

Regimens of Hymenoptera Venom Immunotherapy

DAVID B. K. GOLDEN, M.D.; MARTIN D. VALENTINE, M.D.; ANNE KAGEY-SOBOTKA, Ph.D.; and LAWRENCE M. LICHTENSTEIN, M.D., Ph.D.; Baltimore, Maryland

We studied 64 sting-allergic patients treated with one of three regimens of insect-venom immunotherapy: slow, rush, or a step-function regimen. All regimens had a top dose of 100 μg and a similar cumulative dose. Efficacy was 100% in all regimens. Fifty percent of the patients had at least one large local reaction at a rate of 9.6 reactions/100 injections. Sixteen percent had systemic symptoms at 1.6 reactions/100 injections. Reaction rates did not differ among the groups, but the slow regimen involved twice as many injections as the rush regimen, and thus caused twice the number of reactions. The rush regimen caused a greater and more rapid rise in anti-venom IgG than did the slow regimen, with no difference in IgE levels. We conclude that although equally effective, the rush regimen of venom immunotherapy is associated with a greater immune response and fewer adverse reactions than the slow regimen.

IMMUNOTHERAPY with the venoms of insects of the order Hymenoptera has been shown to specifically protect insect-allergic persons against anaphylactic reactions to the stings of these insects (1-3). It has also been associated with a significant increase in venom-specific serum IgG antibodies (1-7), which have been shown to be associated with immunity in passive transfer experiments (5, 7). In contrast, immunotherapy with whole body extracts that contain little or no venom (8) is clinically no more effective than placebo (1) and is not associated with an increase in circulating venom-specific IgG antibodies (9).

The regimen used in our previously reported controlled trial of venom immunotherapy (1) was relatively rapid. The maximum dose of 100 μg was arbitrarily selected; it is approximately equal to the venom content of two honeybee stings (2) and had been used successfully in our previous uncontrolled studies. This regimen was 95% effective in protecting allergic persons against anaphylaxis from a challenge sting and caused a brisk venom-specific IgG response. Drawbacks to this immunotherapy included frequent adverse local reactions to the injections and the occurrence of an occasional systemic reaction. This type of "rush" immunotherapy differs from the regimens usually advised for clinical use with other allergens (10) in that the initial dose is quite high, doses are rapidly increased, and there is a single end-point associated with objective clinical efficacy. We therefore set out to study various regimens of venom immunotherapy with respect to clinical efficacy, frequency and pattern of adverse reactions, and degree and pattern of immunologic responses.

Materials and Methods

PATIENTS

Sixty-four patients were selected from those presenting and referred to our clinic, on the basis of a positive history of insect-

sting-related anaphylaxis and positive intradermal skin tests with insect venom. In-depth informed consent regarding the possible benefits and risks of venom immunotherapy by varied regimens and of optional supervised in-hospital challenge stings was obtained in accordance with the Johns Hopkins Joint Committee on Clinical Investigation.

VENOMS

All venom preparations used for diagnosis and therapy were provided by Pharmacia Diagnostics (Piscataway, New Jersey) as lyophilized venom extracts, 500 μg /sterile vial. These were reconstituted to 100 μg /mL stock solutions and serially diluted to 10⁻⁴ μg /mL in Pharmacia diluent (sterile saline, 0.03% human serum albumin, mannitol, phenol). The venom originates from Dr. A. Benton and is obtained from honeybees by electric-grid stimulation (11) and from the vespids by individual venom-sac extraction (BENTON AW. Personal communication). Honeybee (HBV), yellow jacket (YJ), yellow hornet (YH), white-faced hornet (WH), and *Polistes* wasp (Pol) venoms were used.

DIAGNOSIS

The historical diagnosis of anaphylaxis was defined as a systemic manifestation of immediate hypersensitivity distant from the site of the sting. These episodes were always and only sting-related. Seven patients had cutaneous signs only (urticaria/angioedema); all others had respiratory or vascular manifestations as well. Of the 64 patients studied, urticaria was reported by 69%, dizziness or hypotension by 67%, throat swelling or hoarseness by 50%, dyspnea by 48%, loss of consciousness by 30%, and wheezing by 8%.

