

Combination of passive and active immunization in honeybee venom immunotherapy

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Immunotherapy with honeybee venom (HBV) is effective under optimal conditions, but many untoward systemic reactions are observed when venom doses are increased, especially with rush protocols. Although there were large interpatient variations, the levels of serum bee venom specific IgG were found to significantly correlate with the protection of the allergic patients. A double-blind, placebo-controlled study was carried out to determine whether passive immunization with fractionated IgG from a beekeeper's serum pool was able (1) to protect patients undergoing a rush immunotherapy program with HBV against untoward systemic reactions, and (2) to observe if the active immunization with HBV could elicit an active IgG immune response toward venom allergens. Hyperimmune IgG was obtained from a pool of sera of beekeepers (Cohn fractions II to III) that was subsequently treated with pepsin for intravenous use. All IgG subclasses were found to be present in the preparation. Twenty-seven patients, highly allergic to HBV, were randomly allocated to two treatment groups. Twenty patients received 10 to 15 gm of fractionated IgG, and seven other patients received saline in a double-blind fashion. The next day, a 3-hour rush protocol attempted to reach a cumulative venom dose of 200 µg; 16/20 patients who received the fractionated IgG reached this dose without any systemic reaction, whereas none of the seven subjects who received saline could attain it, and all patients had a systemic reaction. The active immunization with HBV elicited a significant ($p < 0.05$; Wilcoxon W Test) increase in venom IgG after 1 week of treatment in the patients who reached the maintenance dose. Venom IgG was subsequently increased in this group. In the group of patients who received saline, a similar increase in venom IgG was observed; 21/27 patients were stung by honeybees between 3 weeks and 2 months after the maintenance dose had been reached. None of the patients had a systemic reaction. Passive immunization with IgG was demonstrated to protect, significantly, patients against systemic reactions of active immunotherapy, and the combination of passive and active immunotherapy can be used as an alternative when patients develop an allergic reaction close to the bee season. (J ALLERGY CLIN IMMUNOL 1987;79:947-54.)

Immunotherapy with Hymenoptera venoms is highly effective under optimal conditions,^{1,2} but untoward systemic reactions may occur, mainly when venom doses are being increased.¹⁻³ Immunotherapy with yellow jacket or wasp venom appears to be safer than immunotherapy with HBV,⁴ especially when rush protocols are used.⁴⁻⁶

Abbreviations used

HB: Honeybee
HBV: Honeybee venom
HBV-IgE: Serum honeybee venom-specific IgE
HBV-IgG: Serum honeybee venom-specific IgG

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The mechanisms by which venom immunotherapy operates are still unclear, but it has been suggested that in some, but not all, cases serum venom IgG may have a protective role and may correlate with protection in patients undergoing specific immunotherapy.⁷ The passive infusion of HBV-IgG was demonstrated to protect patients from an HB sting challenge in one study,⁸ whereas no such protective effect was observed in the other study.

