

A double-blind study comparing monomethoxy polyethylene glycol-modified honeybee venom and unmodified honeybee venom for immunotherapy

I. Clinical results

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Thirty-five patients were allocated at random to immunotherapy (IT) in a double-blind way with either monomethoxy polyethylene glycol (mPEG)-modified honeybee venom (HBV) or HBV. The two groups were well matched regarding age, sex, skin sensitivity, HBV-specific serum IgE and IgG antibodies, and history of reactions after a field sting; mPEG-HBV-treated patients received doses that increased more steeply than doses of the HBV-treated patients. The maintenance dose of the former group (200 µg) was greater than that of the latter group (100 µg). During IT, both groups had the same frequency of local swellings after injections. Four patients receiving mPEG-HBV developed one mild systemic reaction (SR) during dose increase, whereas 10 patients receiving HBV demonstrated one or more of these reactions, compelling two patients to stop therapy. Following challenge with a honeybee sting after about 14 weeks of IT, six patients with SR were observed, four in the mPEG-HBV-treated group and two in the HBV-treated group. In the HBV-treated group, three patients were not challenged, one because of an insufficient IgG increase and two other patients because they dropped out of IT before reaching maintenance dose because of repeated SRs. Since the mPEG-HBV is extremely well tolerated during IT and the success rate is not significantly lower than with unmodified HBV, we suggest it as an attractive alternative to HBV for the treatment of HBV hypersensitivity. Increase of the maintenance dose may result in an even better clinical efficacy. (*J ALLERGY CLIN IMMUNOL* 1987;80:252-61.)

The efficacy of IT with Hymenoptera venoms for patients with a history of systemic allergic reactions to Hymenoptera stings has been demonstrated in two controlled prospective trials^{1,2} and suggested in many uncontrolled studies.³⁻¹² At reexposure to a sting of the culprit insect, 71% to 95% of patients receiving IT with venoms no longer develop an SR, and patients who do demonstrate diminished reaction.^{1,2,9,10,13-16}

Abbreviations used

IT:	Immunotherapy
LLR:	Large local reaction
SR:	Systemic reactions
HBV:	Honeybee venom
mPEG-HBV:	Monomethoxy polyethylene glycol modified HBV
ICT:	Intracutaneous skin test
PRU:	Phadebas RAST units
Ab:	Antibody

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Allergic side effects, from LLRs to severe SRs, have been recognized as a major problem of IT with Hymenoptera venoms. In some cases, they cause IT to be stopped. One approach to reduce side effects of IT with allergens is the use of chemically modified

TABLE I. Patient characteristics

	Treatment	
	mPEG-HBV	HBV
No. of patients	17	17
M/F	13/4	15/2
Age mean (range); yr	34.6 (17-70)	34.5 (17-57)
Field sting reaction		
Urticaria, angioedema	5	3
Respiratory	9	10
Shock	3	4
HVB-specific IgE, PRU/ml*	3.1 (0.2-65.8)	3.6 (0.3-27.0)
HBV-specific IgG, U/ml*	60.9 (19-460)	60.9 (19-421)
Skin sensitivity wheal area (mm ²) at ICT with 0.1 µg/ml†	79 (64-98)	100 (85-119)

*Geometric mean value (range).

†Arithmetic mean value (range).

preparations. Chemical modification is supposed to reduce allergenicity, i.e., the ability of the allergen to react with specific IgE Abs but not to destroy immunogenicity, i.e., the ability of the allergen to induce a protective immune response. Lee and Sehon¹⁷ have developed polyethylene glycol-conjugated allergens and have studied their efficacy in animal models. In addition to reduced allergenicity, they observed a suppression of an ongoing IgE response with these preparations.

Various chemical modifications of HBV have been described and studied in animals^{18, 19}; mPEG-modified HBV was recently demonstrated²⁰ to have an IgE-suppressing effect in HBV-sensitive mice. The IgG response of mPEG-HBV-treated mice was, however, comparable to that of an HBV-treated control group. In vitro studies on human material, including RAST-inhibition and histamine-release assays,²¹ indicated that mPEG-HBV is about 100 times or more less allergenic than unmodified HBV.