Intradermal skin tests were done on the volar aspect of the forearm with all five venoms and a diluent control. The initial concentration tested was 0.1 μg /mL, and repeat skin tests at either tenfold lower or higher concentrations were done to obtain a 2+ end-point (wheal at least 5 mm, erythema at least 20 mm). We have previously shown that these concentrations of venom encompass the useful range for diagnosis (12, 13). Only one positive reaction occurred in 30 normal adults with no history of anaphylaxis (13). Nonspecific irritative reactions begin to occur at 3 μg /mL and are not uncommon at 10 μg /mL (GOLDEN DBK, VALENTINE MD, LICHTENSTEIN LM. Unpublished observations).

All patients had 2+ skin tests at 1 μg /mL or less except two who were 1+ at 1 μg /mL; both had systemic reactions including hypotension, urticaria, and angioedema. The patients in this study were typical sting-allergic patients who did not differ from patients in previous studies in severity of history, skin test sensitivity, or immunologic findings.

IMMUNOLOGIC STUDIES

Venom-specific IgE antibodies were measured before and during treatment using a specific and sensitive radioallergosorbent test technique described elsewhere (14). Venom-specific IgG (blocking) antibody was quantitated by a double-antibody radioimmunoassay as previously reported (4).

IMMUNOTHERAPY

Patients were randomly assigned to three treatment groups, which did not differ historically in severity of reactions to stings, history of atopy, history of large local reactions to stings, or previous whole body extract immunotherapy. The groups also did not differ in degree of skin test sensitivity or pretreatment levels of venom-specific IgG or IgE antibodies. The three regimens used are shown in Table 1. Regimen I (slow) consisted of 16 weekly doubling injections starting at 0.01 μg (the average

► From the Division of Clinical Immunology, Department of Medicine, The Johns Hopkins University School of Medicine; Baltimore, Maryland.

skin test dose). The maintenance dose was achieved after 14 weeks. Regimen II (rush) involved seven bimonthly treatments starting at 15 μ g (in divided doses at 30-minute intervals). Maintenance dose was reached in 6 weeks. Regimen III (step) also started at 15 μ g and increased stepwise to 25, 50, and 100 μ g with 5-week plateaus at each dose. Maintenance dose was achieved in 11 weeks. The cumulative dose was similar in all regimens. Nine patients were treated with HBV (five HBV only); 58 with one or more vespid venoms (YJ, YH, WH); and 30 with *Polistes* wasp venom (one Pol only).

Challenge stings were done in-hospital with intravenous line established and emergency resuscitation personnel and equipment standing by. Yellow jackets or honeybees, or both, collected locally were used according to the sensitivity and treatment of the individual. The precise identity of the insects was always subsequently established by entomologic examination.

STATISTICAL ANALYSIS

Comparison of large local or systemic reactors with nonreactors for each variable was done by chi-square test on a two-sample contingency table. Comparison of group IgG or IgE titers at each time was done by rank sum test to derive the two-tailed *P* value from the value of *z*.

Results

EFFICACY

Fifty-two patients were stung in-hospital after 18 to 20 weeks of immunotherapy. Four were stung in the field after achieving the full 100- μ g dose, and the insect was definitively identified as the one to which that patient was sensitive. None of these 56 patients reported any systemic symptoms whatsoever.

The eight subjects not stung included three in group I who had not reached maintenance dose after 4 months (two due to systemic and local reactions, one due to a 2-month interruption in therapy); four in group II (two due to illness or cardiac status, one who was treated only with *Polistes* wasp venom, and one whose antivenom IgG was

Table 1. Regimens of Hymenoptera Venom Immunotherapy

Week	Dose		
	I (Slow)	II (Rush)	III (Step)
	μ g		
Initial	0.01	1 + 5 + 10	1 + 5 + 10
1	0.03		25
2	0.1	30	
3	0.25		25
4	0.5	60	
5	1.0		25
6	2.5	100	50
7	5		
8	10	100	50
9	20		
10	30		50
11	40	100	100
12	60		
13	80		100
14	100		
15	100	100	100
16			
17	100		
18			100
19		100	
20	100		
Total	650	605	640

