

17. Smith TF, Aelvoet M, Morrison DC. The effect of bacterial lipopolysaccharide (LPS) on histamine release from human basophils. I. Enhancement of immunologic release by LPS. *Clin Immunol Immunopathol* 1985;34:355.
18. Pruzansky JJ, Grammer RC, Patterson R, Roberts M. Dissociation of IgE receptors from human basophils. I. Enhanced passive sensitization for histamine release. *J Immunol* 1983; 131:1949.
19. Grant JA, DuPre E, Thuesen DO. Complement-mediated release of histamine from human basophils. III. Possible regulatory role of microtubules and microfilaments. *J ALLERGY CLIN IMMUNOL* 1977;60:306.
20. Wyke AM, Impraim CC, Knutton S, Pasternak CA. Components involved in virally mediated membrane fusion and permeability changes. *Biochem J* 1980;190:625.

Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids

III. Efficacy and safety of unfractionated and high-molecular-weight preparations in rhinoconjunctivitis and asthma

Jean Bousquet, MD, PhD,* Hans J. Maasch, PhD, Adnan Hejjaoui, MD,*
Wienia Skassa-Brociek, MD,* Rüdiger Wahl, PhD,**
Henriette Dhivert, MD,* and François-Bernard Michel, MD*
Montpellier, France, and Reinbeck, West Germany**

*Specific immunotherapy with unmodified formalinized allergoids is effective in grass-pollen allergy, but systemic reactions have been observed. A high-molecular-weight formalinized allergoid (HMW-GOID) was fractionated by gel filtration, retaining molecules of >85,000 daltons in the expectation of improving safety without sacrificing efficacy. HMW-GOID and unfractionated allergoid (GOID) had a similar allergenic activity assessed by RAST inhibition, but the HMW-GOID preparation was 65 times less reactive when it was tested by skin prick test than the GOID preparation. A double-blind, placebo-controlled study was carried out in grass pollen-allergic patients with placebo (14 patients), GOID (15 patients), and HMW-GOID (13 patients). An additional group of 18 patients was treated by a rush schedule with a standardized orchard grass-pollen extract. A similar mean cumulative dose was administered with both allergoids. The fractionated allergoid only elicited minor systemic reactions similar to reactions elicited by placebo, whereas 20% of patients treated by GOID and 5.5% of patients receiving the standardized extract had a severe systemic reaction. For rhinitis, conjunctivitis, and asthma, the HMW-GOID and the standardized extract had a similar efficacy, significantly greater than placebo. GOID was less effective than the other two active treatments but was significantly more effective than placebo treatment for asthma and conjunctivitis. (*J ALLERGY CLIN IMMUNOL* 1989;84:546-56.)*

From the *Clinique des Maladies Respiratoires, Hopital L'Aiguelongue, Centre Hospitalier Universitaire, Montpellier, France, and **Allergopharma Joachim Ganzer, Reinbeck, West Germany.

Received for publication Dec. 13, 1988.

Revised March 29, 1989.

Accepted for publication April 10, 1989.

Reprint requests: Jean Bousquet, MD, Clinique des Maladies Respiratoires, Hopital L'Aiguelongue, Centre Hospitalier Universitaire, 34059 Montpellier Cedex, France.

1/1/14809

Abbreviations used

BU:	Biological unit
GOID:	Unfractionated allergoid
HMW-GOID:	High-molecular-weight allergoid
MW:	Molecular weight
PNU:	Protein nitrogen unit
SPT:	Skin prick test
HPLC:	High-performance liquid chromatography

TABLE I. Protocol of specific immunotherapy with GOID and HMW-GOID

Day	AM	PNU
1	9	50
	10	100
	11	200
8	9	300
	10	500
14		700
21		1000
28		1500
35		2000

Four injections of maintenance dose (2000 PNU) before April 1; from April 1 to October 1, bimonthly injection of 1000 PNU.