In a clinical pilot study with HBV-sensitive patients,²² doses of up to 400 µg of mPEG-HBV induced no SR, whereas LLRs were observed. The mean serum level of HBV-specific IgE was reduced below the pretreatment value, even after 6 weeks of IT. At the same time, a significant increase of HBV-specific IgG was observed. Eight of the patients experienced field stings and tolerated them well. The results of this open pilot study indicated that mPEG-HBV could be a promising new approach to IT of HBV sensitivity. We therefore initiated a double-blind trial of IT with mPEG-HBV and HBV, comparing their tolerance, efficacy, and ability to induce an immune response. The clinical results of this study are contained in this article. Results of skin testing and laboratory analyses,

TABLE II. Dose schedules of mPEG-HBV and HBV

Week No.	Vial No.	Doses (µg)	
		mPEG-HBV	HBV
0	1	0.5	0.1
	2	5.0	1.0
	3	10	3.0
1	4	30	5.0
2	5	60	10
3	6	100	20
4	7	200	40
5	8	200	65
6	9	200	100
7	9	200	100
8			
9	9	200	100
10			
11			
12	9	200	100
13			
14		Challenge	
15			
16	9	200	100
Monthly	9	200	100

including crossed radioimmuno-electrophoresis and estimation of subclass specific-IgG Abs, will be presented and discussed in a second article.²³

MATERIAL AND METHODS

Patients

The study included 35 white patients, 24 in Bern and 11 in Johannesburg. One patient treated with HBV interrupted

TABLE III. Systemic side effects during immunotherapy

	Treatment	
	mPEG-HBV	HBV
No. of patients	17	17
Subjective complaints	2	6
Objective symptoms of an allergic SR	2	4
Treatment necessary	0	4
Change of treatment schedule	0	5
Dropouts because of side effects	0	2

IT after only a few weeks of treatment because he went abroad. Data from this patient are therefore excluded from all types of calculations except the calculation dealing with the allergenicity of the two venoms. All patients had developed an SR after a field sting by a honeybee, were skin test positive with HBV at ≤ 0.01 $\mu\text{g/ml}$, and had an HBV-specific IgE titer of >0.35 PRU. The patients were allocated at random to IT with either mPEG-HBV or HBV. The characteristics of the patients allocated into the two groups are presented in Table I. The two groups undergoing IT were well matched regarding sex, age, type of reaction after field sting, HBV-specific IgE, HBV-specific IgG, and skin sensitivity. There were no statistically significant differences between the treatment groups in these variables.

Allergen extracts

Unmodified HBV (Pharmalgen or Reless) was supplied by Pharmacia AB, Uppsala, Sweden (batch No. HA 25034). The investigation preparation mPEG-HBV (Pharmacia AB) (batch No. D 810310), was produced by coupling mPEG succinate of molecular weight, 5700 daltons, to HBV according to the mixed anhydride method.²⁴ The chemical properties of the mPEG-HBV are described in more detail elsewhere.²² Only one batch of mPEG-HBV was used in this study. The standardization of such extracts has been described previously.^{21, 24} The allergenic activity was estimated by RAST inhibition to avoid batch-to-batch differences in potency. The protein content of the modified and unmodified product was similar. The content of mPEG and of free aminogroups was also checked to assure consistency between batches. Furthermore, the change of specific IgG to allergens has been investigated during IT with mPEG-HBV by crossed radioimmuno-electrophoresis.^{22, 30} These investigations have demonstrated that the major allergens are still immunogenic after modification.

Both preparations were freeze-dried, and the same batch numbers were used throughout the study, both for skin testing and IT. For reconstitution and dilution of the freeze-dried preparations, buffered saline with human serum albumin (albumin diluent, Pharmacia AB) was used. Lyophilized and reconstituted preparations were visually identical.

TABLE IV. Results of the sting challenge

	Treatment	
	mPEG-HBV	HBV
Patients	17	14
LLR (>10 cm in diameter, >24 -hr duration)	7	4
Mild subjective complaints	1	3
SRs	4	2
Cutaneous	1	2
Respiratory	1	—
Cardiovascular	2	—

Skin test sensitivity

All patients were tested intracutaneously with both mPEG-HBV and HBV before the start of IT. Before performing the ICT on the patient's back in triplicate, a pretest was performed in duplicate on the volar part of the forearm to select the three lowest concentrations of the venoms eliciting a positive reaction. Together with albumin diluent and histamine (HCl), 1 mg/ml, patients were tested with mPEG-HBV at concentrations of 1, 10, 100, and 1000 $\mu\text{g/ml}$, and HBV at concentrations of 0.001, 0.01, 0.1, and 1.0 $\mu\text{g/ml}$. The use of 1000 times higher concentrations of mPEG-HBV in ICT was based on earlier findings of its decreased allergenicity. Fifteen minutes after performing the ICT, the wheal contours were encircled with a fine-filter tip pen and transferred by means of a transparent tape to a patient data sheet. The wheal areas were calculated with a digitizer connected to a VAX-11/750 computer (Digital Equipment Co., Maynard, Mass.).

