

The effect of immunotherapy on the cutaneous late phase response to antigen

William A. Nish, MD,^a Ernest N. Charlesworth, MD,^a Thomas L. Davis, MD^b
Bonnie A. Whisman, MS,^a Sandra Valtier, BS,^a
Margaret G. Charlesworth, MAS,^a and Kristin M. Leiferman, MD^c
Lackland Air Force Base, Texas, and Rochester, Minn.

Background: This study used the skin chamber model to evaluate prospectively the effect of immunotherapy (IT) on the cutaneous early and late phase response (LPR) to epicutaneous antigen challenge.

Methods: Nine subjects with allergic rhinitis were studied at three time points: before starting IT, after 3 months of IT, and after 6 months of IT. Skin chamber histamine content was measured hourly for 12 hours, and cell counts performed hourly during hours 6 to 12. An intradermal skin test was placed, and the reaction was measured hourly for 12 hours. Skin biopsy specimens were obtained 8 hours after intradermal placement and evaluated for cellular infiltrate and major basic protein deposition. Serum antigen-specific IgG and IgE levels were measured at each time point to confirm physiologic effect of IT.

Results: Six months of IT significantly ($p < 0.05$) decreased both early and LPR skin test reactivity and skin chamber histamine for hours 1 to 3, 4 to 6, and 9 to 12. Skin chamber LPR cellular influx decreased significantly ($p < 0.05$) for neutrophils only. Decrease in LPR histamine after 6 months of IT was significantly correlated with both decrease in mononuclear cells ($R^2 = 0.817$, $p = 0.002$) and decrease in neutrophils ($R^2 = 0.813$, $p = 0.009$). Also significantly correlated were decrease in LPR skin test reactivity, with percent change in skin chamber mononuclear cells ($R^2 = 0.800$, $p = 0.009$) and decrease in early skin test reactivity ($R^2 = 0.675$, $p = 0.01$). Biopsy specimens showed no consistent change in either dermal cellular infiltrate or deposition of major basic protein.

Conclusion: IT significantly attenuates cutaneous histamine release and skin test reactivity and is accompanied by a decrease in skin chamber LPR neutrophil influx without significantly altering the dermal infiltrate at 8 hours. (*J ALLERGY CLIN IMMUNOL* 1994;93:484-93.)

Key words: Immunotherapy, late-phase response, skin chamber, allergic rhinitis

Although the mechanisms by which immunotherapy (IT) promotes clinical improvement in allergic rhinitis are not fully understood, both retrospective¹ and prospective studies^{2, 3} have demonstrated that the size of the cutaneous late phase response (LPR) is decreased after IT. The

Abbreviations used

AU:	Antigen unit
HDM:	House dust mite
IL:	Interleukin
IT:	Immunotherapy
LPR:	Late phase response
MBP:	Major basic protein
MC:	Mountain cedar
OCD:	Optimal challenge dose
PNU:	Protein nitrogen unit

From ^athe Allergy-Immunology Department and ^bthe Department of Dermatology, Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, and ^cthe Department of Dermatology, Mayo Clinic, Rochester.

Supported in part by National Institutes of Health grant AR36008.

Received for publication Dec. 21, 1992; revised June 8, 1993; accepted for publication June 8, 1993.

The opinions expressed are those of the authors and do not necessarily represent the views of either the United States Air Force or the Department of Defense.

Reprint requests: William A. Nish, MD, Allergy-Immunology Service, 301 Fisher St., Suite 401, Keesler Air Force Base, MS 39534-2531.

1/1/49186

skin chamber has been used⁴⁻⁶ as a model for studying the cutaneous LPR to allergen by measuring cells and mediators in chamber fluid during the LPR. With the use of the skin chamber it has previously been shown that a second rise in histamine is concomitant with the cutaneous LPR, analogous to earlier work by Naclerio et al.,⁷ who used a nasal model. This late histamine release

has been associated with an influx of neutrophils, eosinophils, and basophils into the skin chamber.⁴

The purpose of our study was to evaluate the effect of 6 months of high-dose IT on the cutaneous LPR to antigen. We studied subjects with allergic rhinitis to *Juniperus ashei*, commonly known as mountain cedar (MC), and *Dermatophagoides pteronyssinus*, or house dust mite (HDM). MC is a potent seasonal allergen in south Texas, with a characteristic pollen season from November through February, during which time few other species are actively pollinating.⁸ House dust mite is a perennial allergen in the San Antonio area. Subjects chosen were allergic only to MC or HDM, avoiding possible priming effects from other allergens. We measured changes in cellular influx and histamine release into skin chambers in addition to immunohistologically examining LPR skin biopsy specimens taken 8 hours after intradermal skin testing.

METHODS

Patients

Subjects with allergic rhinitis were selected on the basis of history and skin test reactivity to MC (*J. ashei*) or HDM (*D. pteronyssinus*) and the demonstration of a cutaneous LPR. Subjects were skin tested by the investigators to determine the optimal challenge dose (OCD) for skin chamber studies and to document the occurrence of an LPR. The OCD is the dose of antigen that elicited a wheal at least 10 cm² with pseudopods, or 15 cm² with a flare of at least 45 cm². The OCD was determined by titrated intradermal skin testing (0.02 ml), starting with 1 antigen unit (AU)/ml for HDM or 1 protein nitrogen unit (PNU)/ml for MC, and increasing until the OCD was reached or to a maximum dose of 1000 AU/ml or 1000 PNU/ml. Skin test data were documented and measured by the tape transfer technique. Subjects were then instructed in self-measurement of the cutaneous LPR by tape transfer. Subjects who on follow-up visit had a documented cutaneous LPR were invited to enroll in the study. Exclusion criteria included the following: age less than 18 years, pregnancy, IT within 5 years, current use of β -blocking medications, or the presence of concurrent medical problems. The protocol was approved by the Wilford Hall Medical Center Institutional Review Committee.