Table 2. Adverse Reactions During Venom Immunotherapy

	I (Slow)	II (Rush)	III (Step)	Total
Large local reactions				
Subjects, no.	22	22	20	64
Reactors, no.	9	11	12	32
Reactions/ injections, no.	37/450	22/233	31/260	91/943
Reaction rate, %	8.1	9.9	11.9	9.6
Reactions/ reactor, no.	4.1	2.1	2.6	2.8
Systemic reactions				
Subjects, no.	22	22	20	64
Reactors, no.	4	4	2	10
Reactions/ injections, no.	7/450	4/233	4/260	15/943
Reaction rate, %	1.55	1.72	1.54	1.59
Reactions/ reactor, no.	1.75	1.0	2.0	1.5

in doubt at the time); and one in group III due to cardiac status.

ADVERSE REACTIONS

Table 2 shows the observed prevalence and rate of large local reactions. Fifty percent of all patients had at least one large local reaction during immunotherapy, at an overall average rate of 9.6 reactions/100 injections. These patients (reactors) averaged 2.8 large local reactions each. The three groups did not differ significantly in the large local reaction rate, which ranged between 8% and 12%. Because the slow regimen consisted of almost twice as many injections as the rush regimen, it was associated with twice as many reactions per patient.

The patients who had large local reactions did not have a significantly higher prevalence of atopic history, previous whole body extract therapy, or large local reactions to past stings, nor did they differ in severity of history or skin test or in pretreatment antivenom IgE or IgG levels. The peak reaction rate occurred at the 25 μ g-dose level in both the rush and slow groups.

Large local reactions occurred rarely at doses below 15 μ g (4.9/100 injections) or above 50 μ g (6.8/100 injections) and were most frequent at doses in the 15- to 50- μ g range (19.4/100 injections). Giving repeated injections at doses of 25, 50, or 100 μ g (regimen II versus III) caused no increase in the number of reactions because reactions rarely occurred after the first injection at these doses. Such repeat doses also were not associated with any fewer reactions at higher doses.

Table 2 shows the observed prevalence and rate of systemic reactions. About 16% of all patients reported systemic symptoms during immunotherapy, at an overall rate of 1.6 reactions/100 injections. These patients (reactors) averaged one and one-half reactions each. The groups did not differ significantly in reaction rate, but again there is a proportionately larger number of reactions in the slow group, related to the larger number of injections given. Of the 15 reactions observed, only five were electively treated with epinephrine—one patient with hypotension and dyspnea, two with visible flushing, and two with persistent hoarseness. The other 10 reac-