Allergenicity of the mPEG-HBV preparation

From the results of the ICT with both mPEG-HBV and HBV in the 35 patients, we estimated the allergenicity of the former preparation in relation to HBV by means of the parallel line assay.^{25, 26} Plotting of wheal areas against concentration (log scale) results in a straight line.

Application of this procedure to various extracts of an allergen results in parallel lines from which the relative potencies of the allergen can be calculated. The ICT results of 29 patients were accepted for the evaluation, 16 of the mPEG-HBV-treated and 13 of the HBV-treated patients. Six patients were omitted from the calculations because they did not exhibit parallel lines. The geometric mean and the 95% confidence limits of the relative potencies were calculated.

Sting challenge with a living honeybee

In order to evaluate the early outcome of IT, a challenge was planned at week 14, i.e., 2 weeks after the sixth maintenance dose of mPEG-HBV (200 μg) or the fourth maintenance dose of HBV (100 μg), respectively. Because of two early failures, it was later decided that HBV-specific

TABLE V. Clinical and laboratory data in relation to sting challenge

Patient	Treatment	Previous reaction	Specific IgE in PRU/ml		Specific IgG in U/ml		Reaction at challenge
			Pretreatment	Prechallenge	Pretreatment	Prechallenge	
2	HBV	U,R	1.8	7.3	< 20	60	Not done
4	HBV	U,AE,S	46.3	24.2	283	>1000	LLR
6	HBV	AE,R	2.7	1.3	54	101	None
9	HBV	U	65.8	42.3	256	>1000	None
11	HBV	U,R,S	0.3	0.2	129	147	Mild AE
102	HBV	U,R	0.41	0.24	< 20	88	LLR
104	HBV	U,R	1.5	1.9	< 20	260	None
108	HBV	U,R	10.1	9.8	86	220	None
110	HBV	AE,R	6.3	3.7	40	128	LLR
111	HBV	U,GI	7.4	4.1	88	220	None (SS)
114	HBV	U,AE	2.8	2.9	108	168	None
117	HBV	U,GI,R	3.9	2.5	320	600	LLR
120	HBV	U,AE,R	15.5	5.3	20	134	LLR (SS)
122	HBV	GI,S	2.9	1.9	124	188	Mild AE
124	HBV	U,GI,R	3.9	0.84	< 20	106	None (SS)
116	HBV	U,GI,S	2.9	2.3	320	200	Dropped out†
105	HBV	AE,R	1.3	2.7	23	124	Dropped out†
1	mPEG-HBV	U,AE	10.8	9.2	138	527	None
5	mPEG-HBV	U,AE	27.0	23.6	421	>1000	None
7	mPEG-HBV	U,R	0.5	0.8	145	623	Mild U
8	mPEG-HBV	U,R,GI	15.7	8.6	173	394	None
10	mPEG-HBV	U,AE,R	7.1	22.6	< 20	101	None
101	mPEG-HBV	U,R,S,GI	1.5	2.6	26	44	R,S,GI
103	mPEG-HBV	U,R	4.6	2.9	76	180	LLR
106	mPEG-HBV	U,R	0.21 (1.1)*	0.29	< 20	142	R,S
107	mPEG-HBV	AE,R	0.21 (0.83)*	0.27	< 20	108	LLR
109	mPEG-HBV	U,AE,R	8.4	10.3	32	200	LLR
112	mPEG-HBV	U,AE,R	8.6	5.6	< 20	116	LLR
113	mPEG-HBV	U,AE,R	5.1	1.5	22	192	LLR
115	mPEG-HBV	S	0.19 (0.7)*	0.07	220	340	None (SS)
118	mPEG-HBV	U,AE	3.5	2.0	50	132	None
119	mPEG-HBV	R,S,GI	2.7	1.6	40	240	R,GI
121	mPEG-HBV	AE	1.9	0.35	< 20	82	LLR
123	mPEG-HBV	U	8.6	10.8	460	760	LLR

U = urticaria; AE = angioedema; GI = gastrointestinal symptoms; R = respiratory symptoms; S = shock symptoms; SS = subjective symptoms.