In pollen allergy, double-blind, placebo-controlled studies have demonstrated that specific immunotherapy was effective with formalinized allergoids¹⁻⁵ and standardized allergen extracts.⁶⁻¹⁰ However, these allergen extracts and allergoids administered in doses sufficient to demonstrate efficacy were found to elicit systemic reactions in 10% to 30% of treated patients.^{5,7,9,10} In ragweed-pollen immunotherapy, it was observed that high MW polymerized extracts were safe and effective,^{11,12} suggesting that high MW preparations are not only effective but also may be safer than the unfractionated ones.

In the south of France, grass pollens predominate, and patients often are first observed with rhinitis, conjunctivitis, and asthma.¹³ In grass-pollen allergy, it appears that asthma is significantly prevented by immunotherapy,^{6,14,15} although some discrepant results have been published.¹⁶ Specific immunotherapy also appears to be effective in pollen-induced conjunctivitis, but very few clinical studies have been performed.^{6,17,18}

The first goal of the study was to determine if an HWM-GOID could be as effective and better tolerated than a GOID when it is administered in similar doses. We compared these two allergoids in a double-blind, placebo-controlled study and introduced a fourth unblinded group of patients receiving a standardized orchard grass-pollen extract. As we had consistently found during the years that this extract elicited the best clinical effects,^{6,14} it provided a positive control. A second goal of the study was to assess the efficacy of specific immunotherapy in the three major symptoms induced by pollen allergy, that is, rhinitis, conjunctivitis, and asthma.

MATERIAL AND METHODS

Patients

Seventy patients (12 to 46 years of age; mean, 25.2 ± 12.1 years) volunteered to enter the study after informed

TABLE II. Medication scores

Symptoms	Medication	Score
Rhinitis	Nasal cromoglycate	1
	Nasal beclomethasone	2
	Anti-H ₁ histamine (terfenadine, mequitazine)	3
	Oral prednisone	4
Conjunctivitis	Cromoglycate eye drops	1
	Corticosteroid eye drops	2
	Anti-H ₁ histamine (terfenadine, mequitazine)	3
	Oral prednisone	4
Asthma	Inhaled salbutamol	
	200 µg	1
	600 µg	2
	Theophylline (10 mg/kg)	3

consent. All patients had symptoms of rhinitis during the past 2 to 27 years during the grass-pollen season, and more than half also had symptoms of asthma and/or conjunctivitis. All patients had a positive prick test to a 1/100 (wt/vol) of standardized orchard-grass pollen extract (Stallergènes Laboratoires, Fresnes, France). Allergen-induced skin tests are positive when the wheal size is >75% from that of a wheal size induced by a 9% codeine-phosphate positive control solution.⁵⁻¹⁴ All patients had a positive RAST to orchard-grass pollen. None of the patients had received any form of specific immunotherapy to pollen extract.

Immunotherapy

Standardized grass-pollen extract. A standardized and lyophilized orchard-grass pollen (*Dactylis glomerata*) extract (Stallergènes Laboratoires) was prepared and controlled according to methods previously published in detail.^{5,14}

Mixed grass-pollen GOID. A six grass-pollen GOID was prepared by the two-step procedure according to the method of Marsh et al.² from pollens of six different grass species (*Dactylis glomerata*, *Festuca elatior*, *Holcus lanatus*, *Lolium perenne*, and *Poa pratensis*). The methods of preparation and control of the lyophilized allergen extract and lyophilized GOID have been previously published in detail.^{2,14} The residual formaldehyde content was tested by reaction with ammonium acetate, according to the method described in the European Pharmacopoeia,¹⁹ and we found a level of 0.13 µg/ml (130 ppm) of formaldehyde in the lyophilized GOID.