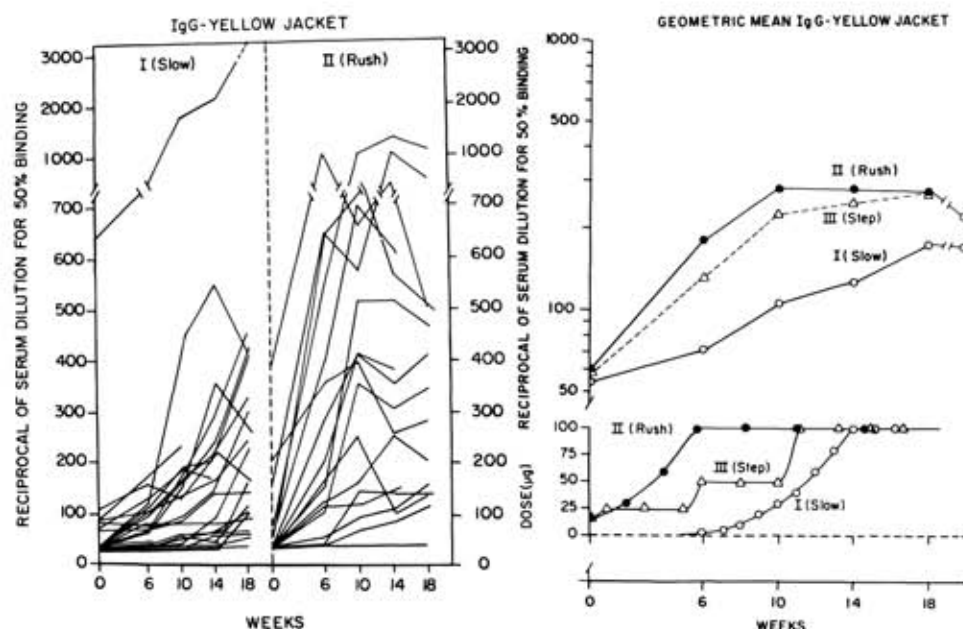


Figure 1 Left. Anti-yellow-jacket (YJ)-venom IgG antibody titers. Time course during venom immunotherapy is shown for each patient in regimen I (*slow*) and regimen II (*rush*). Immunoglobulin G titer is expressed as reciprocal of serum dilution for 50% binding. Right. Time course of anti-YJ-IgG antibody response in each of the three regimens, also showing the venom dose at each time for each regimen. Immunoglobulin G titers are the geometric mean for each group at each time, expressed as the reciprocal of serum dilution for 50% binding. See Table 3 for confidence limits and statistics.

tions included subjective symptoms such as dizziness or lightheadedness, pruritis, and throat discomfort, which did not require therapy. Thus, the incidence of patients with objective systemic signs requiring epinephrine was actually 7.8% at a rate of 0.5 reactions/100 injections.

Patients who had systemic reactions did not differ from those who did not in history of atopy, whole body extract therapy, large local reactions to stings, severity of history or skin test, or pretreatment antivenom IgE or IgG antibodies.

IMMUNE RESPONSE

Since 90% of the subjects were treated with YJ venom, the anti-YJ immune response was analyzed in greatest detail. The immune response and clinical efficacy of HBV immunotherapy have been previously reported (1-3). Patients treated with multiple venoms did not differ in immune response or adverse reactions from patients treated with a single venom.

The production of YJ-venom-specific IgG over time is shown in Figure 1, *left*, for the slow and rush regimens. The rush regimen was associated with a greater and more rapid rise in serum IgG, reaching a peak between 6 and 10 weeks, by the time of the first 100-μg dose. The slow regimen had just begun to produce significant increases in IgG by the time of the first 100-μg dose at Week 14. The time course of the increase of geometric mean anti-YJ-venom IgG titers and of the doses for each regimen is shown in Figure 1, *right*, and illustrates the above observations. Regimen III produced a response intermediate between regimens I and II. The antibody level did not differ significantly from that in the rush regimen and was significantly greater than the slow regimen titers after Week 6 when the dose was increased to 50 μg. This dose caused the most marked increase in IgG, with little increment at the 100-μg dose. The rush regimen was associated with significantly greater IgG levels than was the slow regimen from 6 through 14 weeks (Table 3) and was still

higher at 18 weeks ($P < 0.08$). One patient had IgG titers markedly different from all other patients (see Figure 1). When he is excluded from group I, the IgG levels at 18 weeks for groups II and III are significantly greater ($P < 0.05$) than those of group I.

The YJ-venom-specific IgE antibody response was variable from patient to patient, as shown in Figure 2. Analysis of the geometric mean IgE antibody indicated that titers tended to rise slightly in the first 6 to 10 weeks, at doses up to 25 to 30 μg, and then fall slowly toward pretreatment levels. The regimens did not differ significantly in IgE levels at any time.