Extracts

The same stock extracts of either MC or HDM were diluted for the initial skin testing and for all subsequent skin testing and chamber challenges. MC extract (saline solution with 0.3% human serum albumin; Hollister-Stier, Spokane, Wash.) from one lot with a strength of 9500 PNU/ml was lyophilized and refrigerated. The same investigator reconstituted the extract 1 day before each study with sterile albumin saline solution with

0.04% phenol (Hollister-Stier), and serial dilutions were made each time extract was used. A single vial of standardized extract of HDM (Hollister-Stier) containing 50% glycerin with 10,000 AU/ml was used throughout the course of the study. Serial dilutions were made by the same investigator at appropriate times; identical concentrations and storage conditions were used for each study. Each extract was kept refrigerated at 4° C during the 11-month course of the study. To attempt to rule out any change in potency of extracts with time and to verify identical biologic activity, skin test reactivity to study extracts was periodically tested in two MC- and HDM-allergic patients not involved in the study and not receiving IT. No change in potency was identified for either extract.

Skin chamber studies

Skin chamber studies were performed before the start of IT, and after 3 months and after 6 months of IT. Subjects allergic to MC were studied outside the MC season to avoid any priming effect. HDM is a perennial allergen in the San Antonio area. Conventional high-dose IT^{2,3} was administered, and subjects were allowed to progress to maintenance doses at their own paces. The dose of antigen used for the epicutaneous skin chamber challenge was 10 times the OCD. Administration of all antihistamines was stopped at least 3 days before skin chamber studies. Daily symptom diaries were not maintained because we studied MC-allergic patients out of season and it was not our goal to assess the clinical efficacy of IT.

Twelve hours before the study, superficial skin blisters with a diameter of approximately 0.5 cm were raised on the ventral surface of each forearm. These were formed by use of a heat-suction device⁴ that applied both suction and a steady temperature of 39° C. Blisters were formed at the same time in relation to the start of each study. On the day of the study both blisters were unroofed and any fibrin clot that had formed was gently removed. Metal skin chambers with an interior volume of approximately 500 μ l were secured over the denuded skin surface with tape and ostomy adhesive. The chambers included a side port for adding and withdrawing fluids and a top port for venting. Chambers on both the right and left forearms underwent two 5-minute washes with 0.5 ml of lactated Ringer's solution with 250 μ g/ml heparin before the challenge was commenced. The antigen side was challenged with 0.5 ml of 10 times the OCD of the appropriate antigen (MC or HDM) for 1 hour, whereas the control side was challenged with 0.5 ml of the lactated Ringer's solution for an equal time. After 1 hour the fluid was withdrawn from each chamber, transferred to 1.8 ml polypropylene centrifuge tubes, and replaced with the lactated Ringer's solution on both sides. This same procedure of withdrawing fluid each hour and replacing it with fresh lactated Ringer's solution was followed for both chambers for the duration of the study. Simultaneously with the commencement of the skin chamber antigen challenge, a 0.02 ml intradermal skin test of the OCD of

TABLE I. Changes in serum levels of antigen-specific antibodies with IT in HDM-allergic subjects

Subject	Parameter	Pre-IT	3 mo	6 mo
1	Dose	0	68	1013
	IgG	106	123 (116%)	195 (184%)
	IgE	41	61 (149%)	50 (122%)
2	Dose	0	3.3	317
	IgG	108	95 (88%)	131 (122%)
	IgE	2	26 (1600%)	32 (1300%)
3	Dose	0	26	131
	IgG	70	143 (204%)	187 (267%)
	IgE	15	26 (173%)	20 (133%)
4	Dose	0	48	813
	IgG	94	ND	185 (197%)
	IgE	55	ND	42 (76%)
Mean (SD)	Dose	0	36.3 (24.2)	569 (358)
	IgG	94.5 (15.1)	120 (19.7)	175 (25.4)
	IgE	28.3 (20.9)	37.7 (16.5)	36.0 (11.2)

ND, Not done.

For subjects 1 to 4 antigen doses are expressed as cumulative AU administered; IgE is expressed as percent binding, and IgG in nanograms per milliliter; 3- and 6-month antibody levels are expressed as percentage of pre-IT level in parentheses.

antigen was placed on the proximal portion of the arm, which contained the antigen-challenged skin chamber. The size of the intradermal skin test was measured and documented by tape transfer after the first 15 minutes and then hourly throughout the study. Flare size at each point was subsequently measured with a Vidas planimeter (Kontron Instruments, Everett, Mass.).

Cell counts were performed hourly beginning at hour 6. The polypropylene tubes were centrifuged at 3000 rpm for 5 minutes. The supernatant was withdrawn and frozen at -70°C , then the pellet was reconstituted with 0.5 ml of the lactated Ringer's solution. The tubes were then agitated and 10 μl was withdrawn for cell count with a Neubauer hemacytometer (Reichert Scientific Instruments, Div. of Warner-Lambert Technologies Inc., Buffalo, N.Y.). The remaining cell suspensions were layered onto glass slides by cytocentrifugation at 600 rpm for 5 minutes. The slides were stained with Wright-Giemsa stain. Differential cell counts were performed on 100 cells per slide by a certified medical technologist who was unaware of the origin of the slide.

Skin chamber fluid was pooled into hours 1 to 3, 4 to 6, 7 to 9, and 10 to 12 and stored at -70°C . The fluid was analyzed at a later date for histamine content with a radioenzymatic assay, as previously described,⁹ with a sensitivity of 0.05 ng/ml. All histamine assays for a single subject were performed at the same time to avoid interassay variability.