*Initial IgE value 3 to 8 months before starting the study.

†Dropped out because of side effects.

IgG levels should have reached a value of at least 150 U/ml before the challenge could be performed. If the serum sample of week 9 had not reached this level, the dose of mPEG-HBV was increased by weekly intervals to 300 and 400 µg, and the dose of HBV to 150 and 200 µg. The challenge was performed after five injections of the new maintenance dose. In 14 of the 31 challenged patients, seven patients in each group, the challenge was performed at a HBV-specific IgG level <150 U/ml.

For five patients (two patients receiving mPEG-HBV and three patients receiving HBV), the maintenance dose was increased because of an insufficient IgG response. Before challenge was performed, a venous catheter was im-

planted for rapid application of medication, if this were necessary.

In vitro assays

Blood samples for immunologic assays were drawn at the start of IT and after 4, 9, and 14 (the day of challenge) weeks and 6 and 12 months after the start of IT.

All sera were stored at -20° C until sera were analyzed. The HBV-specific IgE and IgG Abs were analyzed in duplicate according to the manufacturer's directions (Phadebas RAST and Phadebas IgG RAST, respectively). Sera with an IgE level >3.5 PRU/ml were diluted five or 25 times.

For IgE Abs, Phadebas RAST units are interpreted as

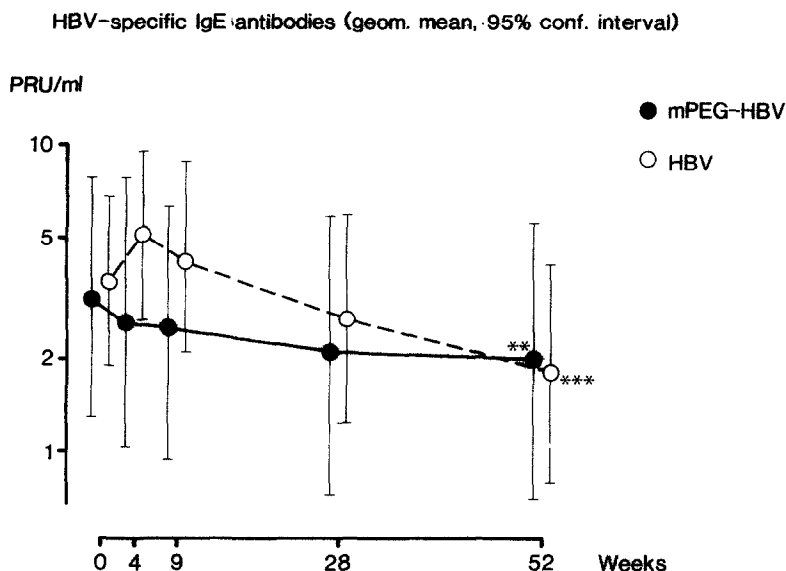


FIG. 1. HBV-specific IgE Ab (geometric mean and 95% confidence interval). ** $p < 0.01$; *** $p < 0.001$.

follows: <0.35 , low or absent; 0.35 to 0.69 , borderline; >0.7 , elevated; >3.5 , highly elevated. For IgG Abs, units per milliliter are interpreted as follows: <20 , low or absent; 20 to 50 , borderline; 50 , elevated. The value of 150 U/ml corresponds to about 5 μ g of IgG Ab per milliliter.

Dose schedule

The dose schedules used in our study are presented in Table II. At the first visit, three injections with increasing doses were administered. At weekly intervals, the dose was increased to the maintenance dose, which for HBV was reached after 6 weeks (100 μ g) and for mPEG-HBV, after 4 weeks (200 μ g). After this time, the intervals were increased successively to 4 weeks. Because of the decreased allergenicity of mPEG-HBV, the dose schedule was more aggressive than that of HBV.

Because the vials with HBV and mPEG-HBV contained 0.12 and 1.2 mg of venom, respectively, it was necessary to reconstitute and dilute with different volumes of diluent. In order to retain the double-blind nature of the study, special personnel delivered vials with patient code number and vial number to the physicians or nurses administering the injections. The same vial number of the two different types of venom always contained the same volume of venom extracts. The reconstitution and dilution of the venoms were performed either the day before or at the day of the injection.

Recording of side effects

Patients were observed for 30 minutes after each injection for the occurrence of side effects. On special outlined data sheets, the patients (Bern) daily recorded the size and duration of local side effects as well as the type and duration of SR. The patients in the other group (Johannesburg) completed the data sheets on their next visit to the hospital.