Discussion

Immunotherapy has traditionally been done on a cautious and empiric basis. Popular regimens begin with doses usually too small to evoke any adverse reaction or immune response, usually less than the positive skin test dose, and the dose is doubled with each weekly injection. The dose is often held constant or reduced if uncomfortable local reactions occur, and considerably reduced if systemic symptoms occur. Persistent reactions of either type may cause immunotherapy to be maintained at suboptimal (and often clinically ineffective) doses, or abandoned altogether. More rapid, high-dose, and persistent regimens are believed to be associated with more frequent and severe adverse reactions and poor patient compliance. The end-point of effective immunotherapy is often unclear and difficult to assess objectively. In recent years the immunologic consequences and mechanisms of immunotherapy have been studied, but the level of understanding remains incomplete. The production of antigen-specific IgG "blocking" antibody has been clearly demonstrated, but questions remain as to its mechanism of action. The possible role of other mechanisms, for example, antigen specific or nonspecific suppression of IgE production, changes in the release of mediators, and direct or soluble-product-mediated changes in cellular

immunity, cannot be evaluated with the available data.

Venom immunotherapy affords a unique opportunity to measure objectively the efficacy and specific immunologic response to varied regimens. Efficacy was uniformly complete in all patients challenged after 4 months of immunotherapy. Only three of 64 patients did not achieve a maintenance dose in that time, one due to a 2-month interruption in therapy and two due to systemic reactions. All of these patients did achieve maintenance dose (after 8 months in one case). Thus, in contrast to traditional advice, all patients can achieve a predetermined maximum dose, even in the face of adverse reactions.

Adverse reactions were of two basic types: large local or systemic. Large local reactions occurred with similar frequency in each group. Although very common, these reactions only rarely prevented the regular increment in dose. Local reactions occurred most frequently at doses of 15 to 50 µg and were much less troublesome at higher and maintenance doses. The slower, lower dose induction phase of regimen I did not decrease the frequency of reactions at higher doses. In fact, because the reaction rate was the same and twice as many injections were required per treatment course in the slow regimen, twice as many reactions occurred in those patients in Group I. The stepwise regimen III showed that large local reactions were avoided by repeating the same dose but occurred with the usual frequency when the dose was increased. These observations call into question the rationale of avoiding increases in dose due to local reactions. A minimal number of reactions per treatment course can be achieved by using a "rush" regimen and progressive dose increments even in the face of local reactions (unless so large as to cross a joint line), since the frequency of local reactions invariably falls off as doses exceeding 30 to 50 µg are achieved. The immunologic correlates of this observation are discussed below.

Adverse reactions of greater concern are systemic reactions. While the overall incidence was 15.6%, objective systemic signs and symptoms requiring epinephrine occurred in 7.8% of patients at 0.5 reactions/100 injections.

Table 3. Anti-Yellow-Jacket-IgG Antibody Response

Time	I (Slow)	II (Rush)	III (Step)
<i>wks</i>			
0	54 (42-71)*	60 (42-87) <i>P</i> < 0.85†	59 (43-80) <i>P</i> < 0.64
6	71 (53-96)	182 (102-325) <i>P</i> < 0.007	130 (91-186) <i>P</i> < 0.007
10	105 (70-158)	283 (175-459) <i>P</i> < 0.003	226 (143-358) <i>P</i> < 0.009
14	125 (79-199)	281 (171-462) <i>P</i> < 0.015	240 (164-353) <i>P</i> < 0.025
18	174 (110-274)	276 (165-462) <i>P</i> < 0.085	263 (182-380) <i>P</i> < 0.106
Excluding one patient	151 (105-217)	<i>P</i> < 0.045	<i>P</i> < 0.05
38	165 (103-263)	214 (138-331) <i>P</i> < 0.45	

* Geometric mean anti-yellow-jacket-IgG (95% confidence limits) expressed as reciprocal of serum dilution for 50% binding.
† Level of significance compared to regimen I.