Serum samples were obtained from each subject during each study for the subsequent measurement of antigen-specific IgG and IgE. For HDM-allergic study subjects serum was sent to a commercial laboratory (Specialty Laboratories, Inc., Santa Monica, Calif.) for determination of HDM-specific IgE and IgG levels by radioallergosorbent assay. For MC-allergic subjects ELISA was performed in our laboratory by standard

techniques for MC-specific IgG, IgG₄, and IgE, as previously described.²

Biopsy specimens

Seven of the nine subjects volunteered to undergo skin biopsies as part of the study. Biopsy specimens were obtained from LPR sites 8 hours after antigen challenge both before IT and after 6 months of IT. The OCD of HDM or MC (0.02 ml) was injected intradermally in the upper arm, and a 4 mm punch biopsy was obtained at the center of the cutaneous LPR 8 hours later. The biopsy specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Sections were examined by a dermatopathologist in a blinded fashion. Differential cell counts on 100 cells were performed on each biopsy specimen. A visual analog scale of 1+ to 4+ was used to assess semiquantitatively the overall density of cellular infiltration. Additionally, biopsy specimens were stained for eosinophil granule major basic protein (MBP) by indirect immunofluorescence with affinity chromatography-purified polyclonal antibody.¹⁰ Both cellular and extracellular MBP localization were judged on a visual analog scale of 1+ to 4+ on the bases of numbers of cells and the extent of extracellular protein deposition.

Statistics

The Wilcoxon signed-rank test (Statview; Abacus Concepts Inc., Berkeley, Calif.) was used to compare changes in the various parameters recorded during the course of the study. Analysis of variance was used to establish statistical significance of pre-IT versus post-IT measurements, and regression analysis was used for comparative analysis of correlation between different measurements. All results were compared after the

TABLE II. Changes in serum of antigen-specific antibodies with IT in MC-allergic subjects

Subject	Parameter	Pre-IT	3 mo	6 mo
5	Dose	0	3.3	316
	IgG	1.6	1.5 (94%)	2.6 (163%)
	IgG ₄	0.022	0.05 (227%)	0.367 (1670%)
	IgE	5.8	3.6 (62%)	4.9 (84%)
6	Dose	0	6.2	201
	IgG	15.1	13.6 (90%)	19.1 (127%)
	IgG ₄	0.072	0.141 (196%)	0.602 (836%)
	IgE	6.8	71.1 (1046%)	61.6 (906%)
7	Dose	0	1.1	46
	IgG	9.9	7.6 (77%)	7.5 (76%)
	IgG ₄	0.006	0.006 (100%)	0.020 (333%)
	IgE	1.8	1.4 (78%)	2.3 (128%)
8	Dose	0	1.7	723
	IgG	8.8	4.7 (53%)	14.9 (169%)
	IgG ₄	0	0	0.448 (∞)
	IgE	3.1	1.8 (58%)	4.9 (158%)
9	Dose	0	2.8	88
	IgG	7.2	7.0 (97%)	9.1 (126%)
	IgG ₄	0.087	0.096 (110%)	0.106 (122%)
	IgE	22.1	10.5 (48%)	31.8 (144%)
Mean (SD)	Dose	0	3.02 (1.77)	275 (243)
	IgG	8.52 (4.36)	6.88 (3.98)	10.6 (5.77)
	IgG ₄	0.037 (0.035)	0.059 (0.054)	0.309 (0.216)
	IgE	7.92 (7.31)	17.7 (26.91)	21.1 (23.0)

Antigen dose is expressed in cumulative PNU administered; units for antibody levels were arbitrarily derived from ELISA standard curve; 3- and 6-month antibody levels are expressed as percent of original values in parentheses.

control chamber values were subtracted from the antigen-challenged chamber values.

RESULTS

A total of nine subjects with allergic rhinitis participated in the study. Five were allergic to MC, and four were allergic to HDM. Eight subjects were allergic to a single antigen; the other subject was allergic to both MC and HDM. Two subjects had mild asthma but did not require chronic antiinflammatory therapy. Only one subject had been previously treated with IT, and that had been discontinued 13 years before entry into this study. Five women and 4 men, with ages ranging from 19 to 45 years, and a mean age of 34 years, participated in the study.

Serum antigen-specific antibodies

Changes in serum HDM-specific IgG and IgE levels, with the cumulative dose of HDM (in AU) for each subject at each study point, are shown in Table I. Levels of antibody at 3 and 6 months are expressed as a percentage of the pre-IT level. Increases in levels of IgG were consistently found; changes in specific IgE were less consistent. Table II shows the results for MC-allergic subjects, in

whom IgG₄ levels were also measured and doses of MC were calculated in PNU. Subjects 7 and 9 had relatively low total cumulative doses of antigen and demonstrated the least increase in IgG and IgG₄ levels.

Skin test reactivity

Mean changes in skin test reactivity during the course of the study are shown in Fig. 1. Note that for the pre-IT study group the cutaneous early phase was maximal at 15 minutes and decreased by 2 to 3 hours. The cutaneous LPR began at 3 to 4 hours, peaked at 8 hours, and declined by 12 hours. The marked diminution in cutaneous LPR after IT is clearly shown in Fig. 1, with lesional areas significantly reduced for hours 4 to 12 ($p < 0.05$). The skin reactivity in the early cutaneous response (hours 1 to 3) was also significantly decreased by IT ($p < 0.05$).

Skin chamber studies

The antigen doses used in the skin chamber challenges were as follows: MC, 100 PNU/ml for three subjects; MC, 1000 PNU/ml for two subjects; HDM, 100 AU/ml for two subjects; and HDM, 1000 AU/ml for two subjects. Cumulative

