

Hymenoptera venom immunotherapy

II. T proliferative and T suppressive activities induced by *Vespula* immunotherapy: Effects on long-term antibody responses

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Thirty-five children who had exhibited major clinical reactions to yellow jacket stings were desensitized during an 8-day rush immunotherapy (RIT). Maintenance treatment was continued for at least 1 year in all cases and for 2 years in 21 cases. Blood samples, obtained before, during, and after RIT, were analyzed for specific T proliferative (TP) and T suppressive (TS) cell activities and for specific IgE, IgG1, and IgG4 levels. TS and TP cell activities increased during RIT ($p < 0.001$) and remained elevated throughout the 2-year follow-up. There were transient rises in specific IgG1 and IgE during RIT, but the levels of both decreased thereafter. Specific IgG4 increased throughout the follow-up period. The data indicate that the levels of specific IgE and specific IgG1 were correlated with TP cell responses (inducers) and TS cells responses (suppressors) and therefore might be controlled by them; data also suggest that TS cell responses should play a role in the IgG4 response. These changes in TS and TP cell responses and antibody responses have been incorporated into a model of antibody-response regulation by cell-mediated immunity in this type of allergy. (*J ALLERGY CLIN IMMUNOL* 1989; 83:572-80.)

Allergy to yellow jacket or honeybee stings leads to life-threatening reactions and occasional deaths. Immunotherapy with pure venoms has been demonstrated to be effective in inducing lower reactivities in restung patients.¹⁻³ Short-time RIT is now generally used to hyposensitize allergic subjects. This method produces fewer reactions than does long-term hyposensitization.^{2, 4-7} It has been demonstrated that immunotherapy with JV induces a decrease in IgE,^{4, 5, 8-13} although there is generally a transient rise in IgE during RIT^{4, 8, 9, 11} and a long-lasting increase in IgG,^{4, 8-14} which mostly involves IgG1 and IgG4.^{4, 11, 15}

The decrease in IgE and the increase in blocking

Abbreviations used

RIT:	Rush venom immunotherapy
BV:	Honeybee (<i>Apis mellifera</i>) venom
JV:	Yellow jacket (<i>Vespula</i>) venom
JV TP:	Yellow jacket T proliferative cell activity
JV TS:	Yellow jacket T suppressive cell activity
sIgE:	Specific IgE
sIgG1:	Specific IgG1
sIgG4:	Specific IgG4
t IgE:	Total IgE
AP:	Alkaline phosphatase
PBS:	Phosphate-buffered saline
BSA:	Bovine serum albumin
OD:	Optical density

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IgG4 are frequently considered to be two of the most relevant parameters of immunotherapy efficacy.^{2, 3, 16, 17} Nevertheless, neither parameter can be used with complete confidence as an indicator for stopping maintenance therapy,^{2, 18} perhaps because of the broad spectrum of individual antibody responses.^{3, 16, 19, 20} This would indicate that there are other factors that may influence a patient's response.^{3, 11, 21}

Cell-mediated responses are undoubtedly among these factors. Immunotherapy has been demonstrated to induce cell-mediated immunity to a variety of antigens. The responses detected include increases in specific TP²²⁻²⁹ and specific TS responses.³⁰⁻³³ Increases in specific TS activity have been observed in response to BV in animals,³⁴ in humans,^{35, 36} and in the study presented in part I, pages 563-71.

This study was therefore carried out to analyze the cell-mediated and humoral responses of allergic subjects receiving RIT to ascertain the changes in both TS and TP activities and in the levels of the major antibodies involved, IgE, IgG1, and IgG4. These data were analyzed and correlated with the status of the patients before RIT to produce a model of the way in which these two parts of the immune system interact. The study was carried out on children allergic to yellow jacket, since, in the Paris region, three times as many children are observed as outpatients with yellow jacket allergies than with honeybee allergies. The results demonstrated that RIT leads to increases in TS and TP activities and that the antibody responses are closely related to these early T cell responses.

MATERIAL AND METHODS

Thirty-five children (eight girls and 27 boys) aged from 3 to 14 years (10.2 ± 2.9) who had exhibited systemic allergic reactions to yellow jacket stings were selected for the study. Only four children also had clinical manifestations with other Hymenoptera stings, three to honeybee, and one child to *Vespa crabro*. All families and children gave informed consent.