Statistical analyses

By using parallel line assay,^{22, 23} we estimated the relative potency (allergenicity) of the mPEG-HBV in relation to HBV as well as changes in skin sensitivity during IT. Geometric means and 95% confidence intervals were calculated.

All other tests of changes from baseline within and between groups were performed with the Student's *t* test. The same method was used for tests of differences in levels between groups.

Ethics

Informed consent was obtained from all patients, and the outline of the study was submitted to and approved by the local ethical committee.

RESULTS

Allergenicity of mPEG-HBV

The allergenicity of mPEG-HBV evaluated by means of parallel line assay was 2% of that of HBV (95% confidence interval: 0.5% to 10%). The mean value of the two treatment groups was 3% (mPEG-HBV) and 2% (HBV), respectively. No difference between the groups was observed.

Dose schedule: tolerance of injections and adherence to schedule

LLRs of >5 cm in diameter occurred with equal frequency in both treatment groups but were not a major problem in any of the patients.

Systemic side effects were more often observed in the HBV-treated group (Table III).

Mild subjective complaints like heat sensation, par-

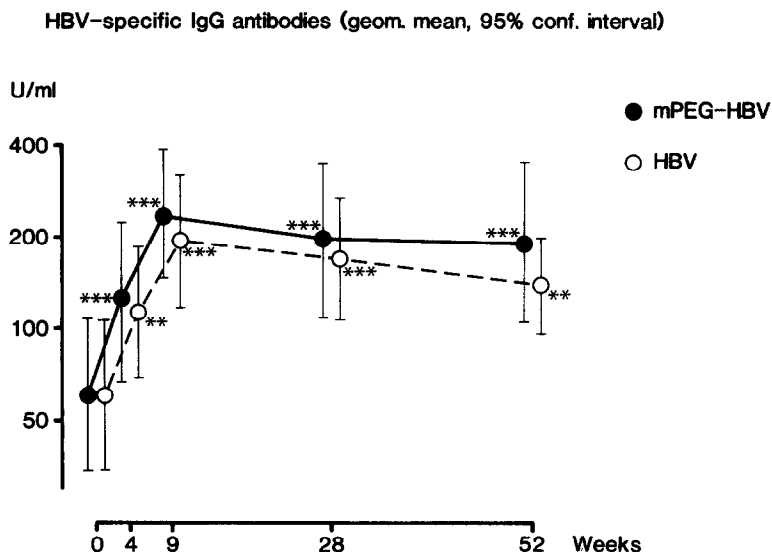


FIG. 2. HBV-specific IgG Ab (geometric mean and 95% confidence interval). ** $p < 0.01$; *** $p < 0.001$.

esthesia or pruritus without objective symptoms were observed in six patients receiving HBV and in two patients receiving mPEG-HBV. Objective symptoms of an allergic SR were observed in four patients receiving HBV and in two patients receiving mPEG-HBV; in the latter two patients, one had a localized angioedema of the upper lid and one patient had mild bronchospasm. Both symptoms occurred relatively late, 40 minutes and 1 hour after injection of 10 and 200 μg of mPEG-HBV, respectively, and did not necessitate medical treatment.

Four patients receiving HBV treatment developed SR. One female patient suffered from repeated mild SR with erythema of the face, palmar pruritus, and a mild dyspnea starting at 20 μg . Another patient developed five SRs, three at 20 μg or lower. The last SR in this patient occurred after the first application of 100 μg . The SR was severe and required adrenalin treatment. Two additional patients had repeated SRs, manifesting with cutaneous symptoms in one patient and cutaneous symptoms and bronchospasm in the other patient. These reactions necessitated treatment with antihistamines and/or adrenalin and, finally, prompted the patients to stop the study.

One interesting finding was that some patients receiving mPEG-HBV developed local pruritus not only at the site of the injection administered that day but also at the site of the injection administered 1 week earlier (alternative upper arm). Patients receiving mPEG-HBV adhered strictly to the dose schedule, whereas five patients receiving HBV, including the two dropouts, had to modify their dose schedule. Two patients receiving mPEG-HBV and three receiving

HBV had to increase their maintenance dose >200 μg and 100 μg , respectively, in an attempt to increase the HBV-specific IgG response before the provocation with a honeybee. One of the patients who increased the dose of HBV to 200 μg still demonstrated an insufficient IgG increase and was therefore not challenged.