Mixed grass pollen HMW-GOID: Preparation. The lyophilized GOID (6.0 gm) was dissolved in 500 ml of 0.004 mol/L of NH₄HCO₃ and sterile filtered (pore size, 0.15 µm). The separation was done with a preparative Sephadex G-100 in a column (Pharmacia, Freiburg, West Germany) calibrated by dextran blue and a mixture of bovine serum albumin (MW, 67,000 daltons), egg albumin (MW, 45,000 daltons), chymotrypsinogen (MW, 25,000 daltons), and cy-

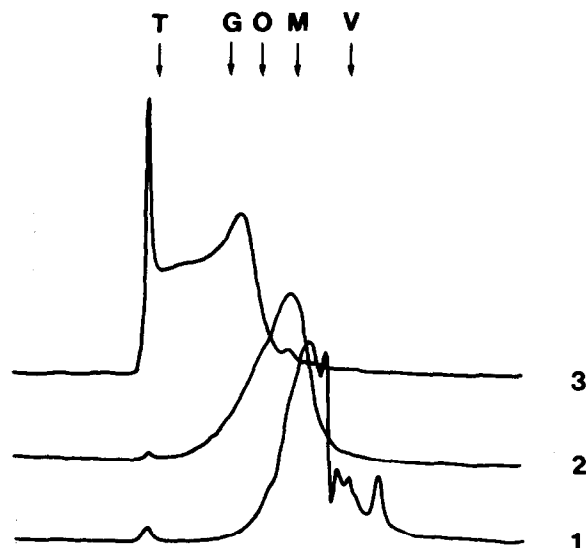


FIG. 1. MW determination of the native mixed grass-pollen allergen (1), GOID (2), and the high MW allergoid, HMW-GOID (3), performed by HPLC. Calibrating substances: V, vitamin B₁₂, 1350 daltons; M, horse myoglobin, 17,000 daltons; O, chicken ovalbumin, 44,000 daltons; G, bovine gamma globulin, 158,000 daltons; T, bovine thyroglobulin, 670,000 daltons. All samples were analyzed on equal protein basis (20 µg).

tochrome c (MW, 12,500 daltons). The actual separation was done at a flow rate of 25 ml/hr. A 0.05 mol/L of phosphate buffer (pH 7.5) was taken as an eluent. All molecules with an MW of >85,000 daltons were pooled and lyophilized. The yield of HMW-GOID was 19.7% of GOID on weight basis. For immunotherapy, HMW-GOID was dissolved in 0.9% saline solution containing 0.4% phenol and standardized in PNUs.

Comparison between the mixed grass-pollen allergen used to prepare allergoids, GOID, and HMW-GOID. The three preparations were tested in parallel to assess their MW distribution and relative allergenicity by means of HPLC, RAST-inhibition, and skin testing.

The MW distributions of these three preparations were compared by HPLC according to a method previously described in detail.²⁰ Briefly, the HPLC system consisted in a BIO-Sil TSK, 250 column connected to a guard column (Bio-Rad Laboratories, Munich, West Germany) (300 by 7.4 mm internal diameter), a ConstaMetric model III pump (Milton Roy, Hasselroth, West Germany), a Rheodyne injection valve, model 7125 (Rheodyne, Inc., Cotati, Calif.), a model D 1203 ultraviolet monitor (254 nm) (Milton Roy), and a model 301 integrator (Milton Roy). The eluent was a pH 6.5 0.5 mol/L of ammonium acetate buffer (Merk Frosst Laboratories, Darmstadt, West Germany) at a flow rate of 0.9 L/min; 20 µg of proteins was injected in 20 µl. The system was calibrated with a calibration kit of Bio-Rad Laboratories (Munich, West Germany) containing a mixture of proteins from 1.35 to 670 kd.

The *in vitro* allergenic activities of both allergoids in comparison with the native allergen were examined by RAST inhibition described previously.²¹ Allergens of the mixed grass-pollen extract coupled to paper disks, according to the method of Ceska et al.,²² were used as a solid phase. A pool of 18 sera from nonhyposensitized patients highly sensitive to grass pollens was used as a source for antigass-pollen IgE. To allow comparison of the allergenic activity, 50 µg of protein per milliliter of each extract was tested.