The severities of the children's reactions to the most recent sting were recorded, and the data were quantified on a scale of 0 (generalized urticaria with moderate dyspnea) to 2 (life-threatening reactions) (average; 0.8 ± 0.9). It was found this severity was related to the number of previous stings (mean 1.2 ± 3.7) ($p < 0.003$).

Half the children were atopic (15/35) and/or belonged to atopic families (20/35). The presence of atopy was demonstrated by positive skin tests to house dust mites in correlation with the patient's history, elevated total serum IgE, and positive family history of atopy.³⁷

Children who had positive skin reactions to at least 0.01 $\mu\text{g/ml}$ of JV (class 2) were selected for the study. Most children (21/35) had positive reactions to 0.001 $\mu\text{g/ml}$ (10/35, class 3) or had positive reactions to 0.0001 $\mu\text{g/ml}$ (11/35, class 4).

Skin tests were performed by intradermal injection of 0.03 ml of JV, as described in part I of this article. A single lot of venom (Y 34250, Stallergenes Laboratories, Paris, France) was used for the entire study.³⁸

A correlation ($p < 0.01$) was found between the skin test reactivity classes (2.8 ± 1.0) and the RAST classes (2.4 ± 1.3).

Immunotherapy

Immunotherapy started generally 6 to 10 months after clinical reactions to a sting had justified immune investigation. For practical purposes treatment began earlier in three children and later (up to 15 months) in three other children.

Children were desensitized with a rush protocol by which maintenance doses (100 μg of JV) were reached by the end of the 8-day treatment period. All children were hospitalized for treatment, and any clinical reactions were carefully noted. The procedure used was similar to that adopted for honeybee desensitization and described in part I of the accompanying article. Increasing doses of venom were administered at 2-hour intervals so that the dose reached 0.0008 μg on the first day, 0.08 μg on the second day, and then at a longer interval reaching 0.8 μg on the third day, and 8 μg , 50 μg , 80 μg , 90 μg , and 100 μg on subsequent days. Maintenance doses were administered twice (days 14 and 28) in the first month and once a month thereafter.

Data for all 35 children covered at least the first 12 months after RIT, whereas 21 children were followed for the full 2-year period.

Measurement of specific TP and TS activities

The techniques used to measure these specific T-lymphocyte activities are fully described in part I of the accompanying article. Mononuclear cells were isolated from cell suspensions with a Ficoll-Hypaque gradient. Cells were then cultured for 2 days in the presence of *Vespula* venom to generate TS cells; a second sample of cells was cultured without antigen to preserve responding cells.

The TS cells were then treated with mitomycin C to stop cell division, and both cell populations (ratio, 1:1) were cultured together in the presence of a series of *Vespula* venom concentrations (0.01, 0.1, and 1 μg venom for 10^5 cultured cells). Each culture received a 4-hour pulse of tritiated thymidine, and the incorporated radioactivity was counted.

The incorporation into responding cell cultures was used to quantify specific TP activity (JV TP) and that of suppressor cells alone was used as a control for mitomycin C treatment. The incorporation into cocultured responding and suppressor cells was used to quantify specific TS activity (JV TS).

TP and TS activities are expressed as indexes (for details, see accompanying part I).

The same lot of *Vespula* venom (Y 34250) was used throughout the study.

t IgE

t IgEs were assayed with the PRIST system (Pharmacia AB, Uppsala, Sweden).^{39, 40} The results are presented as international units per milliliter.

slgE

Serum samples for children selection were measured with the RAST system,⁴⁰⁻⁴² and the responses used to classify the

