well of a 1:1000 dilution of goat antimouse IgG peroxidase conjugate in 10% FBS in PBS was incubated for 1 hour at 37° C. After IgG peroxidase conjugate was aspirated and plates were washed, substrate was added, and plates were developed as described above for IgE antibodies. No transfers were performed. Although IgG subclass assay standardization could be achieved semiquantitatively with myelomas and subclass antibodies in isotypic dilution curves, such an assay could not be considered quantitative because of variable binding of myelomas with immunosorbents and variable primary antibody affinities for homologous myeloma antigens. Results are therefore expressed as net absorbances, yielding semiquantitative comparative data.

The specificities of all assays were proved by appropriate preincubation and direct binding with relevant and irrelevant antigens or myeloma immunoglobulins, as previously reported.¹⁰ The lower limits of the assays were 2 SDs above the mean level of a nonhuman antibody containing serum assayed 10 times.

Statistical analysis

Subject characteristics were compared with Student's *t* test or chi-square analysis. Antibody levels were compared with the Mann-Whitney U test. Symptom-medication scores were compared to treatment groups, skin test category, late cutaneous response, and dose by rank conversion and use of one-way or two-way analysis of variance for repeated measures, nonparametric one-way analysis of variance (Kruskal-Wallis), or Spearman's rank correlation. Antibody levels were compared to treatment groups and skin test category by log conversion and use of one-way or two-way analysis of variance. Antibody levels were compared to late cutaneous response, and symptom-medication scores by Spearman's rank correlation. All *p* values are two tailed. Statistics were computed by use of the StatView 512+ software program (BrainPower, Inc., Calabasas, Calif.) on Macintosh 512k microprocessor (Apple Computer, Inc., Cupertino, Calif.).

RESULTS

Patient characteristics

Twenty-six subjects completed the study with the active treatment arm, and 25 subjects in the placebo-

TABLE I. Subject characteristics with respect to treatment

	Treatment		<i>p</i>
	Active	Placebo	
No.	26	25	NS
Mean age (range)	43.4 (22-69)	47.1 (25-75)	NS
Sex (M/F)	13/13	12/13	NS
MC IgE (U/ml)	18.6	22.1	NS
MC sIgG1 (U/ml)	58.7	61.7	NS
MC sIgG4 (U/ml)	133.2	83.0	NS
Total IgE (IU/ml)	196.5	88.4	NS
Skin test (single positive skin test/multiple positive skin test)	6/20	6/19	NS
First immediate cutaneous response (cm ²)	2.6	2.5	NS
First late cutaneous response (cm ²)	44.0	38.3	NS

NS, Not significant.

TABLE II. Subject characteristics with respect to skin test characteristics

	Single positive skin test	Multiple positive skin test	<i>p</i>
No.	12	39	—
Mean age (range)	46.5 (25-75)	44.8 (22-69)	NS
Sex (M/F)	5/7	20/19	NS
MC IgE (U/ml)	22.0	19.7	NS
MC sIgG1 (U/ml)	47.9	64.6	NS
MC sIgG4 (U/ml)	207.7	85.8	NS
Total IgE (IU/ml)	127.0	133.5	NS
First immediate cutaneous response	2.1	2.4	NS
First late cutaneous response	25.3	37.6	NS
Personal history of atopy (yes/no)	0/12	6/33	NS
Family history of atopy (yes/no)	8/4	28/11	NS

NS, Not significant.

treated arm. The actively treated group contained subjects with six single positive skin tests and 20 multiple positive skin tests, and the placebo-treated group contained subjects with six single positive skin tests and 19 multiple positive skin tests. There were no significant differences in age, sex, MC sIgE, MC sIgG1, MC sIgG4, total IgE, initial immediate cutaneous response, or initial late cutaneous response (Table I).