In vivo allergenicity was assessed by SPTs with a parallel-line bioassay and a modification of the technique according to the method of Dreborg and Grimmer.²³ Five tenfold dilutions of the three extracts and histamine hydrochloride, 1 mg/ml (Stallergènes Laboratories), were tested in duplicate with the modified method according to Pepys,²⁴ described in detail in a previous article.²⁵ Skin tests were done on the volar surface of the forearm in 15 nonhyposensitized patients highly allergic to grass pollens by the same investigator between 9 and 11 AM to avoid possible circadian variations. None of the patients had taken drugs that might modify the performance of skin tests. The wheals were recorded by the Scotch (3M Center, St. Paul, Minn.) -tape technique 15 minutes after the performance of the tests. Although some wheals were very small, all subjects tested had a positive wheal reaction for all extracts tested, and all diameters could be accurately measured. The statistical analysis of skin test was done by parallel-line bioassay.²⁶ Mean wheal diameters were calculated and plotted against log₁₀ of concentration.

Placebo. A placebo was prepared with 0.9% saline containing 0.4% phenol as a preservative and increasing concentrations of histamine dihydrochloride (0.005 to 0.5 mg/ml).

Immunotherapy. A double-blind, placebo-controlled immunotherapy was performed according to the step protocol indicated in Table I for allergoids and placebo. The maintenance dose was chosen according to a previous study in which we had found that 2000 PNU of GOID was effective in reducing symptoms and skin test reactivity and increasing IgG levels.⁵ A coseasonal immunotherapy was administered bimonthly from April 1 to October 1, the dose being reduced by one half.

The standardized orchard grass-pollen extract was administered by a rush procedure according to a 3-day protocol previously published,⁵ and then patients received four maintenance injections of 3000 BU. A coseasonal immunotherapy was administered every 2 weeks between April 1 and October 1, the dose being reduced by one half.

Investigations

Symptom scores. When the patients attended the clinic in April, they were taught the possible symptoms that could occur and the medication that they should take (Table II). The daily symptom score forms were developed previously in our clinic^{5, 14} and included symptoms of rhinitis (rhinorrhea, sneezing, and nasal obstruction), conjunctivitis (watery eyes, redness of the eyes, and pruritus), and asthma

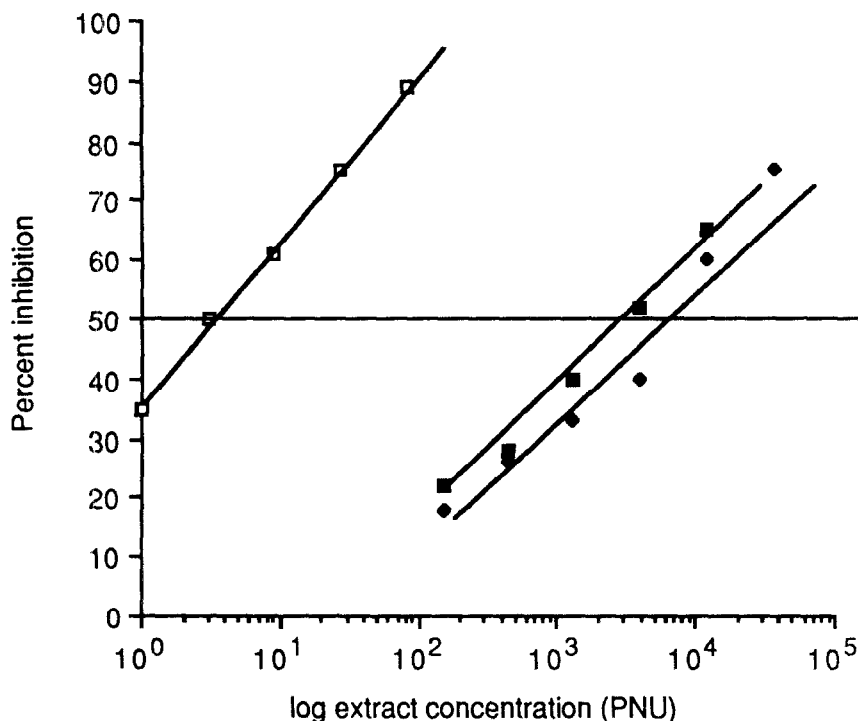


FIG. 2. RAST-inhibition studies of the mixed grass-pollen allergen (□), GOID (◆), and high HMW-GOID (■).