TABLE I. Evolution of TP and TS activities during immunotherapy

Antigen quantity for 10 ⁵ cells	TP activity (TP index)			TS activity (TS index = % of suppression)		
	0.01 µg	0.1 µg	1 µg	0.01 µg	0.1 µg	1 µg
Day 0 (n = 35)	1.3 ± 0.7 ↓ ***	2.0 ± 0.8 ↓ ***	2.7 ± 1.3 ↓ ***	-2.8 ± 26.7 ↓ ***	+1.9 ± 33.0 ↓ ***	+11.4 ± 31.8 ↓ ***
Day 8 (n = 35)	2.2 ± 0.9	3.1 ± 1.3	4.3 ± 2.1	+24.1 ± 23.0	+32.6 ± 27.0	+46.7 ± 20.0
1 yr later (n = 35)	2.2 ± 0.9	3.3 ± 1.7	3.9 ± 2.1	+33.0 ± 30.7	+57.0 ± 21.3	+53.4 ± 18.1
1 yr later (n = 21)	1.9 ± 1.0	3.7 ± 2.0	4.1 ± 1.7	+42.6 ± 21.3	+53.7 ± 21.5	+49.7 ± 20.5
2 yr later (n = 21)	2.6 ± 0.8	3.8 ± 1.5	4.5 ± 2.2	+32.7 ± 18.5	+44.0 ± 22.5	+38.4 ± 17.4

Specific TP and TS activity are reported with the following indexes:

$$\text{TH index} = \frac{\text{cpm of vespula (VTP) cultures} - \text{cpm of TP controls}}{\text{cpm of TP controls}}$$

$$\text{TS activity} = (\text{cpm of VTP cultures} - \text{cpm of TP controls}) - [(\text{cpm of V cocultures} - \text{cpm of control cocultures}) - (\text{cpm of VTS cultures} - \text{cpm of TS control})] / (\text{cpm of VTP culture} - \text{cpm of TP control})$$

$$\text{TS index} = [1 - \text{TS activity}] \times 100$$

TS index represents the percent of suppression measured in coculture system.

****p* < 0.001; significant differences between day 0 and day 8.

children were 0 for sIgE levels between 0.07 and 0.1 IU/ml, 1 for sIgE <0.3 IU/ml, 2 for sIgE <1.0 IU/ml, 3 for sIgE <4.0 IU/ml, and 4 for sIgE levels of 4.0 and above.

A specific enzyme-linked immunofluorescent assay was used to quantify sIgE in serum samples obtained during and after RIT. The technique is derived from the one of Layton and Stanworth⁴³ and Labrousse et al.⁴⁴ and had been modified by one of us (J. S. L.) for fluorescent quantification.

Antigen (10 µg/ml of JV in 0.1 mol/L of carbonate-bicarbonate buffer, pH 9.6) was incubated overnight at +4° C in high-capacity multiwell plates (Immulon II, Dynatech Industries, Alexander, Va.). The plates were extensively washed with Tween-PBS (0.5% Tween 80 in 0.1 mol/L of PBS, pH 7.4) and 100 µl aliquots of dilution serum, diluted 1/20 in BSA-PBS (1 mg/ml BSA in 0.1 mol/L of PBS, pH 7.4), were dispensed, in duplicate, into the wells. The plates were incubated at room temperature for 90 minutes and washed with Tween-PBS; 100 µl (1/5000 in Tween-PBS) of anti-IgE conjugated to AP (ATAB Laboratories, Orimbio, Paris, France) was added to each well, and incubation was continued for a further 90 minutes at room temperature.

The plates were washed once more with Tween-PBS, and 100 µl of fluorescent substrate (10⁻⁴ mol/L of methylumbelliferyl phosphate [Sigma Chemical Co., St. Louis, Mo.] in 1 mol/L of Tris HCl, pH 8.5) was added to each well and incubated for 1 hour at room temperature. The plates were then read in an automatic fluorometer Micro-FLUOR, (Dynatech Industries).

Patients' sera were compared to a positive reference standard of pooled sera from untreated *Vespula* (or honeybee)-allergic patients; 50 sera from nonallergic patients served as negative controls.

One unit of sIgE was arbitrarily defined as the measurable dilution (1/200) of the positive standard for which the following ratio

$$\frac{\text{OD of 1/20 diluted negative controls}}{\text{OD of standardized dilution of positive standards}}$$

was <0.35 in order to mimic RAST results. Patient's serum values were expressed with the same ratio. The results were classified on a scale corresponding to the RAST classes (class 1, 0.35 to 0.7 U/ml; class 2, 0.7 to 3.5 U/ml; class 3, 3.5 to 17.5 U/ml; and class 4, >17.5 U/ml).