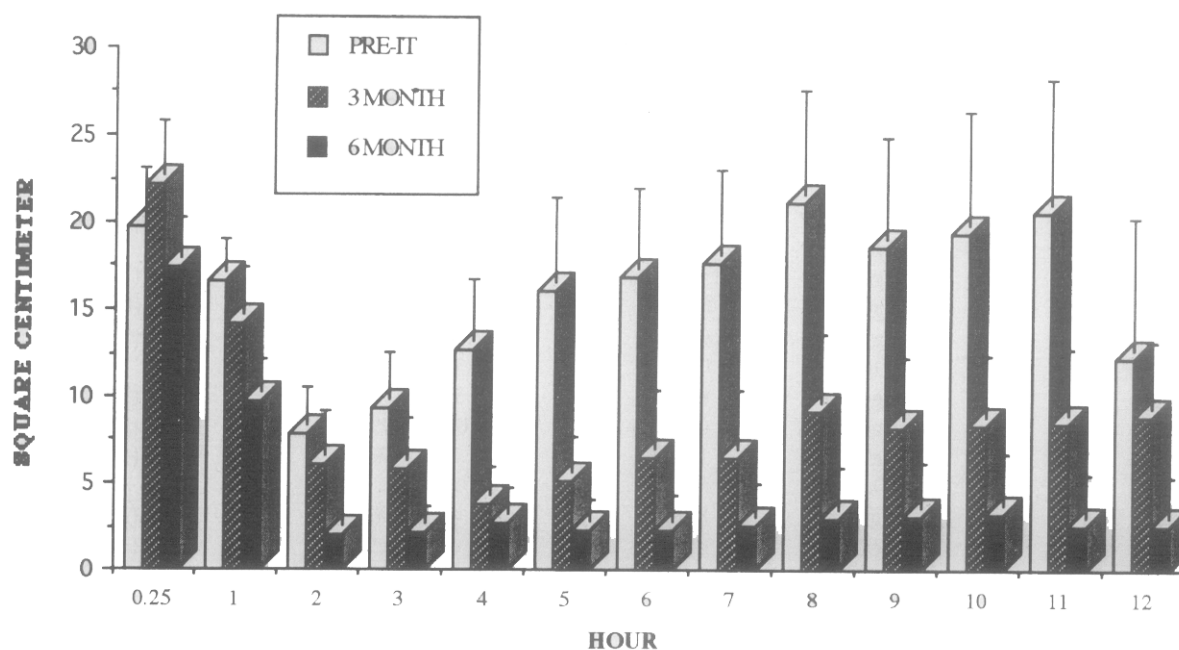


FIG. 1. Change in skin test reactivity over the course of the study. Bars indicate standard error of the mean.

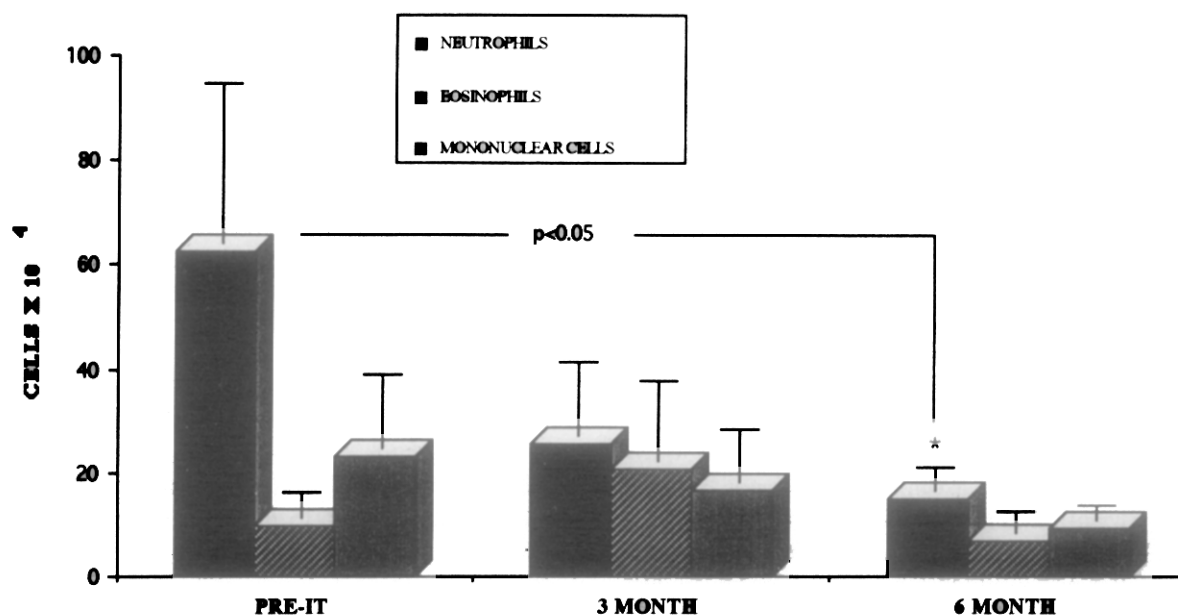


FIG. 2. Changes in total cellular influx into skin chamber during hours 6 to 12 for each cell type during the course of the study.

cell counts for hours 6 to 12 for each cell type are shown in Fig. 2. The numbers of neutrophils decreased with IT at both 3 and 6 months but were significantly decreased ($p < 0.05$) from the pre-IT ($62.8 \pm 30.4 \times 10^4$ cells) numbers to the 6-month ($14.9 \pm 4.79 \times 10^4$ cells) study. Mononuclear cells also decreased ($23.1 \pm 14.5 \times 10^4$,

$16.7 \pm 10.2 \times 10^4$, and $9.35 \pm 2.87 \times 10^4$ cells, respectively) during the course of the study, but the numbers were not significantly different. Eosinophils increased at 3 months ($9.78 \pm 4.66 \times 10^4$ cells before IT to $20.6 \pm 15.4 \times 10^4$ cells), followed by a decrease at 6 months ($6.98 \pm 3.88 \times 10^4$ cells); neither of these changes was