Sting challenge by a honeybee

All seventeen patients in the mPEG-HBV-treated group and 14 in the HBV-treated group were challenged with a sting by a living honeybee. Two patients in the HBV-treated group dropped out before challenge because of repeated SR after treatment injections, and one patient was not challenged because of an insufficient IgG increase (Table IV).

After the challenge, LLRs of >10 cm in diameter and more than 24 hours of duration were observed in seven patients receiving mPEG-HBV and five patients receiving HBV. Four patients, one receiving mPEG-HBV and three receiving HBV, had mild subjective complaints, including a sensation of heat, paresthesia, pruritus, or pressure in the head. No objective symptoms were observed. Six patients, four receiving mPEG-HBV and two receiving HBV, developed definite SR. One of the patients receiving HBV IT developed erythema of the face, tachycardia, and mild angioedema of the lips that occurred about 6 minutes after the sting. After intravenous injection of clemastine, the patient recovered completely within 1 hour. The other patient developed a mild angioedema that was not treated. Three patients receiving mPEG-HBV developed more severe SR, and one pa-

Ratio HBV-specific IgG/IgE antibodies (geom. mean, 95% conf. interval)

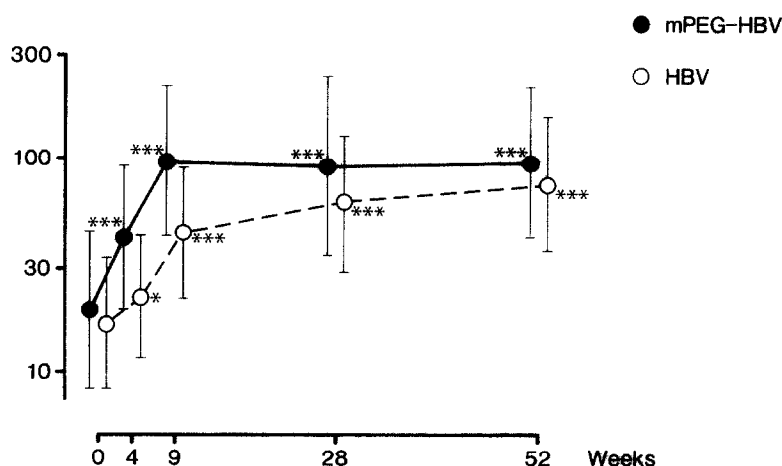


FIG. 3. The ratio of HBV-specific IgG/IgE Ab (geometric mean and 95% confidence interval).
* $p < 0.05$; *** $p < 0.001$.

tient had mild urticaria. In one patient, tachycardia developed within 4 minutes, followed by wheezing and dyspnea. In spite of adrenalin, subcutaneously, clemastine, intravenously, and oxygen administered by a nasal tube, erythema of the face and conjunctivitis developed, followed by nausea and recurrent vomitings. The patient recovered after 40 minutes. The second patient complained of generalized itching within 5 minutes, followed by dyspnea, fall in blood pressure to 75/40 mm Hg, and tachycardia. After treatment with adrenalin, clemastine, and prednisolone, the patient recovered completely within 1 hour. The third patient became dyspneic 6 minutes after the sting, followed by a fall in blood pressure, and tachycardia. In spite of the injection of adrenalin, clemastine, and prednisolone, the blood pressure dropped to unmeasurable values, and the patient lost consciousness and became incontinent. After repeated injections of adrenalin and infusion of 500 ml of plasma protein solution, the patient regained consciousness, and blood pressure rose slowly and was normalized 1 hour after the sting.

Table V illustrates that all those patients developing SR had experienced severe reactions with respiratory and/or shock symptoms before treatment.

Serum HBV-specific IgE Abs

The HBV-specific IgE Ab developed differently in the two treatment groups (Fig. 1). There was a successive decrease in the group treated with mPEG-HBV. After 1 year, the HBV-specific IgE Ab level was significantly decreased ($p < 0.01$). The group treated with HBV demonstrated an initial increase in HBV-specific IgE Ab followed by a contin-

uous decrease, resulting in significantly lower levels after 1 year of IT ($p < 0.001$). In the mPEG-HBV-treated group, 5/17 patients had an increased level after 4 weeks of IT, but levels were lower in all patients after 1 year of IT compared to levels observed at the start of IT. In the group treated with HBV, 8/17 patients had higher HBV-specific IgE levels after 4 weeks of IT than at the start of IT. After treatment for 1 year, four of the patients still had increased levels.