TABLE I. Characterization of patients

Patient	Age (yr)	Sex	Allergic reactions				HBV-IgE (PRU/ml)	HBV-IgG (U/ml)	Date of rush	Systemic reaction	
			Severity*	nb	Last reaction	Skin test (μ g/ml)				During rush (μ g)†	During maintenance
1	53	F	4 (coma)	3	8/82	0.00001	19	30	3/83	0	0
2	23	F	2	2	9/82	0.1	4	10	4/83	0	0
3	21	M	3	1	7/82	0.01	12	241	4/83	0	0
4	20	F	3	1	6/82	0.1	6	22	5/83	0	0
5	54	M	4 (coma)	2	9/82	0.00001	45	33	5/83	10	0
6	27	M	4	3	9/83	0.001	10	10	5/83	0	0
7	28	F	3	2	7/82	0.01	7	165	5/83	0	0
8	43	M	3	3	7/82	0.01	13	35	6/83	0	0
9	32	F	4	3	6/83	0.0001	22	118	6/83	0	0
10	28	M	4	8‡	6/83	0.00001	23	689	7/83	130	0
11	27	M	4 (coma)	4‡	8/83	0.0001	168	649	8/83	130	0
12	63	M	4 (coma)	3	8/83	0.00001	4	70	8/83	0	0
13	22	M	4	4‡	5/82	0.001	189	578	8/83	130	0
14	53	F	3	10	7/82	0.1	5	36	8/83	0	0
15	13	M	3	3	6/83	0.001	7	794	8/83	0	0
16	32	F	3	4	7/79	0.01	150	307	9/83	0	week 2
17	45	M	4	3‡	6/83	0.0001	2	146	10/83	0	0
18	43	F	3	3	8/83	0.01	5	157	10/83	0	0
19	45	F	4	2	8/83	0.001	9	77	10/83	0	0
20	28	F	3	2	8/82	0.01	8	133	10/83	0	0
21	35	F	3	2	6/83	0.0001	7	37	7/83	70	0
22	26	M	3	2	8/82	0.001	17	188	7/83	70	0
23	42	M	4	2‡	5/83	0.0001	5	51	7/83	30	0
24	12	F	4	2	7/83	0.00001	170	1075	10/83	10	week 2
25	31	M	4	2	6/83	0.001	7	33	10/83	10	0
26	25	M	4	3‡	7/83	0.001	12	45	10/83	10	0
27§	31	F	4	5‡	4/83	0.001	5	20	10/83	30	—

Patients in group I are Nos. 1 to 20; patients in group II are Nos. 21 to 27.

*Severity of reactions according to Mueller.²⁴

†When a systemic reaction occurred during the rush protocol, the cumulated dose of venom received by the patients is indicated.

‡All systemic reactions were not as severe as the last one that is indicated.

§Patient 27 did not agree to continue the specific immunotherapy after the rush.

In Europe, most allergic systemic reactions to HBV occur in members of families of beekeepers,¹⁰ and since honey is often processed in the family house or nearby, it is therefore important to attempt to protect the patients as soon as possible after the allergic reaction.

A double-blind study was carried out and combined passive immunotherapy with fractionated IgG from a beekeeper's serum pool, and active immunotherapy was used to (1) decrease the duration of the first phase of immunotherapy, (2) reduce the number and the severity of untoward reactions during venom immunotherapy, and (3) achieve rapid protection of the patients. For this purpose only HBV was used because HBV immunotherapy is more difficult to achieve than yellow jacket venom immunotherapy.

MATERIAL AND METHODS

Patients

Twenty-seven patients ranging in age from 12 to 63 years (33.41 ± 12.9 years) were included in the study after informed consent. All were allergic to HBV and presented severe or extremely severe anaphylactic reactions. They all had positive skin tests to HBV and had HBV-IgE. Many patients had presented more than one systemic reaction (Table I).

Fractionation of IgG

Immunoglobulins were fractionated from a pool of 50 beekeepers' sera containing 175 U/ml of HBV-IgG (RAST, Pharmacia Diagnostics AB, Uppsala, Sweden) and 79 kU/L of total IgE. Serum HBV-IgE (PRIST, Pharmacia Diagnostics AB) from the pool was slightly positive (3 PRU/ml) before fractionation. Cohn fraction II to III was

prepared according to the cold ethanol method described by to prevent immunoglobulin aggregation. Kistler and Nitschmann.¹¹ For intravenous use, the fraction II to III was then acidified to pH 4, and a small amount of pepsin was added;^{12, 13} glucose and glucosamine were used. After neutralization, the final product was lyophilized.

Fractionated immunoglobulin was tested for hepatitis-associated antigens, the Veneral Disease Research Laboratory test was nonreactive, the immunoglobulin was free of aggregates or of components of the contact activation system, and pyrogenicity was in the normal range according to the European Pharmacopaea.