When the subjects were divided according to skin test, there were 12 in the single positive skin test group and 39 subjects in the multiple positive skin test group,

reflecting the percentage in our general clinic population. There were no significant differences in age, sex, initial specific antibody titers, total IgE, immediate or late cutaneous responses, or personal or family histories of atopy (Table II).

Symptom-medication scores

Results of IT are presented in Tables IIIA and IIIB. Symptom-medication scores were significantly lower in the actively treated group versus the placebo-treated group, 57.0 versus 129.9; *p* = 0.0001. There were no significant differences in symptom-medication

TABLE IIIA. Summary of responses to IT

		Active					
Patient No.	Skin test	% Change	% Change				Total symptom-medication scores
		Immediate cutaneous response	Late cutaneous response	% Change	% Change	% Change	
				MC IgE	MC IgG1	MC IgG4	
1	SPST	48.72	−100.00	5.41	438.64	669.23	32
2	SPST	6.25	−84.62	56.63	2580.00	1190.20	129
3	SPST	−52.54	−76.47	−73.85	4720.00	1003.94	35
4	SPST	20.66	−100.00	−6.58	1213.33	246.48	59
5	SPST	ND	ND	−50.15	249.12	98.69	34
6	SPST	−22.29	−100.00	−53.46	3403.85	391.37	50
7	MPST	−8.37	122.12	−5.53	516.67	346.01	161
8	MPST	123.81	−64.04	−30.99	708.54	1044.81	59
9	MPST	ND	ND	−1.53	295.16	1133.33	20
10	MPST	−13.70	−100.00	−13.35	561.54	441.37	87
11	MPST	−6.88	−50.77	200.42	232.05	−165.79	61
12	MPST	−2.52	−100.00	−54.10	909.52	523.19	52
13	MPST	−43.44	−100.00	190.19	1506.25	921.43	21
14	MPST	−14.57	−76.40	150.79	750.00	265.31	39
15	MPST	−35.53	−79.22	1625.40	1707.27	640.51	211
16	MPST	.64	−100.00	19.63	275.81	372.50	20
17	MPST	66.19	−80.92	85.31	5676.47	1018.63	49
18	MPST	118.79	−89.69	−7.36	2800.00	2709.62	4
19	MPST	−6.12	−100.00	ND	812.66	444.77	11
20	MPST	16.32	−63.99	70.10	491.67	734.19	102
21	MPST	−44.50	−100.00	279.00	5195.24	100.81	25
22	MPST	−4.57	−100.00	65.32	2135.00	1688.46	73
23	MPST	−56.36	−89.89	43.34	990.00	652.53	55
24	MPST	.48	−100.00	−39.37	443.10	450.62	7
25	MPST	37.38	−100.00	−23.53	1438.67	965.00	69
26	MPST	ND	ND	87.56	3937.50	1016.00	18
Mean		5.559	−79.74	100.43	1691.85	727.04	57

SPST, Single positive skin test; MPST, multiple positive skin test; ND, not done.

score for subjects with single positive skin tests versus subjects with multiple positive skin tests, 87.5 versus 94.4; $p = 0.6648$ (Table IV).

Reduction in the late cutaneous response

IT resulted in no significant change in the immediate cutaneous response. However, the late cutaneous response was significantly reduced; actively treated mean versus placebo-treated mean was 8.59 cm² versus 33.37 cm, respectively; $p = 0.0001$ (Figs. 1 and 2). The size of the late cutaneous response after IT, the change in the late cutaneous response, and the percent change in the late cutaneous response were all significantly correlated with symptom-medication scores, with the highest correlation being with percent change in the late cutaneous response, $r_s = -0.566$ and $p < 0.0002$ (Table V). The late cutaneous response was also significantly related to the postseason

levels of MC sIgG1 and MC sIgG4. When postseason levels were compared to percent change in late cutaneous response, MC sIgG1 was $r_s = 0.626$ and $p < 0.0002$, and Mc sIgG4 was $r_s = 0.549$ and $p < 0.0002$. Cumulative dose of MC antigen was significantly related to second late cutaneous response, change in late cutaneous response, and close to significance to percent change in late cutaneous response (Table VI). When subjects were grouped according to the size of the late cutaneous response at the end of MC season, mean symptom-medication scores for those subjects with no measureable late cutaneous responses was 45, mean symptom-medication score for those subjects with late cutaneous responses between 1 and 20 cm² was 106, and mean symptom-medication score for those subjects with late cutaneous responses >20 cm² was 121. The difference was highly significant, $p = 0.0057$. There was a significant difference