Serum HBV-specific IgG Abs

The HBV-specific IgG Abs (Fig. 2), which at the start of the study were similar in the two treatment groups, had increased significantly in both groups, even 4 weeks after starting IT, and remained significantly increased during the whole year of IT. The IgG level remained somewhat higher throughout the investigation period in the group treated with mPEG-HBV compared to the group treated with HBV. There were three and two patients, respectively, in the two treatment groups who had an unchanged HBV-specific IgG level after 4 weeks receiving IT, and one patient receiving HBV with a very high level at the start demonstrated a decreased level of HBV-specific IgG. In addition, in each of the two groups, there was one single patient who had a lower level after treatment of 1 year compared to the starting level. Both patients had pretreatment IgG titers of >400 U/ml.

The ratio of HBV-specific IgG/IgE Abs

In both groups after 4 weeks of treatment, a significantly increased ratio was observed, and this continued throughout the 1 year of IT (Fig. 3). Initially,

this increase was more pronounced in the group treated with mPEG-HBV in which the maximal ratio of 95 was reached after 9 weeks of IT and remained at that ratio during the first year. The group treated with HBV developed a continuous rise to a ratio of 75 after 1 year of IT. There were no significant differences between the groups, neither in level nor in changes from the start of IT.

Serum HBV-specific Abs in relation to sting challenge

Prechallenge levels of HBV-specific IgE and IgG Abs in relation to the result of the sting challenge are presented in Table V. Although most of the successfully treated patients demonstrated a distinct increase of HBV-specific IgG Ab at week 14, this level did not reliably predict the outcome of the challenge. In this regard some of the successfully treated patients (patients 6, 10, and 118) had HBV-specific IgG Ab levels <150 U/ml, whereas three of the failures (patients 7, 119, and 122) had levels >150 U/ml. No relationship between prechallenge HBV-specific IgE and results of the challenge could be found.

DISCUSSION

In an uncontrolled pilot study²² involving 14 HBV-sensitive patients, IT with mPEG-HBV was well tolerated and appeared to be effective. In this controlled, double-blind trial with 34 HBV-sensitive patients, its therapeutic effects and tolerance were therefore compared to the routinely used unmodified HBV preparation.

The *in vivo* allergenicity of mPEG-HBV, evaluated by means of ICT, was 2% of that of HBV. This figure is 10 to 20 times higher than that found with *in vitro* methods with sera and leukocytes from about 10 patients. The different methods used and the larger number of patients in this study may have contributed to the difference. However, clinically, a considerably reduced allergenicity could be demonstrated that justified a higher starting dose, a steeper dose increase, and a higher maintenance dose of the mPEG-HBV.

Since one of the presumed advantages of a modified allergen is the possibility of a rapid dose increase, treatment schedules were rather aggressive. This explains the relatively high incidence of side effects in HBV-treated patients. Large local reactions were observed with equal frequency in both treatment groups but did not cause alterations of the treatment schedule in any of the patients. This finding disproves the suggestion from the pilot study²² that the mPEG-HBV should induce more local side effects. SRs were more frequently observed and more severe in HBV-treated patients. In five patients receiving HBV, the dose schedule had to be modified, and two patients dropped

out because of repeated allergic side effects, whereas adherence to the dose schedule was complete in mPEG-HBV-treated patients. Thus, in spite of a larger maintenance dose and a steeper dose increase, mPEG-HBV was much better tolerated than unmodified HBV.

After the sting challenge with a honeybee, 4/17 patients receiving mPEG-HBV (24%) and 2/14 receiving HBV (14%) developed objective symptoms of an SR. In addition, one patient receiving mPEG-HBV and three patients receiving HBV had minor subjective complaints that did not require any medical treatment and that could have been due to anxiety or hyperventilation as well as to allergic reactions. Those patients developing generalized reactions, all had a history of respiratory or shock reaction before IT but did not have any particular features in their pretreatment or prechallenge HBV-specific IgE or IgG Ab levels. However, an extremely low specific IgG level in one patient (patient 101) and a low specific IgE level in another patient (patient 106) were observed in their prechallenge sera.