IgG represented >97% of total proteins. IgG subclasses were measured by radial immunodiffusion in agar plates containing specific antisera and by use of the World Health Organization International Reference plasma pool 67/97 as a calibrator.¹⁴ It was found that IgG₁, IgG₂, and IgG₃ were increased tenfold when the immunoglobulins were compared with the beekeepers' serum pool, and IgG₄ was increased fivefold. Specific HBV-IgG was increased ninefold (1309 U/ml, Pharmacia IgG RAST). Total serum IgE was increased to 146 kU/L and HBV-IgE was undetectable.

Methods used

Venoms obtained from Stallergènes Laboratories (Fresnes, France) are standardized by means of the titration of hyaluronidase and phospholipase A₂ as well as RAST inhibition. These venoms were used for treatment and skin tests.

Skin tests were performed by intradermal test according to the method of Zelesnick et al.¹⁵

Serum HBV-IgE was titrated by the Phadebas RAST (Pharmacia Diagnostics AB). Since venoms used for treatment and in vivo tests were obtained from a different company, we checked the concordance of RAST discs prepared with venoms of both origins. Twenty individual sera were tested, and there was a highly significant correlation ($r = 0.96$), indicating that both venoms were similar. Since many sera were out of range, all were titrated pure and diluted 1/5 and 1/10. The results are presented in peripheral resistant units per milliliter. All samples were titrated in duplicate, and results were averaged. All determinations on each patient were done at the same time.

HBV serum IgG was titrated by the classic Pharmacia IgG RAST (Pharmacia Diagnostics AB). All samples were titrated in duplicate, and results were averaged. All determinations on each patient were done at the same time.

Protocol

Passive immunization with fractionated IgG was performed by intravenous infusion of 10 to 15 gm of IgG. This amount was estimated from the quantity of HBV-IgG infused and the volume of blood of the patients to increase the IgG level by 60 to 80 U/ml of serum. Five volunteers ranging in weight from 58 to 72 kg who had neither HBV-IgG nor HBV-IgE received hyperimmune globulins, and the titration of HBV-IgG 24 hours after infusion revealed that their level had increased from 60 to 80 U/ml (Pharmacia

TABLE II. Rush immunization with HBV

	Time	HBV (μ g)
Day 1	9:00	1
	9:30	10
	10:00	20
	10:30	40
	11:00	60
	11:30	70
Day 7	9:00	70
	9:30	70
	10:00	70
Day 21		200
Day 21		200
Day 21		200
Day 28		200
Day 42		200
Day 56		200
Monthly maintenance dose		200

IgG RAST) when 12.5 gm were injected. Thus, children <50 kg received a dose of 10 gm, and 15 gm were infused in adults. One gram of IgG was infused in 25 to 30 minutes, and the infusion rate was decreased when a febrile reaction occurred. Fractionated immunoglobulin or saline was administered in a double-blind fashion by different doctors from doctors who performed immunotherapy. When a pyrogen-like reaction occurred, aspirin was administered by the intravenous route.

Active immunization with HBV was started the next day. Patients were admitted to an intensive care unit, and blood pressure and pulse rate were serially recorded every 3 minutes with a Holter system (Space Laboratories, Inc., Bellevue, Wash.). When the systolic or diastolic blood pressure decreased by >30 mm Hg and the pulse rate increased by >30/min, or if other systemic symptoms such as urticaria, angioedema, or asthma occurred, the venom injection was stopped. Venom was administered at 30-minute intervals to reach a cumulative dose of 200 μ g in 3 hours (Table II). A maintenance dose of 200 μ g was chosen according to previous data,¹⁰ which suggested that this monthly maintenance dose induces a significantly higher protection than 100 μ g. Then, at weekly intervals for the next 3 weeks, the same cumulative dose was injected. Booster injections were administered at 28, 42, and 56 days after the rush protocol and thereafter every month.

When the cumulative dose of 200 μ g of HBV could not be reached because of a systemic reaction, the HBV dose that did not induce a systemic reaction was administered the next day and subsequently increased every day to reach the maintenance dose. When the cumulated dose of 200 μ g of HBV was reached, the initial protocol was then used.