Measurement of sIgG1 and sIgG4

A similar enzyme-linked immunofluorescent assay technique was used to measure sIgG1 and sIgG4. The serum samples were diluted 1/50 with BSA-PBS for sIgG1 quantification and 1/10 for sIgG4 and incubated with mouse anti-IgG1 or mouse anti-IgG4 (100 µl of 1/2500 dilution) (Seward, Merck, Paris, France). The plates were washed with Tween-PBS, and 100 µl of sheep antimouse IgG (F(ab')₂ antibody (1/5000 dilution in Tween-PBS) conjugated to AP (Sigma Chemical Co.) was added to each well. The sheep antimouse IgG-AP conjugate was adsorbed with human IgG before use to prevent any direct interaction with human IgG. The plates were incubated for 90 minutes

TABLE II. Evolution of antibody levels during immunotherapy

	T IgE (IU/ml)	sIgE (U/ml)	sIgG1 (U/ml)	sIgG4 (U/ml)
Day 0 (n = 35)	140 ± 152	2.4 ± 5.1	0.7 ± 0.9	2.3 ± 1.6
Day 8 (n = 35)	160 ± 114	3.7 ± 5.9	1.4 ± 1.1*	2.6 ± 2.7
1 year later (n = 35)	140 ± 99	2.6 ± 5.0	0.7 ± 0.2	4.95 ± 2.4
1 year later (n = 21)	139 ± 101	2.6 ± 5.3	0.7 ± 0.2	5.2 ± 2.4†
2 years later (n = 21)	130 ± 91	0.9 ± 0.7‡	1.5 ± 0.8‡	8.0 ± 3.6*

Differences were calculated with Student's *t* test for paired experiments.

Statistical differences between day 0 and follow-up testing:

**p* < 0.05.

†*p* < 0.01.

‡*p* < 0.001.

at room temperature and washed with Tween-PBS. Fluorescence was developed and quantified with the AP substrate 4-methylumbelliferyl phosphate as for IgE measurement.

The results are expressed as for sIgE with the following ratio:

$$\frac{\text{OD for diluted patient's serum}}{\text{OD for standardized dilution of positive standard}}$$

The positive reference standard was a serum pool from recently desensitized patients allergic to *Vespula* (or honey-bee). The same negative controls were used. One arbitrary unit was similarly defined with 1/750 dilution of reference standard for sIgG4 and 1/500 dilution of the same standard for sIgG1. Such dilutions were arbitrarily determined to permit a ratio <0.75 when all negative controls were compared to the reference standard.

Patients were arbitrarily assigned to one of the following classes: class 1, 0.75 to 1.25 U/ml; class 2, 1.25 to 2.5 U/ml; class 3, 2.5 to 3.7 U/ml; and class 4, >3.7 U/ml.

Protocol

Blood samples were obtained from all the children included in the study on the first day of RIT (day 0), before beginning treatment, a few hours after the end of RIT (day 8), and once a year thereafter. TS and TP activities were measured on fresh heparinized blood on the day of sampling. The sera were then aliquoted and frozen (−80°C). Aliquots were thawed for sIgE, sIgG1, sIgG4, and t IgE assays. All assays of single type (e.g., IgE) were done on the same day, and the assay was run blind on coded samples.

Statistical analysis

Differences between the values of each parameter at different times were analyzed by Student's *t* test for paired values. Changes in immune parameters were compared with

Pearson's ranking test. These comparisons were also made for the changes in each parameter with respect to all the values quantified before treatment, including skin tests and the patients' histories.

RESULTS

Levels before treatment

Cell-mediated immunity responses. TP activity was quite low before treatment (Table I); four of the children had a TP index of ≥ 2.5 for the lowest venom concentration (0.01 $\mu\text{g}/10^5$ cells), five, a TP index of ≥ 3 for the middle concentration (0.1 $\mu\text{g}/10^5$ cells), and nine children, a TP index of ≥ 3.3 for the highest concentration (1 $\mu\text{g}/10^5$ cells). Generally TS activity was not detectable before treatment, but some children exhibited measurable suppressive activity at that time (>30% of inhibition for five children at the smallest dose, seven for the middle dose, and nine children at the highest dose).

TP activity before treatment was correlated with previous reactions to yellow jacket stings (*p* < 0.015), demonstrating that TP activity before treatment was probably related to the individual susceptibility to venom.