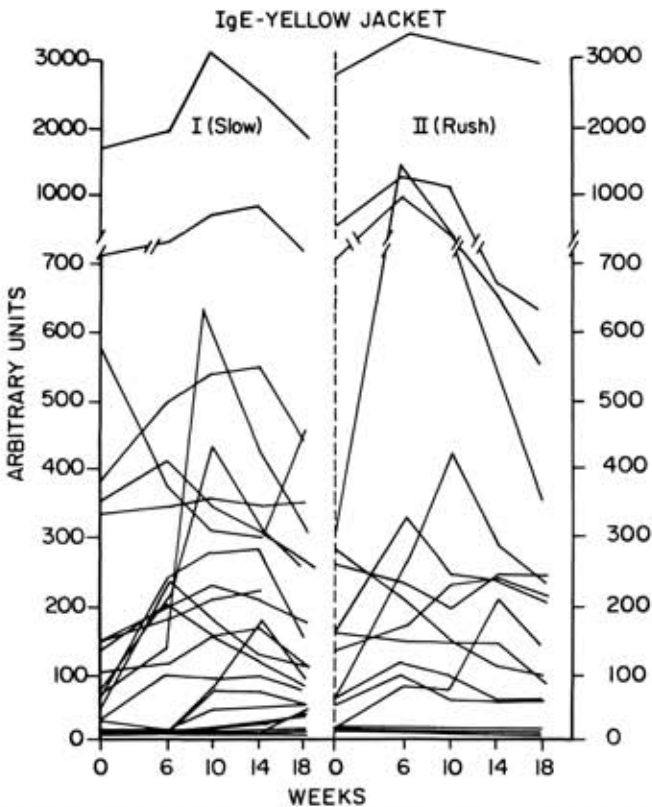


Figure 2. Anti-yellow-jacket (YJ)-IgE antibody titers expressed as arbitrary radioallergosorbent test (RAST) units. Time course of IgE antibody response during venom immunotherapy is shown for each patient in regimen I (slow) and regimen II (rush).

All reactions were easily and quickly reversed. The subsequent dose was either held at the same level or decreased depending on the severity of reaction. Systemic symptoms almost invariably occurred very early in therapy, usually during the first or second week and at doses less than 2.5 µg. Again, the slow regimen did not decrease the rate of systemic reactions but did cause almost twice as many reactions, since twice as many injections were given. Significant increases of serum venom-specific IgG did not occur until the dose exceeded 25 µg in all groups.

By 10 weeks, 50 µg doses in regimen III caused YJ-IgG titers not significantly less than did 100 µg in regimen II. In regimen III no significant increment in YJ-IgG was associated with increasing the dose from 50 µg to 100 µg. This would suggest that 50 µg is an adequate dose of YJ venom to produce an optimal IgG response, but the clinical efficacy of this dose remains to be established. The YJ-IgG response after three doses of 100 µg was significantly less in regimen I (18 weeks) than in regimen II (10 weeks), suggesting the period of low-dose antigen administration was associated with a suppressed IgG response to higher antigen doses. That systemic reactions rarely occurred at doses above 25 µg may be related to the observation that a significant IgG response is first noted at that dose. Similarly large local reactions occurred much less frequently at doses exceeding 50 µg, the dose that elicited an optimal IgG response. The significantly lower IgG levels in the slow regimen (I) com-

pared to the rush regimen (II) at doses of 50 to 100 μ g might be expected to be associated with more large local reactions. In fact, those rates of large local reactions were 8% (group I) and 4.3% (group II), respectively. This suggests that adverse reactions continue to occur until immunologically effective doses are administered, and thus reduction of dose may be an inappropriate maneuver after such adverse reactions.

These studies indicate that venom immunotherapy can be effectively done with the fewest adverse reactions using a "rush" regimen to rapidly achieve maximum doses. Such a regimen is safe in a controlled medical environment and is associated with an optimal immune response. Questions still remain regarding relative indications for venom immunotherapy and the clinical implications of apparent cross-reactivity among the vespid venoms. These are reviewed elsewhere (15-17), and remain under investigation.

ACKNOWLEDGMENTS: The authors thank Ms. Kate Ballantine, Ms. Barbara Cleaves, Ms. Laverne Grove, Ms. Debbie Mellinger, and Ms. Yaiffa Niv for their technical and clinical assistance.

Grant support: by grant AI 08270 from the National Institute of Allergy and Infectious Diseases.

This paper is Publication #379 of the O'Neill Research Laboratories, The Good Samaritan Hospital, Baltimore, Maryland.