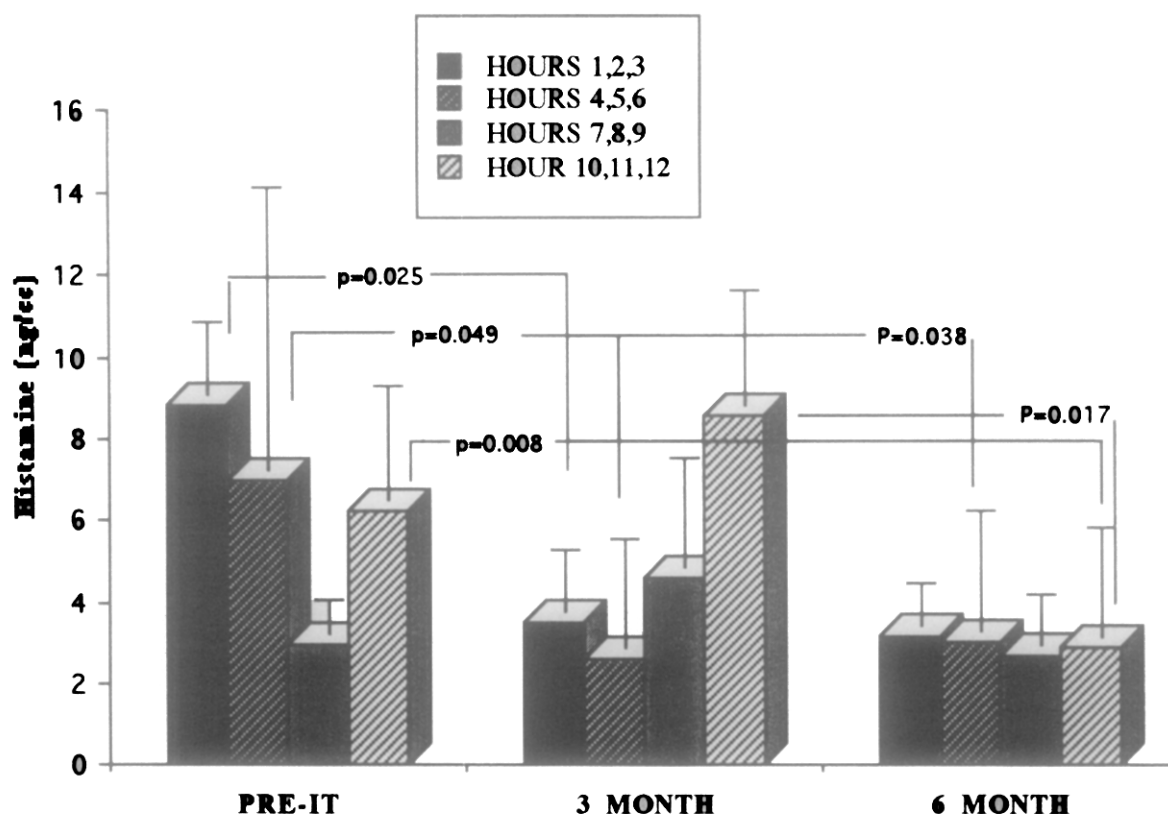


FIG. 3. Changes in skin chamber histamine release during the course of the study.

statistically significantly different from the pre-IT counts. The greatest change in neutrophil influx occurred in the latter hours of the LPR and was significantly decreased before IT versus after IT at hours 9 to 12.

After IT the histamine content in skin chambers decreased significantly in both the early reaction and the LPR (Fig. 3). During hours 1 to 3 histamine levels decreased significantly ($p \leq 0.025$) during the course of the study from the pre-IT levels (mean, 9.43 ± 1.84 ng/ml) to 3-month levels (3.5 ± 1.5 ng/ml; $p < 0.025$), to 6-month levels (2.15 ± 0.37 ng/ml; $p < 0.03$). During hours 4 to 6 histamine levels also decreased from pre-IT levels (6.05 ± 1.78 ng/ml) to 3-month levels (2.62 ± 0.94 ng/ml; $p < 0.05$), to 6-month levels (1.76 ± 0.64 ng/ml; $p < 0.04$). Finally, for hours 10 to 12, histamine levels markedly decreased from pre-IT levels (3.62 ± 1.39 ng/ml) to 6-month levels (1.97 ± 1.08 ng/ml; $p = 0.008$). Histamine levels showed a slight increase for hours 10 to 12 at 3 months (5.04 ± 2.81 ng/ml) followed by a significant decrease from 3 to 6 months ($p = 0.017$).

Regression analysis revealed several interesting correlations. The observed decrease in early skin

test reactivity correlated significantly ($R^2 = 0.675$, $p < 0.01$) with decrease in LPR skin test reactivity, suggesting a relationship between the early mast cell-mediated events and the more complex events of the LPR. Furthermore, the decrease in LPR skin test reactivity correlated significantly ($R^2 = 0.8$, $p = 0.009$) with decrease in percentage of mononuclear cells but not with eosinophils or neutrophils. Finally the decrease in LPR histamine release after IT correlated with both the decrease in mononuclear cells ($R^2 = 0.817$, $p = 0.002$) and the decrease in neutrophils ($R^2 = 0.813$, $p = 0.002$).

Biopsy

Seven of nine subjects agreed to undergo biopsies during the study. Table III shows changes in cellular infiltration of lesions at 8 hours. All biopsy specimens were examined in a blinded fashion. No consistent changes in the cell density or cellular composition were observed with IT. Three of the seven subjects converted to predominantly mononuclear cell infiltration with IT, but two other subjects showed a predominantly mononuclear cell infiltration before IT.

The biopsy specimens were also examined by

TABLE III. Effect of IT on cutaneous LPR biopsy cellular infiltrate and MBP deposition

Subject	Total white blood cells present (%)						Infiltrate density	Change in cells	Change in MBP	
	Neutrophil		Mononuclear		Eosinophil					
	Pre-IT	Post-IT	Pre-IT	Post-IT	Pre-IT	Post-IT				
1	33	30	33	40	33	30	+2 to +3	+1 to +2	Decrease	None
3	1	20	98	50	1	30	+1	+2 to +3	Increase	Decrease
4	1	40	99	20	0	40	+1	+3	Increase	Increase
5	20	1	50	99	30	0	+2— +3	+1	Decrease	None
7	33	30	33	35	33	35	+2— +3	+3	None	None
8	33	1	33	98	33	1	+2— +3	+1	Decrease	Increase
9	60	10	20	60	20	30	+2— +3	+2	None	Decrease

1+ to 4+ scale indicates overall cell density on a visual analog scale.

indirect immunofluorescence for the presence of MBP in a blinded fashion (Table III). No consistent changes in MBP staining were observed during the course of the study.