The observed treatment failure rate for both preparations is in the range of published data.^{1, 2, 9, 10, 12-16, 27} In comparing our success rate of 76% and 86% with the 95% to 100% reported in some studies,^{1, 6, 10, 11, 15, 28} it must be considered that different risk groups were investigated. The risk at reexposure is higher in adults rather than in children,³ in honeybee rather than in yellow jacket allergy,²⁹ and in patients with severe rather than with mild systemic reactions.^{3, 28, 29}

Our adult patients with mostly severe SR to honeybee stings are therefore a high-risk group when these patients are compared to the patients of the high success rate studies mentioned above. In these studies, mainly yellow jacket-sensitive patients were treated.^{1, 6, 28} A considerable proportion of patients had a history of mild SRs only,^{1, 6, 28} or mostly children were investigated.^{3, 10}

In untreated patients or those receiving whole body extract with a history of severe SR after honeybee stings, a reexposure to a honeybee sting induced an SR in 60% and 75% of the patients, respectively.^{2, 29} Both treatment with mPEG-HBV and unmodified HBV thus were accompanied by a marked decrease of the expected incidence of SR in untreated patients and hence can be considered as effective. SRs were somewhat more frequently observed and more severe in patients receiving mPEG-HBV. It must be considered, however, that two of the possibly most sensitive patients in the HBV-treated group were not challenged because they retired from the study after repeated allergic side effects of treatment injections.

The IgE response to IT was slightly different in the

two treatment groups. Mean values of HBV-specific IgE declined steadily in the mPEG-HBV-treated group, whereas the usual initial increase was observed in the HBV-treated group. In both treatment groups however, there were some individuals who demonstrated an initial increase and other individuals who did not.

After 1 year of IT, HBV-specific IgE levels were significantly reduced in both treatment groups. Although there is some suggestion of an initial suppression of the IgE response by IT with mPEG-HBV, the difference between IgE levels after HBV IT is only marginal and completely disappears during further IT. The IgG response to HBV was strong and very similar in both treatment groups, indicating that mPEG-modification did not reduce the immunogenicity of HBV.

After two failures among the first 20 patients, it was decided to double the maintenance dose in patients with a HBV-specific IgG level <150 U/ml, corresponding approximately to the postulated protective level of 5 µg/ml,¹⁵ although we were aware of the limited predictive value of specific IgG Ab in this situation.^{2, 9, 29} However, we were anxious for the safety of our patients, and no better criterion was available. Table V demonstrates clearly that HBV-specific IgG Ab estimated before the challenge does not predict the outcome of the latter and thus confirms previous publications.^{2, 9, 29} The only common feature of the four treatment failures is that all had a history of severe systemic reactions, including cardiovascular and/or respiratory symptoms.

In conclusion, in this double-blind, controlled IT trial in 34 HBV-sensitive patients comparing mPEG-HBV with unmodified HBV, we demonstrated that IT with mPEG-HBV (1) was better tolerated than IT with HBV, (2) induced a comparable IgG immune response to HBV, and (3) was clinically effective.

The three treatment failures receiving mPEG-HBV with rather severe SRs are, however, disturbing. Since treatment with mPEG-HBV induces specific IgG to the relevant HBV allergens,³⁰ covering or destruction of HBV allergens by the modifying process appears unlikely. The dosage of mPEG-HBV may, however, have been insufficient.

Since allergenicity of mPEG-HBV is at least 50 times lower than that of HBV, the maintenance dose of mPEG-HBV can be considerably increased, and this may result in even better clinical efficacy.

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Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects

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To determine the importance of airway inflammation for late asthmatic reactions induced by toluene diisocyanate (TDI), we investigated whether prednisone prevented airway inflammation by modifying the associated airway inflammatory reaction. We measured FEV₁ before and at regular intervals after exposure to TDI and performed bronchoalveolar lavage at 8 hours after TDI in two groups of subjects with previously documented late asthmatic reactions, in one group, after no treatment, and in the other group, after treatment with prednisone (50 mg/day for 4 days). After no treatment, each subject developed a late asthmatic reaction, an increase in airway responsiveness, polymorphonuclear leukocytosis, and increased albumin in bronchoalveolar lavage. By contrast, after treatment with prednisone, no subject developed a late asthmatic reaction or an increase in airway responsiveness, and the number of leukocytes and the concentration of albumin were normal in bronchoalveolar lavage. These results suggest that late asthmatic reactions induced by TDI may be caused by airway inflammation and that prednisone may block the inflammation by inhibiting the inflammatory reaction of the airway induced by TDI in sensitized subjects. (J ALLERGY CLIN IMMUNOL 1987;80:261-7.)

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