(wheezing and shortness of breath). The severity of symptoms was scored from 0 to 5. Patients were asked to take drugs only if they had symptoms. In case of nasal symptoms, they were asked to take first nasal cromoglycate and then, if symptoms were still present, to add nasal beclomethasone, then H₁ blocker, and finally, oral corticosteroids. For conjunctivitis, ocular cromoglycate was taken first, and the H₁ blocker was added if symptoms persisted. For asthma, inhaled salbutamol was administered at a dose of 200 µg, then the dose was increased to 600 µg, and, if this were necessary, long sustained-released theophylline was added. In the latter case, the patient was asked to return to the clinic. Patients completed daily forms during the months of May and June. The forms reported nasal, ocular, and bronchial symptoms for 12 hours, along with all the possible medications that could be used. The symptoms and medications were scored, and average symptom and medication scores were calculated for each patient for rhinitis, conjunctivitis, and asthma.

SPTs. SPTs were performed according to the modified method of Pepys²⁵ with a hypodermic needle on the volar surface of the forearm. None of the patients had any factor that might modify the performance of skin tests.²⁶ Threefold dilutions from 8 to 50,000 BU/ml were tested in parallel with a 9% codein-phosphate solution, and the end point titer was calculated as the threshold concentration eliciting a wheal similar to the wheal elicited by the 9% codeine-phosphate solution, as previously published.^{5, 14, 26}

Serum-mixed grass pollen-specific IgG. Serum-specific IgG was titrated according to the method of Puttonen and Maasch.²⁷ RAST disks were prepared according to the method of Ceska et al.²² with the mixed grass-pollen extract. IgG coupled on the disk was revealed by radiolabeled *Staphylococcus aureus* protein A. The results are presented as percent binding of total added counts.

Serum-mixed grass pollen-specific IgE. Serum-specific IgE was titrated by Phadebas RAST (Pharmacia Diagnostics AB, Uppsala, Sweden) with the same RAST disks as those prepared for the titration of specific IgG. The results are presented as percent binding of total added counts.

Design of the study

All patients were treated the same year and randomly assigned to one of the four treatment groups: placebo (14 patients), GOID (15 patients), HMW-GOID (13 patients), and standardized orchard grass-pollen extract (18 patients). Patients placed in the three former groups were enrolled in a double-blind study, whereas patients placed in the fourth group received the extracts in an open fashion because of the different modality of administration.

Before immunotherapy and between April 1 and April 21 (i.e., before the pollen season), patients had a skin test titration and the titration of serum grass-pollen IgG and IgE. During the season, all patients had follow-up by a symptom-medication score that they filled in every day from May 1 to June 30, but because of a late onset of the season (first