No significant differences were found between MC-allergic and HDM-allergic subjects for any of the responses in skin chamber or biopsy studies.

DISCUSSION

Several studies have previously shown a decrease in the size of the cutaneous LPR with IT. In an early retrospective study Pienkowski et al.¹ examined the effect of IT in a group of IT-treated ragweed-allergic patients compared with an untreated group. In the untreated ragweed-allergic patients a cutaneous LPR developed in 94%, whereas only one third of the treated group displayed a cutaneous LPR. In their study the size of the LPR correlated inversely with the serum level of IgE antiragweed antibody. The first prospective study examining the effect of IT on the cutaneous LPR was by Fling et al.² in MC-allergic subjects treated with IT and compared with a control group treated with placebo. Suppression of the cutaneous LPR in the IT-treated group was demonstrated and correlated significantly with both the cumulative dose of MC antigen received and with MC-specific IgG₄. A follow-up study of MC-allergic subjects by Parker et al.³ also confirmed correlation of cutaneous LPR suppression with postseasonal level of MC-specific IgG₁ and IgG₄ and with improvement of symptoms. There seems to be a consensus that IT decreases the cutaneous LPR more than the cutaneous early response, but the mechanisms remain obscure.

The allergen-induced cutaneous LPR is charac-

terized by an influx of cells, including basophils, eosinophils, mononuclear cells, and neutrophils.¹¹⁻¹³ The effect of IT on the cutaneous LPR cellular influx has not been previously evaluated. Therefore we used the skin blister chamber model to examine prospectively the effect of IT on skin chamber histamine and inflammatory cell influx in addition to LPR skin test reactivity and LPR skin biopsies.

Cutaneous LPR biopsies have been performed by several investigators previously. Charlesworth et al.⁴ demonstrated an increase in basophils, eosinophils, and mononuclear cells in the skin LPR after antigen challenge compared with non-challenged control sites. Frew et al.¹⁴ showed activated CD4⁺ T lymphocytes and activated eosinophils are present in cutaneous LPR biopsy specimens, but cutaneous LPR diameter at 6 hours correlated best with the number of activated eosinophils rather than with the number of infiltrating T cells.¹⁵ They also demonstrated inhibition of cutaneous LPR T-cell and eosinophil infiltration with IT.¹⁶ Lower numbers of infiltrating CD4⁺ T cells were significantly correlated with lower eosinophil counts after IT.

We were not able to demonstrate any consistent quantitative changes in the number of inflammatory cells in biopsy specimens of the cutaneous LPR. Because we performed biopsies at a single point in time, it is possible that serial biopsies at multiple time points of the late phase might demonstrate a more obvious change. Despite the lack of uniform histologic changes in the LPR cellular infiltrate, all subjects demonstrated a marked diminution in the size of their skin test LPRs. Skin biopsy specimens of the LPR demon-

strated no consistent change in differential cell counts or density of cellular infiltrate, although three subjects did convert to a predominantly mononuclear cell infiltrate.

Cellular influx in LPR skin chambers demonstrated a significant decrease with IT for neutrophils only, whereas monocytes did not decrease significantly and eosinophils tended to increase at 3 months followed by a decline to less than the pre-IT levels at 6 months. We found that IT significantly decreased skin test reactivity to antigen at hours 4 to 12 and early skin test reactivity at 1 to 3 hours. The decrease in LPR skin test reactivity was found to correlate significantly with the decrease in early skin test reactivity. The decrease in LPR skin test reactivity also correlated significantly with percent decrease in mononuclear cells, and the decrease in LPR histamine production with IT correlated significantly with both the decrease in mononuclear cells and the decrease in neutrophils. Comparison of the cellular data for LPR skin biopsy versus LPR skin chamber demonstrates differences. There is a time lag between appearance of cells initially in the dermis and their migration into the skin chamber, as demonstrated in previous LPR biopsy studies of Charleworth et al.⁴ This is partly explained by the fact that the appearance of cells in the dermis depends not only on chemotactic factors but also on cellular adhesion molecules on endothelial cells (such as endothelial-leukocyte adherence molecule-1¹⁷) and their respective ligands on the infiltrating cells. Cells enter the skin chambers more as a result of passive diffusion and less as a result of interaction of cellular adhesion molecules.

A recent study by Iliopoulos et al.¹⁸ used the nasal model to evaluate the effect of IT on nasal early reactions and LPR. Twenty-one subjects with allergic rhinitis to ragweed were treated with active extract for 8 months and compared with 20 subjects treated with placebo, with the use of the nasal challenge model. This study found no significant differences in cellular influx into the nose with antigen. Mononuclear cells tended to decrease but not significantly. A study by Furin et al.¹⁹ also used the nasal model to study a cohort of ragweed-allergic subjects and found a significant decrease in eosinophil influx, compared with untreated control subjects, only after 3 years of IT. The effect of IT on late-phase cellular influx has also been studied in the lung. Rak et al.²⁰ demonstrated that IT attenuated the seasonal rise in

bronchoalveolar lavage fluid eosinophils and eosinophil cationic protein in a group of patients with birch pollen-allergic asthma treated with IT compared with an untreated group. Our findings of the effect of IT on the cutaneous LPR were similar to those of both Iliopoulos et al.¹⁸ and Furin et al.¹⁹ for the nasal LPR. The lack of significance for changes in cellular influx with IT, other than for neutrophils, suggests that IT may achieve clinical efficacy by mechanisms other than simply modifying the number of inflammatory cells infiltrating the dermis in the LPR.

Leiferman et al.²¹ have shown that by the third hour after an intradermal antigen challenge there was extensive deposition of MBP, which persisted for up to 56 hours. Although MBP deposition was found in all of our biopsy specimens, no consistent changes were detected after IT. The MBP data confirmed the routine hematoxylin-eosin histologic cell counts, in which no consistent changes in tissue eosinophil numbers were noted. Again, these data suggest that factors that determine LPR skin test reactivity are complex and not solely dependent on the character of the cellular infiltrate.