Humoral immunity. Eight children had t IgE levels ≥ 170 IU/ml, seven being either atopic (four children) or belonging to atopic families (five children). Seven children had sIgE ≥ 3.5 U/ml (Table II). Only three of these children were atopic. The initial levels of sIgG4 and of sIgG1 were low (Table II), but two children had sIgG1 ≥ 1.3 U/ml, and two other children had high sIgG4 (>3.7 U/ml). The two children who exhibited high sIgG4 levels before treatment had been stung several times during the preceding

TABLE III. TP and TS activity variations during rush therapy in relation to clinical manifestation during RIT

	Manifestations					
	No clinical manifestations (n = 11)		Only local reactions (n = 18)		Systemic reactions (n = 6)	
	Day 0	Day 8	Day 0	Day 8	Day 0	Day 8
TP activity (TP index)						
0.01 µg	1.40 ± 0.68*	2.14 ± 0.87	1.25 ± 0.66†	2.28 ± 0.98	1.63 ± 0.73	1.78 ± 0.80
0.1 µg	1.79 ± 0.39‡	3.42 ± 0.76	1.95 ± 0.75†	3.07 ± 1.07	2.37 ± 1.07	2.43 ± 1.51
1 µg	3.13 ± 1.17‡	5.76 ± 1.87	2.81 ± 1.66*	3.56 ± 2.10	2.21 ± 0.24*	4.08 ± 1.95
TS activity (% of suppression)						
0.01 µg	-20.8 ± 31.6†	+20.5 ± 26.3	-2.3 ± 19.3†	+26.3 ± 26.8	-21.8 ± 13.1*	+3.2 ± 28.4
0.1 µg	-4.7 ± 28.4†	+32.2 ± 25.5	-9.8 ± 34.7†	+26.8 ± 34.9	-7.3 ± 39.8	+1.8 ± 46.6
1 µg	+21.6 ± 27.1*	+45.1 ± 24.6§	+0.7 ± 37.0†	+42.9 ± 31.9	+4.1 ± 55.0	+7.9 ± 37.6

Differences were calculated with Student's *t* test for paired experiments.

Statistical evolution for each group during RIT between the two values (day 0 and day 8) of the group:

**p* < 0.05.

†*p* < 0.01.

‡*p* < 0.001.

Statistical differences between the group of children who exhibited systemic reactions and the other groups:

§*p* < 0.03.

||*p* < 0.05.

years and had had several systemic reactions before treatment.

Evolution of immune parameters during rush treatment

Cell-mediated immunity. TP and TS activities increased considerably during rush therapy (*p* < 0.001 for TP activity and *p* < 0.0002 for TS activity at the three antigen concentrations tested) (Table I). Increases in both activities were negatively correlated with initial levels (*p* < 0.035 for TP activity and *p* < 0.001 for TS activity), indicating that rush therapy induces TP activity in patients with low initial TP activity and TS activity in patients with low initial TS activity.

The increase in TS activity was negatively correlated with the atopic status of the patients (*p* < 0.03), indicating that the 15 atopic children had a smaller increase in TS activity during rush therapy (13.8 ± 19.5, 18.5 ± 15.0, and 26.3 ± 20.7 for each antigen concentration).

There was no correlation between skin test reactivity or RAST positivity before treatment and initial levels or therapy-induced increases in TP or TS activity.

Six children had minor systemic reactions sufficient to cause delays in the injection schedules of three children so that maintenance dose levels were not reached until day 10. All systemic reactions appeared

for injection ≥60 µg of venom. RIT was complete within 8 days for the remainder of the children. Eighteen children had local reactions (which appeared for various doses 14 to 80 µg), and 11 children had no clinical reactions during RIT (Table III).

The six children who developed systemic reactions had smaller increases in TP activity (*p* < 0.05 for 0.01 and 1 µg, *p* < 0.02 for 0.1 µg TP indexes compared to nonreactors, and *p* < 0.05 for 0.01 µg and *p* < 0.02 for 0.1 µg compared to local reactors) and smaller increases in TS activity (*p* < 0.075 for 0.1 µg TS index compared to the two other groups and *p* < 0.03 for 1 µg compared to local reactors) during RIT. At the end of rush therapy, they had lower TS activities (*p* < 0.05 compared to local-reactors and *p* < 0.03 compared to nonreactors for 0.1 µg TS activity).

That it is possible to construct an approximate dose-response relationship between the magnitude of the TS increase and the clinical reactions during RIT is presented in Table III; for example, children with systemic responses had a 25% increase in TS, children with local reactions had a 29% TS increase, and children with no reaction had a TS increase of 41% at the lowest antigen concentration.