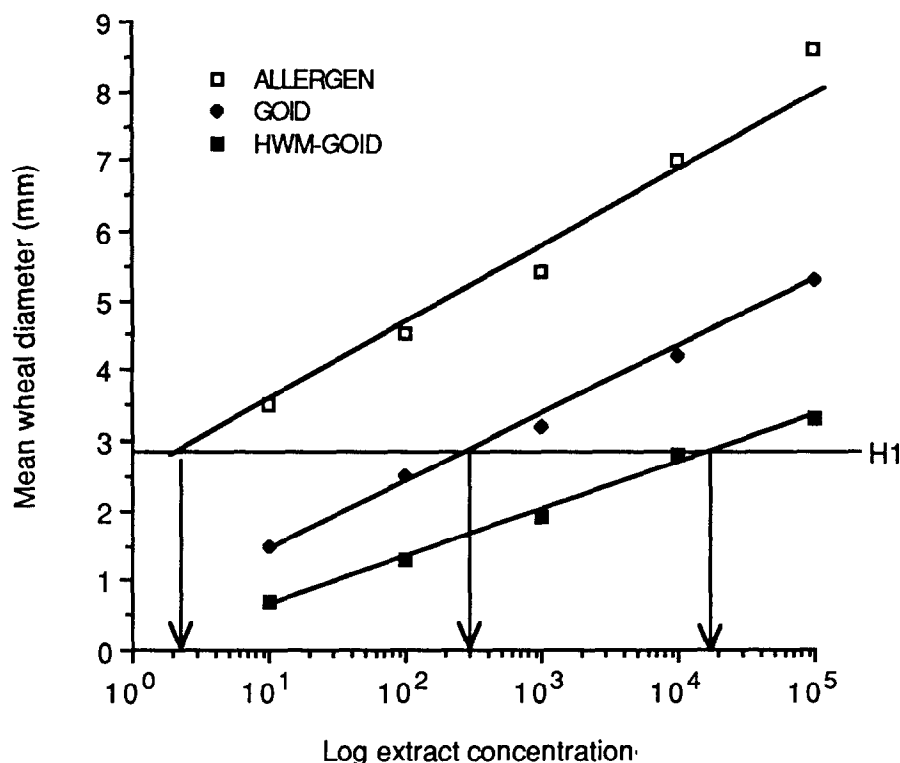


FIG. 3. SPT studies of the mixed grass-pollen allergen (□), GOID (◆), and HWM-GOID (■). H1, mean wheal diameter induced by histamine hydrochloride, 1 mg/ml.

TABLE III. Characteristics of the patients in the four groups

	Placebo	GOID	HWM-GOID	Standardized extract
No. of patients	14	15	13	18
Age (yr)	23.4 ± 11.1	26.2 ± 9.7	27.6 ± 10.6	23.9 ± 13.1
Skin test end point	6.1 ± 1.5	6.6 ± 1.35	6.5 ± 0.9	6.15 ± 1.3
Specific IgE (% binding)	20.5 ± 11.1	22.7 ± 14.1	27.1 ± 13.1	27.6 ± 9.0
Rhinitis				
% Patients with rhinitis	100	100	100	100
Duration of symptoms (yr)	8.7 ± 5.1	9.5 ± 5.9	9.15 ± 9.5	9.0 ± 10.7
Symptom severity (score)	3.7 ± 0.6	3.2 ± 0.8	3.7 ± 0.5	3.4 ± 0.6
Conjunctivitis				
% Patients with conjunctivitis	71.4	86.7	92.3	66.7
Duration of symptoms (yr)*	8.2 ± 3.3	8.2 ± 5.2	9.1 ± 4.9	8.0 ± 9.2
Symptom severity (score)*	3.7 ± 0.5	3.1 ± 0.7	3.4 ± 0.7	3.0 ± 1.1
Asthma				
% Patients with asthma	64.2	60.0	76.9	55.5
Duration of symptoms (yr)*	7.5 ± 5.1	9.6 ± 5.4	8.7 ± 6.2	8.0 ± 9.2

*Only patients suffering from the symptom were taken into consideration.

symptoms occurring between May 21 and 25), symptom scores were only analyzed in June.

Statistical analysis

Statistical analysis was performed with nonparametric tests, the Wilcoxon W test for paired data, and the Mann-Whitney U test for unpaired comparisons.²⁸ Linear regres-

sion analysis was used for RAST inhibition and skin test data.