► Requests for reprints should be addressed to David B. K. Golden, M.D.; The Johns Hopkins University School of Medicine at The Good Samaritan Hospital, 5601 Loch Raven Boulevard; Baltimore, MD 21239.

Received 6 November 1979; revision accepted 14 February 1980.

References

- HUNT KJ, VALENTINE MD, SOBOTKA AK, BENTON AW, AMODIO FJ, LICHTENSTEIN LM. A controlled trial of immunotherapy in insect hypersensitivity. *N Engl J Med*. 1978;299:157-61.
- LICHTENSTEIN LM, VALENTINE MD, SOBOTKA AK. A case for venom treatment in anaphylactic sensitivity to Hymenoptera sting. *N Engl J Med*. 1974;290:1223-7.
- BUSSE WW, REED CE, LICHTENSTEIN LM, REISMAN RE. Immunotherapy in bee-sting anaphylaxis: use of honeybee venom. *JAMA*. 1975;231:1154-6.
- SOBOTKA AK, VALENTINE MD, ISHIZAKA K, LICHTENSTEIN LM. Measurement of IgG-blocking antibodies: development and application of a radioimmunoassay. *J Immunol*. 1976;117:84-90.
- SOBOTKA AK, LESSOF MH, LICHTENSTEIN LM. Absolute blocking antibody levels in passively and actively treated honey-bee-sensitive patients. *J Allergy Clin Immunol*. 1976;57:247. Abstract.
- LIGHT WC, REISMAN RE, SHIMIZU M, ARBESMAN CE. Clinical application of measurements of serum levels of bee venom-specific IgE and IgG. *J Allergy Clin Immunol*. 1977;59:247-54.
- LESSOF MH, SOBOTKA AK, LICHTENSTEIN LM. Effects of passive antibody in bee venom anaphylaxis. *Johns Hopkins Med J*. 1978;142:1-7.
- WYPYCH JI, REISMAN RE, ELLIOTT WB, STEGER RJ, ARBESMAN CE. Immunological and biochemical evaluation of the potency of whole insect body extracts. *J Allergy Clin Immunol*. 1979;63:267-72.
- REISMAN RE, LIGHT WC, WYPYCH JI, ARBESMAN CE. Immunological studies of the effect of whole body insect extracts in the treatment of stinging insect allergy. *J Allergy Clin Immunol*. 1976;57:547-53.
- PATTERSON R, LIEBERMAN P, IRONS JS, et al. Immunotherapy. In: MIDDLETON E, REED CE, ELLIS EF, eds. *Allergy: Principles and Practice*. St. Louis: C. V. Mosby Co.; 1978:877-98.
- BENTON AW, MORSE RA, STEWART JD. Venom collection from honey bees. *Science*. 1963;142:228-9.
- ZELEZNICK LD, HUNT KJ, SOBOTKA AK, VALENTINE MD, TIPPETT MMS, LICHTENSTEIN LM. Diagnosis of Hymenoptera hypersensitivity by skin testing with Hymenoptera venoms. *J Allergy Clin Immunol*. 1977;59:2-9.
- HUNT KJ, VALENTINE MD, SOBOTKA AK, LICHTENSTEIN LM. Diagnosis of allergy to stinging insects by skin testing with Hymenoptera venoms. *Ann Intern Med*. 1976;85:56-9.
- SOBOTKA AK, ADKINSON NF JR, VALENTINE MD, LICHTENSTEIN LM. Allergy to insect stings: IV. Diagnosis by radioallergosorbent test (RAST). *J Immunol*. 1978;121:2477-84.
- LICHTENSTEIN LM, VALENTINE MD, SOBOTKA AK. Insect allergy: state of the art. *J Allergy Clin Immunol*. 1979;64:5-12.
- YUNGERLING JW. The sting-revisited. *J Allergy Clin Immunol*. 1979;64:1-2. Editorial.
- REISMAN RE. Stinging insect allergy. *J Allergy Clin Immunol*. 1979;64:3-4. Editorial.