Evaluation of the treatment

In patients who could reach the maintenance dose within 1 day, serum HBV-IgG and HBV-IgE were serially mea-

TABLE III. Comparison of the two groups of patients

	Group I	Group II	Statistical difference
Age (yr)	13 to 63 (35.1 ± 13.7)	12 to 42 (28.8 ± 9.4)	NS
Sex (% M)	50	58	NS
Severity of reactions according to ²⁴ (%)			
Grade II	5	0	NS
Grade III	45	28	
Grade IV	30	62	
Coma	20	0	
No. of reactions	1 to 10 (3.3 ± 2.15)	2 to 5 (2.5 ± 1.15)	NS
HBV skin tests (μg/ml)	0.1 to 0.000001	0.001 to 0.00001	NS
HBV-IgE (PRU/ml)	2 to 189 (35.4 ± 58.7)	5 to 170 (31.8 ± 61.0)	NS
HBV-IgG (U/ml)	10 to 794 (215.5 ± 252)	20 to 1075 (207.1 ± 386)	NS

NS = not significant.

sured for up to 2 months. Blood was drawn before the injection of fractionated immunoglobulins 24 hours after their infusion every week for the next 3 weeks and 2 months after the beginning of the treatment.

In patients who could not reach the maintenance dose, HBV-IgE and HBV-IgG were analyzed before immunotherapy for a cumulated venom dose of 600 μg corresponding approximately to the third week of treatment and 2 months after the rush. Skin tests were performed before and 2 months after the beginning of the treatment. Twenty-one patients were stung between 3 weeks and 2 months after the beginning of the treatment.

Statistical analysis of the data

Nonparametric tests were used. For paired data, Wilcoxon W test was used, and for unpaired data, Mann Whitney U test was used. Spearman R_s was used for correlations.

RESULTS

Randomization of patients

Twenty patients received passive and active immunotherapy (group I), and seven patients received saline and active immunotherapy (group II). Three other patients received saline infusion but did not wish to be immunized with HBV. They were excluded from the study.

There was no statistical difference between the two groups as indicated in Table III. Serum HBV-IgG ranged from 10 to 794 U/ml in group I and from 37 to 1075 U/ml in group II.

Infusion of fractionated immunoglobulins

No patient who received saline presented any untoward reaction. By contrast, 15/20 patients who had an infusion of fractionated immunoglobulin presented

a mild generalized reaction characterized by chills, nausea, flushing, increased body temperature, and/or a pseudoinfluenza syndrome, but none had symptoms of pyrogenic shock, since blood pressure and pulse rate did not vary. These symptoms occurred after 75% to 95% of the fractionated immunoglobulin had been infused. With a treatment of 500 mg of aspirin by intravenous route, the infusion could be continued. No reaction made it necessary to stop the treatment.

Rush immunotherapy

In group I, 16/20 patients could reach a cumulated venom dose of 200 μg within 3 hours (Fig. 1) without any systemic reaction or any significant variation of the pulse rate or blood pressure.

Four patients presented a systemic reaction requiring epinephrine for cumulated venom doses of 20 to 130 μg. All patients had generalized urticaria, mild hypotension, and tachycardia. Two patients also had laryngeal edema. Three of these patients had, initially, very high HBV-IgG levels (649, 578, and 689 U/ml) and HBV-IgE levels (23 to 189 PRU/ml), whereas the other patient had had a very severe allergic reaction after being stung and presented very high IgE titers (45 PRU/ml). Three to four sessions of immunotherapy were required to reach a cumulative venom dose of 200 μg.

All subjects had a large local reaction that started by the end of the rush protocol and lasted for 12 to 48 hours.