RESULTS

Characteristics of both allergoids

HPLC chromatogram of the three extracts is illustrated in Fig. 1. The native allergen (1) and GOID

TABLE IV. Doses of allergoid received and reactions observed during the treatment

	Placebo	GOID	HMW-GOID	Standardized extract*
Immunotherapy				
Mean maximal dose (PNU)		1453 ± 753	1,969 ± 496	665 ± 223
Mean cumulative dose (PNU)		9096 ± 6304	13,735 ± 6355	3678 ± 567
Local reactions (%)				
5 cm diameter	0	66.6	41.6	72.2
% Systemic reaction per patient				
Minor and without treatment	14.2	26.6	15.3	11.0
Severe and requiring treatment	0	20.0	0	5.5
% Systemic reaction per injection				
Minor and without treatment	1.4	5.3	3.1	3.2
Severe and requiring treatment	0	3.4	0	1.1

*For the standardized extract, 1 PNU equals 3.9 BU.

TABLE V. Symptoms and medications of patients observed during the month of June

	Placebo	GOID	HMW-GOID	Standardized allergen
Rhinitis				
Days with symptoms	26.5 ± 8.6	20.9 ± 10.0	9.15 ± 9.5*†	9.0 ± 10.7*†
Symptom score	63.5 ± 54.6	38.1 ± 27.4	20.4 ± 18.1‡	14.8 ± 22.9‡†
Medication score	53.7 ± 54.1	33.1 ± 41.0	30.5 ± 32.8	22.9 ± 39.1‡
Conjunctivitis				
Days with symptoms	22.8 ± 12.1	11.9 ± 11.3§	1.3 ± 2.1*†	2.25 ± 4.6‡†
Symptom score	44.9 ± 36.8	18.3 ± 20.6§	1.3 ± 2.1*†	3.4 ± 3.5‡†
Medication score	40.1 ± 40.0	19.1 ± 24.7	5.7 ± 17.3‡	5.7 ± 10.7‡
Asthma				
Days with symptoms	18.1 ± 11.1	7.5 ± 10.4§	7.0 ± 7.4§	4.2 ± 5.7‡
Symptom score	20.7 ± 11.0	11.6 ± 23.2	8.4 ± 19.7§	4.7 ± 5.6‡
% Patients treated with antiasthma drugs	44	22	30	30
Medication score	1.56 ± 1.3	0.6 ± 1.1	0.5 ± 0.9	0.6 ± 0.7

Statistical difference from placebo by Mann-Whitney U test.

* $p < 0.001$.†Statistical difference from GOID by Mann-Whitney U test, $0.05 < p < 0.01$.‡ $0.01 \leq p < 0.005$.§ $0.05 < p < 0.01$.

||Only patients who suffered from the symptom the previous year(s) were considered.

(2) consisted mainly in molecules <44,000 daltons, whereas HMW-GOID (3) consisted in larger molecules.

Results of RAST inhibition are illustrated in Fig. 2. The dose-response curves were linear ($r = 0.98$ to 0.99) and parallel ($F = 3.2$ and 5.6 ; the dose-response curve of the native allergen taken as the reference) so that the relative potencies measured at 50% inhibition could be compared. We found that the allergenic activities of GOID and HMW-GOID were reduced by 1563- and 1054-fold, respectively, when allergoids were compared to the native allergen.

Skin test results are illustrated in Fig. 3. Dose-response curves were linear for all three extracts ($r = 0.98$ to 0.99) and parallel ($F = 1.53$ and 4.6 ; the dose-response curve of the native allergen taken as the reference) so that the relative allergenic potencies could be calculated. We observed that the allergenic activities of GOID and HMW-GOID were reduced by 130- and 8500-fold, respectively, when allergoids were compared to the native allergen.

Homogeneity between groups

The characteristics of patients placed in the four groups are indicated in Table III. All parameters stud-