Humoral immunity. The only borderline statistically significant change during RIT was an increase in the level of sIgG1 (*p* = 0.054) (Table II). All other changes (t IgE, sIgE, and sIgG4) were nonsignificant.

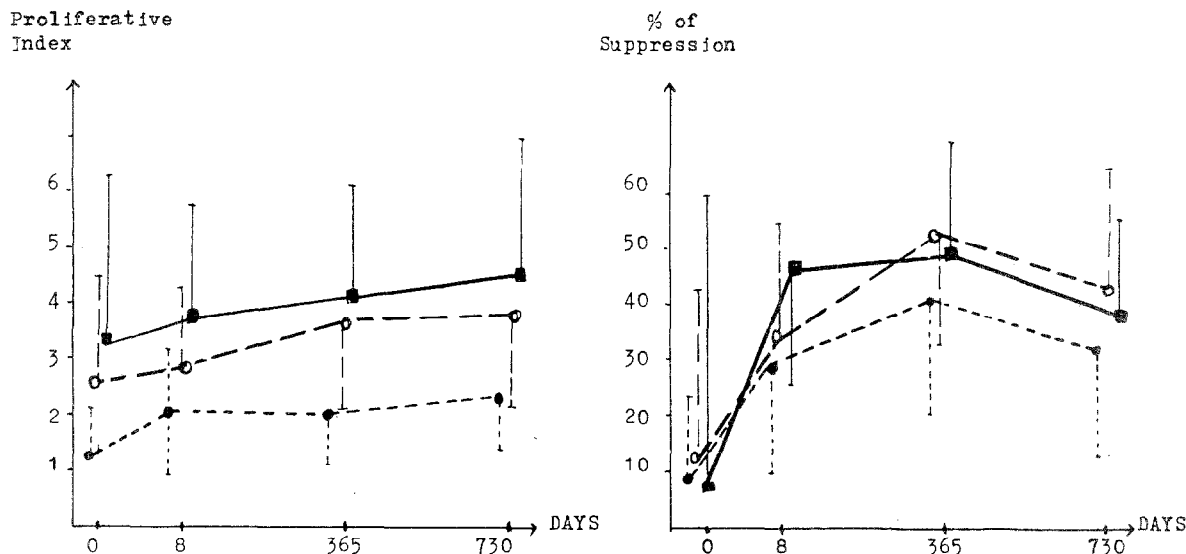


FIG. 1. Evolution of specific TP and TS activities during immunotherapy. Both parameters quantifying cell-mediated immunity (TP and TS specific JV activities) were evaluated to quantify immunotherapy effectiveness in 21 children during a 2-year follow-up. Each curve represents the mean evolution $\pm$ SE (represented by vertical lines for each tested antigenic concentration) for TP and TS (percent of suppression) indexes; 0.01 $\mu\text{g}/10^5$ cells $\bullet$ --- $\bullet$; 0.1 $\mu\text{g}/10^5$ cells $\circ$ — $\circ$; 1 $\mu\text{g}/10^5$ cells $\blacksquare$ — $\blacksquare$.

There was, however, a negative correlation between these nonsignificant changes and the initial levels of each parameter ($p < 0.001$ for t IgE, $p < 0.009$ for sIgE, $p < 0.07$ for sIgG1, and $p < 0.11$ for sIgG4), indicating that the larger changes occurred in patients in which the initial values had been low.

The nonsignificant increase in IgE during RIT was negatively correlated with the increase in TS activity ($p < 0.006$ for t IgE and $p < 0.04$ for sIgE), indicating that the increase in TS activity may play a role in the inhibition of the IgE increase. sIgE decreased in only two patients (-18.0 and -7.3 U/ml) who had very high initial sIgG4 levels (14.2 and 3.7 U/ml); these values contribute to the nonsignificance of the sIgE increases during RIT.

After RIT

Cell-mediated immunity. The TP and TS indexes remained elevated and close to the day 8 values throughout the 2 years of posttreatment follow-up (Table I; Fig. 1). There were small decreases in TS activity at the highest ($p < 0.06$) and the lowest ($p < 0.15$) venom concentrations tested at 2 years after follow-up (Table I).