No patients placed in group II could reach the cumulated venom dose of 200 μg (Fig. 1). They had either a decrease in blood pressure of >30 mm Hg or a systemic reaction for cumulated venom doses rang-

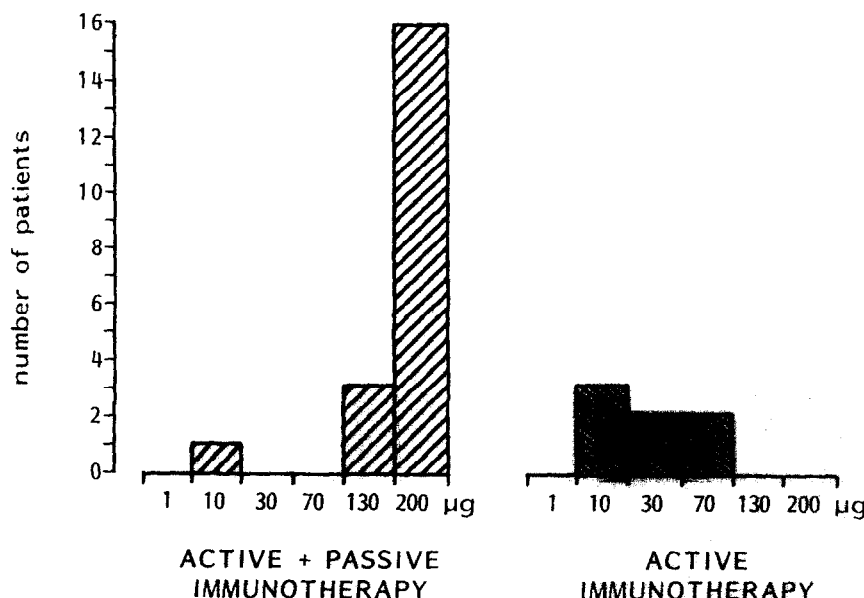


FIG. 1. Cumulative venom dose reached by patients submitted to rush immunotherapy with HBV. All patients who could not reach a cumulative venom dose of 200 µg had a systemic reaction.

ing from 10 to 70 µg. Epinephrine was required in five cases. Three to seven sessions of immunotherapy were needed to reach the cumulated venom dose of 200 µg. All patients had large local reactions.

Maintenance immunotherapy

One patient in group I and one other patient in group II presented an urticarial side reaction while they were receiving immunotherapy. These reactions did not require any specific treatment.

Evolution of serum HBV-IgE

Large interpatient variations were observed in all three groups. Overall, mean values did not change significantly throughout the study.

Evolution of serum HBV-IgG

Mean results are presented in Fig. 2. It can be observed in group I that HBV-IgG was significantly ($p < 0.001$) increased after the infusion of fractionated IgG. In patients who could reach the cumulative dose of 200 µg of HBV in 1 day, a mean increase of 67 U/ml was observed. The immunologic effects of the active immunization were already detectable 1 week after the rush because HBV-IgG was significantly ($p < 0.05$) increased (210 to 256 U/ml). HBV-IgG continued to rise significantly ($p < 0.02$) between days 7 and 14 (256 to 462 U/ml) and thereafter reached a plateau. In patients placed in group I and who could not reach the cumulative dose of 200 µg of HBV in 1 day, HBV-IgG increased 3 weeks

after the beginning of the rush and then reached a plateau.

In group II, HBV-IgG was significantly increased 3 weeks after the beginning of the rush ($p < 0.001$). There was a significant ($p < 0.05$) difference between HBV-IgG in groups I and II, but after 2 months, the difference between the two groups was slight and nonsignificant.

HBV-IgG levels during the first 3 weeks correlated with the initial level ($R_s = 0.89$), and this correlation was less evident after 2 months ($R_s = 0.62$).

Evolution of skin tests

No significant difference could be observed when skin tests performed before treatment were compared with skin tests conducted 2 months later.

HB sting challenge

Twenty-one (15 in group I and six in group II) of 27 patients had a natural sting challenge between 3 weeks and 2 months after the cumulative dose of 200 µg was reached. No systemic reaction occurred, but most patients had a large local reaction. Many of these decided therefore to continue HBV immunotherapy by means of four to six HB stings a month.