TABLE IIIB. Summary of responses to IT

Patient No.	Skin test	Placebo					Total symptom-medication score
		% Change	% Change	% Change	% Change	% Change	
		Immediate cutaneous response	Late cutaneous response				
1	SPST	-7.10	33.85	5.41	205.08	110.74	52
2	SPST	-39.63	5.08	187.13	246.15	188.89	63
3	SPST	-49.20	-73.57	364.30	5540.00	1107.69	105
4	SPST	-101.92	346.15	24.47	135.14	36.92	248
5	SPST	56.42	136.45	-3.64	223.44	173.23	164
6	SPST	20.95	-100.00	5.85	223.08	135.51	79
7	MPST	64.72	-39.42	22.97	253.15	-53.36	118
8	MPST	22.22	-63.78	64.84	204.47	55.51	170
9	MPST	-12.64	39.24	59.33	225.93	164.46	67
10	MPST	-16.41	18.75	20.93	185.71	116.77	43
11	MPST	40.57	3.51	122.28	207.11	-59.57	207
12	MPST	-38.52	-73.01	224.17	850.00	190.00	131
13	MPST	-35.94	-65.80	61.39	268.97	118.42	187
14	MPST	97.56	36.67	1694.96	166.67	123.48	179
15	MPST	-35.26	-67.58	64.00	516.00	171.00	202
16	MPST	-10.02	11.16	-11.20	175.76	113.68	158
17	MPST	ND	ND	9.89	179.03	66.83	51
18	MPST	-22.67	10.81	26.77	219.57	123.58	135
19	MPST	-35.10	-0.46	-37.73	158.02	98.60	127
20	MPST	-7.58	-26.49	87.38	291.19	110.48	76
21	MPST	-28.37	-77.19	313.61	831.88	46050.00*	92
22	MPST	ND	ND	-14.91	180.49	ND	188
23	MPST	-22.29	-74.63	1198.78	401.64	803.00	162
24	MPST	71.24	112.47	40.53	240.00	56.25	182
25	MPST	-44.09	-25.53	63.00	211.34	134.00	62
Mean		3.209	2.91	183.78	493.59	163.45	130

SPST, Single positive skin test; MPST, multiple positive skin test; ND, not done.

*Outlier excluded from analysis.

between the first group (0 cm²) and the second (1 to 20 cm²) and the third groups (21 + cm²), $p < 0.05$ for both groups, but no significant difference between groups two and three (Fig. 3). Cumulative dose of MC antigen was also significantly related to symptom-medication scores, $r_s = 0.314$ and $p = 0.0264$ (Table VI).

Antibody levels

Postseason levels of MC sIgG1 were not significantly related to symptom-medication scores, whereas postseason levels of MC sIgG4 were $r_s = -0.397$ and $p = 0.005$ (data not presented).

Postseason levels of MC sIgE were significantly lower in actively treated subjects than in control subjects, $p = 0.0001$. Both MC sIgG1 and MC sIgG4 levels were significantly higher in actively treated subjects than in control subjects, $p < 0.0001$. Both MC

sIgG1 and MC sIgG4 levels rose in the placebo-treated subjects, presumably because of natural exposure to MC pollen, $p = 0.0479$ and $p = 0.0024$, respectively.