Several mediators have been demonstrated to be released during the cutaneous LPR, including histamine⁴ and leukotriene C₄.²² Additionally, there are emerging data that several cytokines are also released into skin chambers during the LPR, including interleukin (IL)-6,²³ IL-8,²⁴ and IL-1.¹⁷ In the nasal model, tosylarginine methyl ester esterase activity and kinins have been found during the LPR,²⁵ which may enhance vasopermeability of serum-derived proinflammatory proteins. With the nasal challenge model IT has been shown to significantly decrease the release of histamine and tosylarginine methyl ester esterase activity,^{13, 26, 27} kinins,¹³ and the production of prostaglandin D₂²⁵ after allergen challenge. Wang and Hsieh²⁸ found that IT decreased antigen-stimulated histamine release in plasma and supernatant from cell cultures of polymorphonuclear and mononuclear cells in children with asthma and also decreased cell culture supernatant leukotriene C₄ while increasing supernatant prostaglandin E₂.

Our study demonstrates a decrease in both early (hours 1 to 3) and late (hours 4 to 6 and 10 to 12) histamine release after high-dose IT. Brunet et al.²⁹ demonstrated a decrease in spontaneous and allergen-induced production of histamine releasing factor from mononuclear cells in IT-

treated patients with allergic rhinitis. Liao and Hsieh³⁰ found similar results in IT-treated children with asthma. Kuna et al.³¹ showed a decrease in allergen-stimulated mononuclear cell histamine releasing factor in a group of IT-treated adults with asthma and further noted that only those patients with clinical improvement also demonstrated a decrease in spontaneous histamine release. A decrease in allergen-induced histamine releasing factor production in patients treated with IT is one possible explanation for the decrease in cutaneous LPR histamine production that we found. It is also possible that the decrease in LPR histamine may simply represent an attenuation of the number of basophils present in the late phase because we did not use specific stains for basophils. However, because the amount of histamine released from basophils and mast cells can be influenced by many variables, including histamine releasing factor³² and histamine release inhibitory factor,³³ it is possible that the absolute numbers of mast cells and basophils are less important than the factors controlling histamine release. The amount of skin chamber histamine during hours 7 to 9 increased from the first to the second study, then decreased back toward baseline levels by the third study. This may indicate an increase in histamine releasability for this time period during the buildup phase of IT, with decrease in releasability with further IT.

Changes in serum antigen-specific IgE and IgG with IT in our study were consistent with those found by previous investigators. Van Metre et al.³⁴ demonstrated an initial rise in ragweed-specific serum IgE with IT, followed by a failure of the customary rise during the allergic season. After 4 to 5 months of IT, 9 of 15 subjects demonstrated a rise in antigen-specific IgE, and 6 of 15 a decline, but all had an increase in allergen-specific IgG. In our study antigen-specific IgE was decreased from baseline in only two of nine subjects after 6 months, but antigen-specific IgG was increased in eight of nine subjects and antigen-specific IgG₄ increased in all five MC-allergic subjects in whom it was tested. Our findings agree with those of other investigators who have observed that the efficacy of IT is not dependent solely on a decrease in antigen-specific IgE.

In summary, our data confirm results of previous studies showing a decrease in size of the cutaneous LPR. Skin chamber LPR cellular influx decreased significantly only for neutrophils, whereas cutaneous LPR biopsy specimens showed no consistent changes for either the cellular infil-

trate or the deposition of MBP. To our knowledge this is the first time that IT has been shown to significantly decrease both early and late histamine release. This suggests that IT might be exerting a downregulating effect on both the mast cell (presumably the source for early histamine) and the basophil, which may contribute to the late histamine release. Kay et al.¹⁶ in the United Kingdom have shown a decrease in CD4+ T cells and activated eosinophils after 8 months of IT. These authors have hypothesized that IT may suppress the function of TH₂ helper cells and their cytokine products rather than having a direct quantitative effect on the nonlymphocytic inflammatory cells. Further studies are needed to examine the effect of IT on cellular activation, mediator release, and cytokine production.

REFERENCES

1. Pienkowski MM, Norman PS, Lichtenstein LM. Suppression of late-phase skin reactions by immunotherapy with ragweed extract. *J ALLERGY CLIN IMMUNOL* 1985;76:729-34.
2. Fling JA, Ruff ME, Parker WA, et al. Suppression of the late cutaneous response by immunotherapy. *J ALLERGY CLIN IMMUNOL* 1989;83:101-9.
3. Parker WA, Whisman BA, Apaliski SJ, Reid MJ. The relationships between late cutaneous responses and specific antibody responses with outcome of immunotherapy for seasonal allergic rhinitis. *J ALLERGY CLIN IMMUNOL* 1989;84:667-77.
4. Charlesworth EN, Hood AF, Soter NA, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Cutaneous late-phase response to allergen: mediator release and inflammatory cell infiltration. *J Clin Invest* 1989;83:1519-26.
5. Charlesworth EN, Iliopoulos O, MacDonald SM, Kagey-Sobotka A, Lichtenstein LM. Cells and secretagogues involved in the human late phase response. *Int Arch Allergy Immunol* 1989;83:50-3.
6. Charlesworth EN, Kagey-Sobotka A, Norman PS, Lichtenstein LM. Effect of cetirizine on mast cell mediator release and cellular traffic. *J ALLERGY CLIN IMMUNOL* 1989;83:905-912.
7. Naclerio RM, Proud D, Togias AG, et al. Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;313:65-70.
8. Fein BT, Kamin PB. A ten year survey of the hay fever plants and important atmospheric allergens in San Antonio, Texas, metropolitan area. *J Allergy* 1962;33:141-52.
9. Brown MJ, Ind PW, Causon R, Lee TH. A novel double-isotope technique for the enzymatic assay of plasma histamine: application to estimation of mast cell activation assessed by antigen challenge in asthmatics. *J ALLERGY CLIN IMMUNOL* 1982;69:20-4.
10. Peters MS, Schroeter AL, Kephart GM, Gleich GJ. Localization of eosinophil granule major basic protein in chronic urticaria. *J Invest Dermatol* 1983;81:39-43.
11. Dolovich J, Hargreave FE, Chalmers R, Shier KJ, Gauldie J, Bienenstock J. Late cutaneous allergic responses in isolated IgE-dependent reactions. *J ALLERGY CLIN IMMUNOL* 1973;52:38-46.