DISCUSSION

It was only possible to achieve 1 day of rush immunotherapy with HBV when the active immunization was preceded by a passive infusion of HBV-IgG. Eighty percent of the patients received a

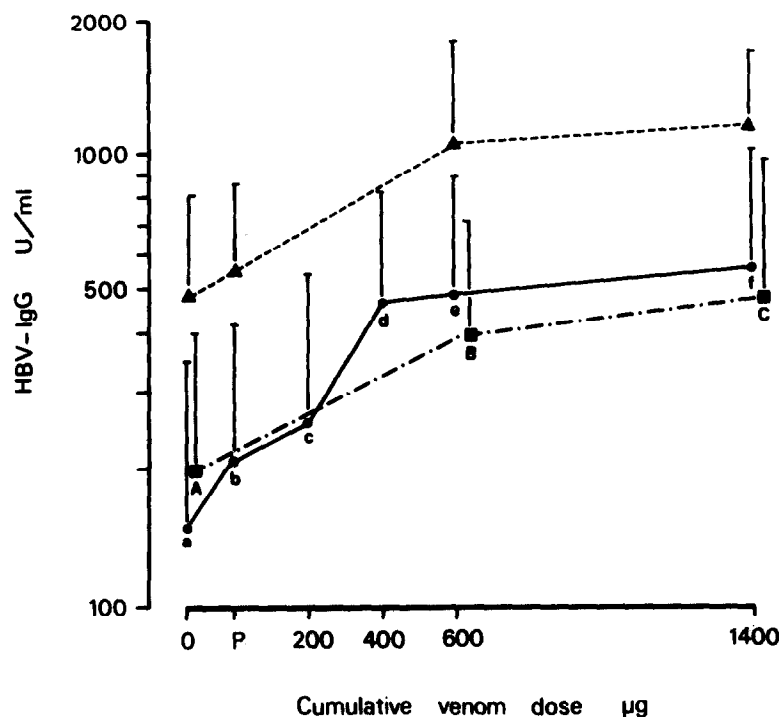


FIG. 2. Evolution of serum HBV-IgG: patients in group I who could reach the maintenance dose (●); patients in group I who could not reach the maintenance dose (▲), and patients in group II (■). $p_{a/b} < 0.001$; $p_{b/c} < 0.05$; $p_{c/d} < 0.02$; $p_{d/e}$:ns; $p_{e/f}$:ns (Wilcoxon W test); $p_{A/B} < 0.001$; $p_{B/C}$:ns (Wilcoxon W test); $p_{A/A}$:ns; $p_{B/B} < 0.05$; $p_{C/C}$:ns (Mann-Whitney U test) (P, after passive immunization).

cumulative dose of 200 µg of HBV without any symptom of systemic reaction. By contrast, all subjects presented a large local reaction for single doses of HBV, ranging from 40 to 70 µg. Moreover, all patients stung by an HB were protected.

The preparation of fractionated immunoglobulin was performed according to a classic procedure,¹¹⁻¹³ and all quality-control tests for intravenous immunoglobulin were done according to the European Pharmacopaea. The final preparation contained >97% of IgG. HBV-IgG antibody content was increased nine-fold. The four IgG subclasses were found to be present in the preparation. Total serum IgE was slightly increased after fractionation, but HBV-IgE could not be detected, possibly because of RAST interference by IgG.¹⁶

From 10 to 15 gm of fractionated immunoglobulin was infused at a low infusion rate to reduce potential side reactions that occurred in 75% of the patients; however, they were always minimal or mild. The only treatment required was aspirin and a reduced infusion rate. It was never necessary to stop the infusion. Such reactions are common with infused immunoglobulin¹⁷ and cannot be compared with systemic anaphylactic reactions that are occasionally observed during HBV

immunotherapy. The long-term safety of their use may be questioned, but it is likely that a single infusion of immunoglobulins is harmless.