No significant differences in the responses of MC sIgE, MC sIgG1, or MC sIgG4 could be detected in our subjects when subjects were divided into groups with single positive skin tests or multiple positive skin tests (data not presented).

DISCUSSION

Allergic rhinoconjunctivitis caused by MC pollen is a significant problem during the winter months in San Antonio. Pollen counts of $>10,000/\text{m}^3$ can accompany northern fronts from the densely wooded areas north and west of the city. MC pollen differs chemically from most other known allergens, having a carbohydrate content of 80% to 90%, and its major

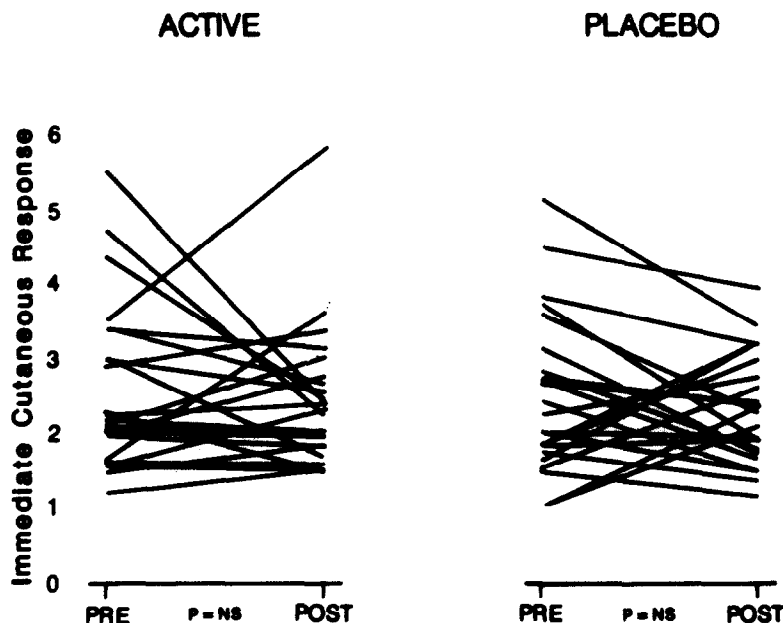


FIG. 1. The sizes of the immediate cutaneous response in the actively treated subjects versus the placebo-treated subjects before the start of IT (*PRE*) and after the pollen season (*POST*). Mean *POST* immediate cutaneous responses in actively and placebo-treated subjects were 2.4 cm² and 2.3 cm², respectively; *p* = NS (not significant).

TABLE IV. Effects of treatment and skin test status on Symptom-medication scores

	Single positive skin test	Multiple positive skin test	Totals
Active	56.5	57.2	57.0*
Placebo	118.5	133.5	129.9*
Total	87.5†	94.4†	92.8

**p* < 0.0001.

†*p* = not significant.

allergen having a relatively high-molecular-weight of 40,000 daltons.¹¹ It has been postulated that this high level of exposure and these unique characteristics of the antigen account for sensitivity in a distinct group of patients.⁴ From previous studies,^{4,5} these patients would be classified as nonatopic, based on personal and family history and skin tests if it were not for their single sensitivity to MC. The existence of relatively large numbers of individuals in this group with a single positive skin test appears to be unique to MC sensitivity. In contrast to earlier retrospective studies, we found no significant differences in the age, sex, total IgE, or personal or family history of atopy between patients with multiple positive skin tests and single positive skin tests in this prospective study. There were no significant differences in the levels of total IgE, MC sIgE, MC sIgG1, or MC sIgG4 between the two groups at entry into the study, nor were there any differences in their responses to IT, based on

changes in these antibody levels or comparison of peak season symptom-medication scores. Analysis of the characteristics of the patient population of Pence et al.⁶ also reveals no significant differences in age, sex, skin reactivity, RAST, or total IgE. We have previously demonstrated that the only detectable IgG responses to MC IT were from subclasses 1 and 4⁵; we therefore did not measure MC sIgG2 or MC sIgG3. Although our findings confirm the existence of a group of MC-sensitive patients with single positive skin tests based on prick skin testing, the absence of any immunologic or clinical differences in patients with multiple positive skin tests casts doubt on the usefulness of recognizing patients with single positive skin tests as a distinct group for any clinical reason, such as predicting response to therapy.