12. Solley GO, Gleich GJ, Jordan R, Schroeter AL. The late phase of the immediate weal and flare skin reaction: its dependence upon IgE antibodies. *J Clin Invest* 1976;58:408-20.
13. DeShazo RD, Levinson AI, Dvorak HF, David RW. The late phase skin reaction: evidence for activation of the coagulation system in an IgE-dependent reaction in man. *J Immunol* 1979;122:692-8.
14. Frew AJ, Corrigan CJ, Maestrelli M, et al. T lymphocytes in allergen-induced late-phase reactions and asthma. *Int Arch Allergy Appl Immunol* 1989;88:63-7.
15. Frew AJ, Kay AB. The relationship between infiltrating CD4+ lymphocytes, activated eosinophils, and the magnitude of the allergen-induced late phase cutaneous reaction in man. *J Immunol* 1988;141:4158-64.
16. Durham SR, Varney V, Gaga M, Frew AJ, Jacobson M, Kay AB. Immunotherapy and allergic inflammation. *Clin Exp Allergy* 1991;21(suppl 1):206-10.
17. Bochner BS, Charlesworth EN, Lichtenstein LM, et al. Interleukin-1 is released at sites of human cutaneous allergic reactions. *J ALLERGY CLIN IMMUNOL* 1990;86:830-9.
18. Iliopoulos O, Proud D, Adkinson NF, et al. Effects of immunotherapy on the early, late, and rechallenge nasal reaction to provocation with allergen: changes in inflammatory mediators and cells. *J ALLERGY CLIN IMMUNOL* 1991;87:855-66.
19. Furin MJ, Norman PS, Creticos PS, et al. Immunotherapy decreases antigen-induced eosinophil cell migration into the nasal cavity. *J ALLERGY CLIN IMMUNOL* 1991;88:27-32.
20. Rak S, Bjornson A, Hakanson L, Sorenson S, Venge P. The effect of immunotherapy on eosinophil accumulation and production of eosinophil chemotactic activity in the lung of subjects with asthma during natural pollen exposure. *J ALLERGY CLIN IMMUNOL* 1991;88:878-88.
21. Leiferman KM, Fujisawa T, Gray BH, Gleich GJ. Extracellular deposition of eosinophil and neutrophil granule protein in the IgE-mediated cutaneous late phase reaction. *Lab Invest* 1990;62:579-89.
22. Talbot S, Atkins P, Goetzl EJ, Zweiman B. Accumulation of leukotriene C4 and histamine in human allergic skin reactions. *J Clin Invest* 1985;76:650-6.
23. Lee CE, Neuland ME, Teaford HG, et al. Interleukin-6 is released in the cutaneous response to allergen challenge in atopic individuals. *J ALLERGY CLIN IMMUNOL* 1992;89:1010-20.
24. Jordan-Wagner DL, Dixon PA, Valtiere S, Charlesworth EN. Cytokines in the cutaneous late phase response to antigen: IL-8 present, TNF-alpha absent [Abstract]. *J ALLERGY CLIN IMMUNOL* 1992;89:306.
25. Creticos PS, Adkinson NF, Kagey-Sobotka A, et al. Nasal challenge with ragweed pollen in hay fever patients: effect of immunotherapy. *J Clin Invest* 1985;76:2247-53.
26. Creticos PS, Marsh DG, Proud D, et al. Responses to ragweed-pollen nasal challenge before and after immunotherapy. *J ALLERGY CLIN IMMUNOL* 1989;84:197-205.
27. Hedlin G, Silber G, Schieken L, et al. Attenuation of allergen sensitivity early in the course of ragweed immunotherapy. *J ALLERGY CLIN IMMUNOL* 1989;84:390-9.
28. Wang JY, Hsieh KH. The effect of immunotherapy on the in vitro productions of histamine, prostaglandin E2 and leukotriene C4 in asthmatic children. *Asian Pac J Allergy Immunol* 1989;7:199-24.
29. Brunet C, Bedard PM, Lavoie A, Jobin M, Hebert J. Allergic rhinitis to ragweed pollen II: modulation of histamine-releasing factor production by specific immunotherapy. *J ALLERGY CLIN IMMUNOL* 1992;89:87-94.
30. Liao TN, Hsieh KH. Altered production of histamine-releasing factor (HRF) activity and responsiveness to HRF after immunotherapy in children with asthma. *J ALLERGY CLIN IMMUNOL* 1990;86:894-901.
31. Kuna P, Alam R, Kuzminska B, Ryzniacki J. The effect of preseasonal immunotherapy on the production of histamine-releasing factor (HRF) by mononuclear cells from patients with seasonal asthma: results of a double-blind, placebo-controlled, randomized study. *J ALLERGY CLIN IMMUNOL* 1989;83:816-24.
32. Warner JA, Pienkowski MM, Plaut M, Norman PS, Lichtenstein LM. Identification of histamine releasing factor(s) in the late phase of cutaneous IgE-mediated reactions. *J Immunol* 1986;136:2583-7.
33. Grant JA, Alam R, Lett-Brown MA. Histamine-releasing factors and inhibitors: historical perspectives and possible implications in human illness. *J ALLERGY CLIN IMMUNOL* 1991;88:683-93.
34. Van Metre TE Jr, Adkinson NF Jr, Amodio FJ, et al. A comparison of immunotherapy schedules for injection treatment of ragweed pollen hay fever. *J ALLERGY CLIN IMMUNOL* 1982;69:181-93.