All the patients who received pretreatment with saline and 20% of patients who had an immunoglobulin infusion could not reach the cumulative dose of 200 µg of HBV. They had mild to severe systemic reactions requiring epinephrine in 80% of cases. It is therefore clear that the infusion of fractionated immunoglobulin protects the patient from a venom injection, and this study confirms previous data.⁸ The dose of 200 µg of HBV is not used routinely, but this is not because patients received 200 µg instead of 100 µg that made the reactions more frequent, since in our experience, patients had systemic reactions between 100 and 200 µg. Some patients who received fractionated immunoglobulin had a systemic reaction during the rush procedure. These patients appear to have particular immunologic patterns because all had high or very high HBV-IgE titers and three of them a very high HBV-IgG titer (649, 689, and 578 U/ml). From studies of beekeepers¹⁰ and from prospective studies on venom efficacy,^{7, 18} it is suggested that a venom IgG titer ranging from 150 to 400 U/ml should protect most subjects; however, there are some investigators

who have demonstrated that HBV-IgG levels are not directly related to the degree of protection.¹⁹ This was not the case in three of four patients, and the relatively low amount of HBV-IgG injected was unlikely to elicit any protection. Thirty-six other patients have now received fractionated immunoglobulin before rush immunotherapy (unpublished data). Five patients could not reach the HBV cumulative dose of 200 µg. They all had HBV-IgG >400 U/ml and very high HBV-IgE titers, confirming therefore that patients with HBV-IgG >400 U/l should not be candidates for passive and active immunotherapy. The reasons for the protective activity of fractionated immunoglobulin cannot be completely elucidated from this study because HBV-IgG only forms a fraction of all infused IgG.

In the future it will be interesting to extend this study by use of immunoglobulin subclasses to determine whether there may be better protection with a lower amount of infused IgG. Moreover, it may be desirable to use purified HBV-IgG or IgG subclasses, but their industrial preparation is still subject to many problems, particularly a high pyrogenicity.

The HBV-IgG response was raised in most subjects 1 week after the rush procedure and in all patients after 2 weeks. Since the half-life of IgG ranges from 8 to 21 days in normal individuals,²⁰ it appears that the production of HBV-IgG by the patients occurs rapidly after the active immunization. All patients treated with HBV alone had a significant increase in HBV-IgG, and there was no significant difference between the two groups. Mean levels of HBV-IgE did not vary as might have been suspected because mean variations usually appear after a longer period of treatment. These observations indicate that the active immune response is not modified by the passive infusion of IgG. HBV skin tests did not vary significantly in either group, and this finding contrasts with those observed in mite or pollen^{21, 22} rush immunotherapy. However, from studies of beekeepers,¹⁰ it appears that the HBV dose is too low to decrease skin tests in treated patients.

Twenty-one patients were stung by an HB either in the clinic or accidentally. No patient presented a systemic reaction, demonstrating that rush immunotherapy with passive immunization or alone elicits not only an immune response but also protects the patients within days. These findings are of importance, since in Europe most HBV-allergic patients (83% in our practice) are members of the families of beekeepers. Very often the honey farm is close to their home, and they represent very high-risk patients. It is therefore important to protect the patients rapidly. Rush immunotherapy appears to be efficient, but alone, it elicits

a very high rate of systemic reactions (see Evaluation of the treatment). The pretreatment with fractionated immunoglobulin makes it possible to reduce sharply the incidence of these untoward reactions and may be an alternative to active treatment alone.

According to our current experience, we consider that only some HBV-allergic patients are candidates for active and passive immunotherapy: (1) Patients with HBV-IgG >400 U/ml (Pharmacia IgG RAST) before treatment should not receive hyperimmune globulins. (2) Members of families of beekeepers, living in areas surrounded by bees who presented a systemic reaction during the bee season are the best candidates because they should be protected as soon as possible. (3) Patients who experience recurrent systemic reactions during immunotherapy with HBV and whose HBV-IgG is <400 U/ml may receive hyperimmune IgG. Müller et al.²³ have used this method and demonstrated that most patients could tolerate the maintenance dose of 100 µg of HBV. However, after a few weeks, some patients relapsed when the protective effect of infused IgG disappeared.

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