Retrospective analysis of patients with single positive skin tests versus patients with multiple positive skin tests, as was done by Ramirez⁴ and by us in our

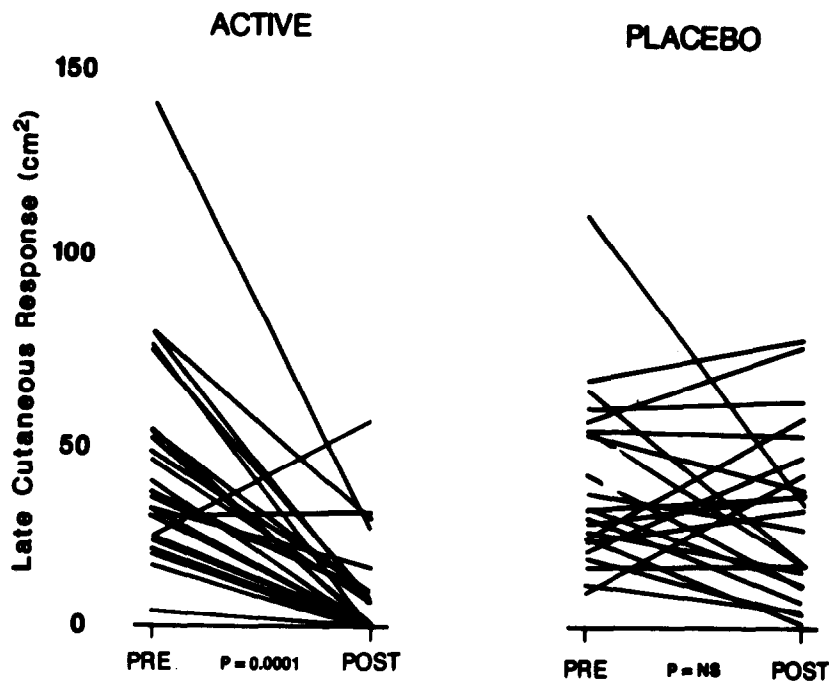


FIG. 2. The sizes of the late cutaneous response in the actively treated subjects versus the placebo-treated subjects before the start of IT (*PRE*) and after the MC pollen season (*POST*). Mean *POST* late cutaneous responses in actively and placebo-treated subjects were 33.4 cm² and 8.5 cm², respectively; *p* = 0.0001.

TABLE V. Relationship of percent change in late cutaneous response with symptom-medication score and MC sIgG subclass levels

	<i>r</i>	<i>Z</i>	<i>p</i> Value
Symptom-medication score	−0.566	3.800	<0.0002
MC sIgG1	0.626	4.199	<0.0002
MC sIgG4	0.549	3.680	<0.0002

TABLE VI. Relationship of cumulative dose of allergen to percent change in late cutaneous response, symptom-medication score, and rank

	<i>r</i>	<i>r</i> ²	<i>p</i> Value
% Change late cutaneous response	0.283	0.080	0.0571

	<i>r</i> _s	<i>Z</i>	<i>p</i> Value
Symptom-medication score	0.312	−2.207	0.0232
Rank	0.314	2.220	0.0264

first study,⁵ may be influenced by an age-dependent bias. Our clinic and IT population tends to have a preponderance of older military retirees who come to live in San Antonio late in life and are exposed to MC pollen for the first time. Incidence of new atopic

diseases increases for 10 years after settlement in a new area.¹² Older subjects who begin allergic symptoms tend to have fewer positive skin tests and a lower incidence of a positive family history of atopy.¹³ Finally, skin test reactivity in terms of numbers and size

of skin tests correlates linearly with IgE, and total IgE tends to fall with older age.¹⁴ Our prospective study that enrolled subjects as they were first observed, eliminated the age bias that would have appeared in a retrospective chart review and demonstrated no differences between subjects with single positive skin tests and multiple positive skin tests, other than their skin tests. This finding was supported by an analysis of the subjects of Pence et al.⁶ in their pioneering study.