Humoral immunity. sIgE levels declined during the first year of follow-up, reached the initial levels a year after therapy, and continued to decline thereafter (Fig. 2). The decline in sIgE was more pronounced during the second year of follow-up, reaching significance ($p < 0.01$).

The decrease in t IgE measured at the 2-year follow-up was correlated with the increase in TS activity during rush therapy ($p < 0.01$ after 2 years). This decline was similar for atopic and nonatopic children. The decrease in sIgE after 2 years ($p < 0.01$) was also correlated with increased TS activity during rush therapy. There was no correlation between the changes in TP activity during RIT and the sIgE levels 2 years after treatment. However, there was a positive correlation ($p < 0.05$) between the increase in the TP activity during RIT and the sIgE level at 1 year after treatment, indicating that large increases in the TP activity tend to result in a delayed decrease in sIgE.

The sIgG4 levels increased during the maintenance treatment (Fig. 2). This increase was significant during the first year ($p < 0.001$) and the second year ($p < 0.05$) of follow-up. The increased sIgG4 after 1 year was also correlated with the increase in TS activity observed during rush treatment ($p < 0.05$). The opposing changes in sIgE and sIgG4 were correlated after 1 ($p < 0.01$) and 2 years ($p < 0.03$).

Thus, during the 2 years of follow-up, sIgE decrease and sIgG4 increase are correlated with TS activity increase during RIT.

The sIgG1 levels had returned to baseline 1 year after RIT ($p < 0.001$) but increased again thereafter ($p < 0.01$) (Fig. 2). The increase in sIgG1 at the second year of follow-up was correlated with the increase in TP activity during RIT ($p < 0.004$), but not with the TS activity variations.

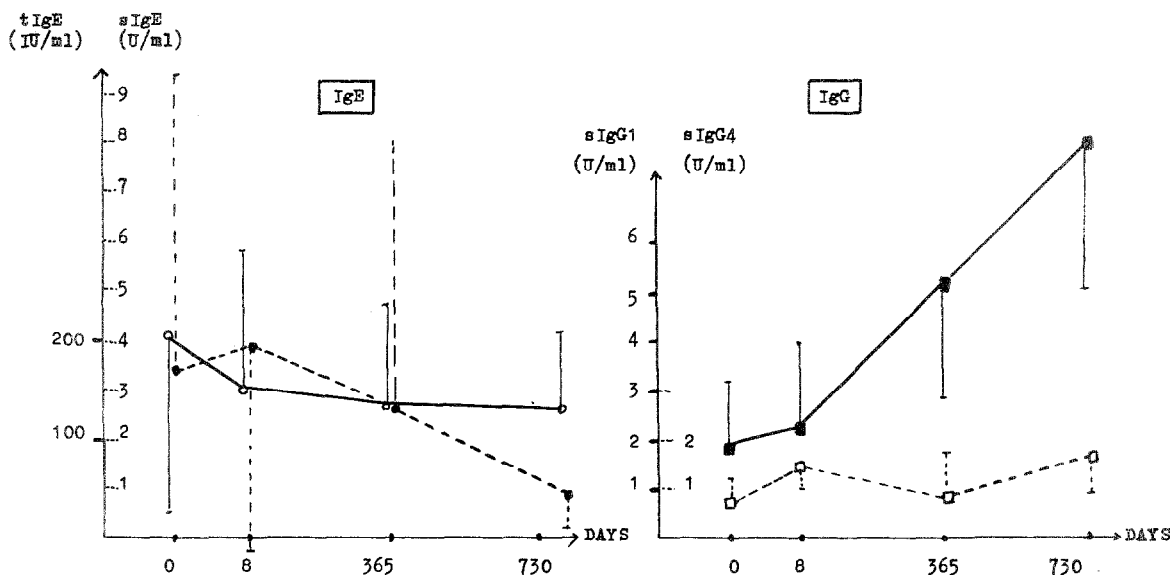


FIG. 2. Evolution of humoral immunity during immunotherapy. During a 2-year follow-up, total IgE (○—○), sIgE (●—●), sIgG1 (□—□), and sIgG4 (■—■) were evaluated in 21 children allergic to JV. The curves represent the mean evolution for each parameter $\pm$ SE (represented by vertical lines).

DISCUSSION

This study examines the changes in both cell-mediated and humoral parameters of children allergic to *Vespula* venom during and after rush immunotherapy. The primary objective was to measure *Vespula*-specific TP and TS activities and to correlate them with the measured levels of specific antibodies (sIgE, sIgG1, and sIgG4). These humoral changes are generally considered to be the main parameters that are influenced by specific immunotherapy.^{2, 3, 16, 17} There is evidence⁴⁵ that cell-mediated changes control and induce antibody production, and it has been demonstrated (see part I article) that there is a correlation between the cell-mediated and humoral responses to RIT in children allergic to honeybee venom. The present study was undertaken to extend and confirm this initial observation on a larger population of 35 children having a *Vespula* venom allergy.

The transient increases in sIgE and of sIgG1 and the sustained increased in sIgG4 observed after *Vespula* RIT are in agreement with earlier studies,^{4, 8-13} whereas opposing changes in sIgE and sIgG4 have been reported during venom therapy.⁴⁶ Correlation between these antibody variations as a result of RIT have only been reported for honeybee allergy⁴⁶ and not for yellow jacket allergy, perhaps because of the small numbers of patients treated for JV allergy.

RIT induced high levels of JV TP and of JV TS activities. Similar high levels of cell-mediated activities have been reported for ragweed allergy,³⁰ other

pollen allergies,³⁰⁻³³ and honeybee allergy³⁴⁻³⁶ (see part I article). However, the published studies on changes in TP activity induced by immunotherapy are conflicting. Positive results were obtained for pollen^{22-27, 30} and mite^{28, 29} allergies, but negative results were found in other studies.⁴⁷⁻⁴⁹ Therapy for honeybee-venom allergy does induce increases in TP and TS activities that persist for at least 1 year after RIT (part I article); the present study indicates that the activities remain high for up to 2 years after JV RIT.

TP activity requires numerous venom injections to be measurable. It has been reported that before treatment, TP activity can only be detected in multistung patients.^{21, 50} This is confirmed by our results. Furthermore, we have demonstrated that significant TP activity is induced by the numerous injections of RIT. TP activity is related to specific antibody responses, namely, to sIgE before treatment.^{21, 50} Our study demonstrates a direct role of TP activity on antibody responses. High increase during RIT is related to long-lasting sIgE and sIgG1 increases.

The changes in sIgE are also correlated with TS activity; there was a direct relationship between the decrease in sIgE after RIT and the increase in TS activity during RIT.

TS activity may influence the antibody responses. A significant correlation was observed between the increase in TS activity during RIT and the subsequent increase in sIgG4. Although a decrease in sIgE associated with increased TS activity has been reported

in a case of ragweed allergy,³² this is, to our knowledge, the first evidence of a correlation between TS activity and sIgG4.

The correlations between the increase in TS activity during RIT and both the sIgE decrease and the sIgG4 increase observed at 2 years after RIT suggest that changes in TS activity play a central role in subsequent changes in antibody levels. The interrelationship between TS activity and these two antibodies are probably of great importance because those antibodies are considered to be clinically important.^{2, 3, 7, 16}

The correlation analyses also indicated that the clinical manifestations during RIT varied with TS activity. Those children whose TS activity increased most during RIT had very small clinical reactions, or even none.

All these results suggest that TS activity might play a key role in the control of clinical manifestations in children with JV allergy, either directly or via specific antibody responses.

As the decrease in sIgE is related to the increase in TS activity and the persistence of sIgE is related to the increase in TP activity during RIT, there is a clear indication that TS and TP activities have opposing roles in the production of sIgE, with TP fostering an increase and TS fostering a decrease.

The accompanying study of RIT in patients allergic to honeybee venom provides similar data. TS and TP activities increased during RIT and remained high thereafter. In both studies, sIgG4 increased progressively after therapy. In contrast, sIgE exhibits a progressive decrease in BV allergy, whereas a transient rise is observed in JV allergy.

The observed differences between the honeybee and yellow jacket studies may point to there being different protective mechanisms for these two Hymenoptera. Further investigations are necessary to explain such differences in Hymenoptera allergies.

The data on JV RIT can be used to construct a tentative model of the role of cell-mediated immunity (TS and TP activities) in controlling antibody, mainly sIgE and sIgG4, in these allergies. TS activity appears to be the major driving force in the response.

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