Our evaluation of the efficacy of IT for MC pollinosis confirms the clinical experience of most allergists in our area of the country and the work of Pence et al.⁵ Patients actively treated with MC extract had significantly lower symptom-medication scores during the peak pollen season compared to placebo-treated patients. The two treatment groups were well matched by end point titration, and there were no significant differences in numbers of patients with single positive skin tests/multiple positive skin tests, age, sex, or antibody levels on initiation of IT. We found a significant correlation between percent change of the late cutaneous response and symptom-medication score, $r_s = -0.566$ and $p < 0.0002$, and we also found that subjects with no measureable late cutaneous response at the end of the MC-pollen season had less than half the symptom-medication scores of the subjects with a measureable late cutaneous response, indicating that suppression of the late cutaneous response was associated with lower symptom-medication scores. This suppression of the late cutaneous response was also significantly associated with the postseason levels of both MC sIgG1 and MC sIgG4.

Suppression of the late cutaneous response to ragweed by IT with ragweed extract has been documented in a retrospective study.¹⁵ This effect on the late cutaneous response was demonstrated to correlate with the level of ragweed-specific IgG, but not IgE. The relationships between size of late cutaneous response, specific antibody levels, and symptom relief could not be examined. A previous study from our institution demonstrated, prospectively, that suppression of the late cutaneous response to MC could be attained by IT with MC extract and that this suppression correlated with the production of MC sIgG4, but not MC sIgG1.⁷ In that study, however, there was no significant difference in the symptom-medication score between IT and placebo-treated patients, probably because of low levels of MC pollen that year, and likewise no relationship between symptom-medication scores and specific antibody levels or suppression of late cutaneous response. The significant relationship of MC sIgG1 with suppression of the late cutaneous response in this study may be due to the use of a monoclonal antibody

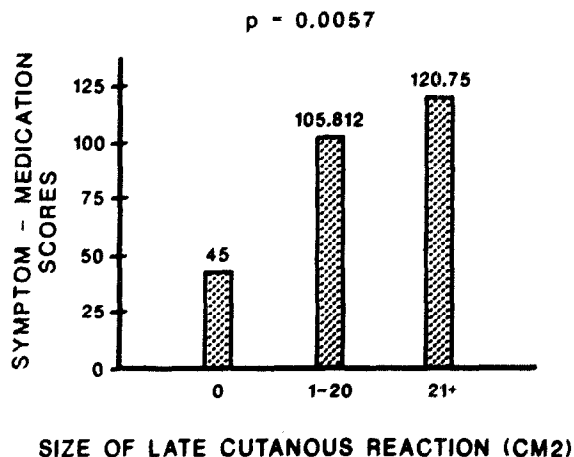


FIG. 3. Mean symptom-medication scores stratified into three groups based on size of late cutaneous response after the MC-pollen season. The p value refers to the overall difference as determined by nonparametric one-way analysis of variance (Kruskal-Wallis). The mean symptom-medication score of the first group (0) was significantly smaller than the mean symptom-medication score of the second (1-20) and third groups (21+), $p < 0.05$ in both cases. The mean symptom-medication score of the second group (1-20) was not statistically different from the third group (21+).

with better affinity than the monoclonal antibody used in our previous study.⁷ This study then is the first to document in a prospective, controlled manner a relationship between antigen-specific IgG, suppression of the late cutaneous response, and clinical improvement with IT.

Previous studies have found a modest change in the immediate cutaneous response to allergen during the course of IT with titrated prick and intradermal skin testing.^{16, 17} We probably failed to observe a change in the immediate cutaneous response because the large amount of injected allergen pushed our dose response up to the shoulder of a hypothetical sigmoid relationship between dose of allergen and size of the immediate wheal.

The mechanism by which IT causes symptom relief in rhinitis is poorly understood. Among the immunologic changes resulting from IT are generation of IgG blocking antibody and suppression of the seasonal rise in antigen-specific IgE.¹⁸ Our study is in agreement with other studies that demonstrate a correlation between blocking antibody level and clinical improvement,^{19, 20} although lack of predictive accuracy of antibody level for improvement in a given patient has led some investigators to doubt a causal relationship between the two. The concentration of blocking antibody rises rapidly in the first few months of therapy, reaching a plateau despite continued antigen admin-

istration.¹⁹ The magnitude of this increase is dose dependent,²⁰ and our association of dose administered with specific IgG subclass levels confirms this relationship. We observed blunting of the seasonal rise of MC sIgE in our actively treated patients, and we observed significant rises in both MC sIgG1 and MC sIgG4 in our placebo-treated patients (although we observed much less than in the actively treated patients), presumably because of natural exposure to MC pollen.

Several authors have proposed that the late reaction to allergen is more clinically relevant than the immediate reaction.^{21, 22} Studies of nasal and cutaneous reactions suggest a relationship between the two reactions. The profile of mediators released, as measured by skin blister technique and nasal washings, also suggests a common pathogenesis for these responses.²³⁻²⁵ Several studies document the dependence of the late cutaneous response on antigen-specific IgE and the ability of antigen-specific IgG to block this reaction in an animal lung model.²⁶⁻²⁸ Atkins et al.,²⁹ however, were unable to correlate the intensity of the late cutaneous response with bronchial reactivity, demonstrating the late cutaneous response correlated with specific IgE, whereas the bronchial reactivity was most closely associated with neutrophil chemotactic activity. Our study demonstrates a significant improvement in symptom-medication scores with IT, and this improvement was closely associated with suppression of the late cutaneous response and generation of antigen-specific IgG4 subclass antibodies. This finding lends support to the theory that similar immunologic mechanisms regulate the late cutaneous response and the nasal late-phase response to antigen. The correlation of symptom relief and suppression of late cutaneous response in our study suggests that measurement of suppression may help predict clinical response to IT. A dose-response study would be necessary to determine whether, in fact, the lowest level of IT required to significantly suppress the late cutaneous response will achieve significant symptom relief. We have induced late cutaneous responses in >70 MC-sensitive patients without any significant problems. However, we advise caution in attempting this maneuver with other allergens, since untoward systemic reactions may be induced.

In summary, this prospective, double-blind, placebo-controlled study demonstrated that IT caused significant symptom relief for MC pollinosis. We failed to find any clinical or immunologic differences between MC-allergic patients with multiple positive skin tests and single positive skin tests, and these two groups did not differ in their clinical or immunologic responses to IT. Symptomatic improvement was

closely associated with suppression of the late cutaneous response to MC and the generation of MC sIgG4.

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Correction

The following corrections apply to the article, "Effect of combined maternal and infant food-allergen avoidance on development of atopy in early infancy: A randomized study," by Zeiger et al. that appeared on page 72 of the July 1989, vol 84, no. 1 issue of *THE JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY*.

1. In Table VIII, page 85, the terms beneath "Predictive capacity of cord blood IgE >0.5 IU/ml" were in improper order and should read:

Positive predictive value (%)
 Negative predictive value (%)
 Sensitivity (%)
 Specificity (%)
2. On page 84 in the right column, the first line should begin "sensitivity and equal specificity" and in the third line the word "capacity" should replace "sensitivity."
3. On page 87 in the right column, second paragraph and third line, the words "positive predictive values" should replace "sensitivities."