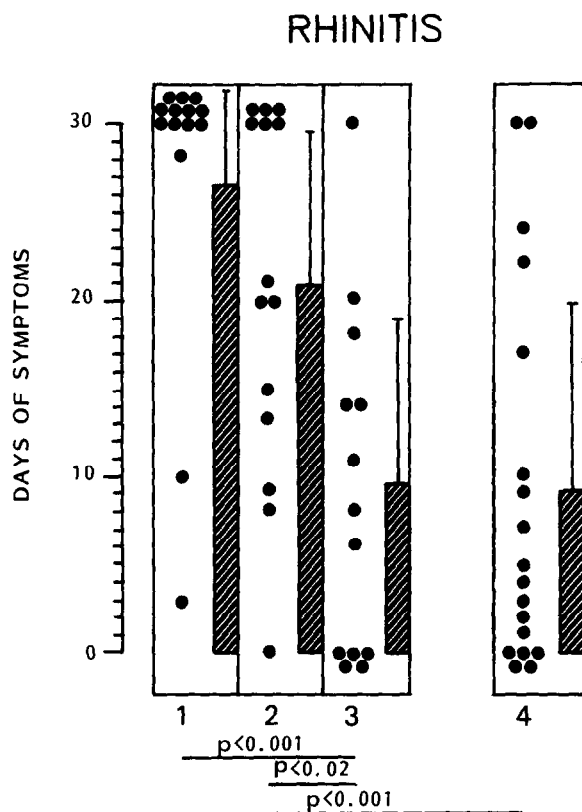


FIG. 4. Number of days of nasal symptoms of patients observed during the month of June. Group 1, placebo; group 2, GOID; group 3, HMW-GOID; and group 4, standardized orchard grass-pollen extract. Statistical analysis by Mann-Whitney U test.

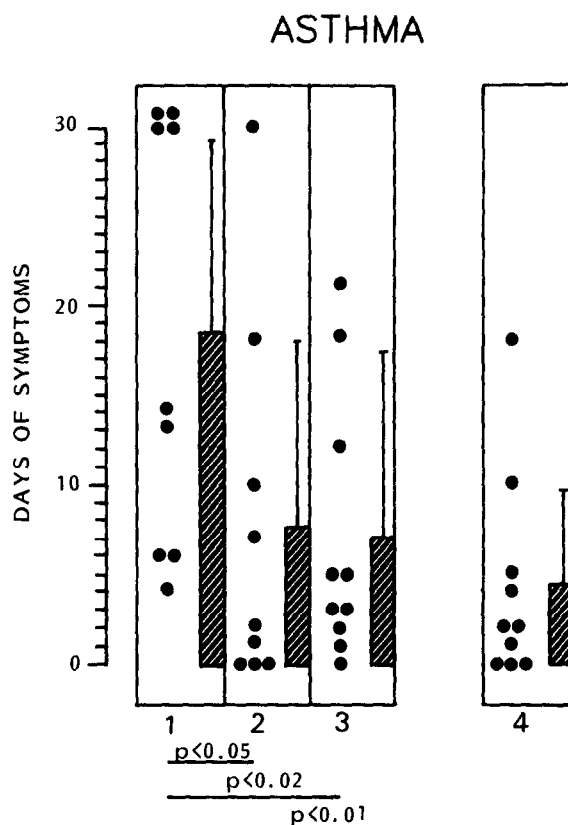


FIG. 5. Number of days of bronchial symptoms of patients observed during the month of June. (Only patients who had been suffering from asthma the previous years were considered.) Group 1, placebo; group 2, GOID; group 3, HMW-GOID; and group 4, standardized orchard grass-pollen extract. Statistical analysis by Mann-Whitney U test.

ied were comparable in these groups and not significantly different.

Protocol of immunotherapy and side reactions

Most patients were able to reach the maintenance dose, but two patients in the GOID group did not because of systemic reactions. The mean maximal dose and the mean cumulative doses were slightly lower in the GOID-treatment group than in the HMW-GOID-treatment group (Table IV). In the standardized allergen-treatment group, all patients reached the maintenance dose.

Large local reactions of >5 cm in diameter and lasting for more than 24 hours were observed in more than half of the patients placed in the GOID-treatment or the standardized extract-treatment groups and 41.6% of patients of the HMW-GOID-treatment group.

Systemic reactions of minor importance, such as flushing of the face, were observed in all groups, including the placebo-treatment group and appeared

to be nonspecific. Severe systemic reactions were absent in the placebo- and the HMW-GOID-treatment groups. Systemic reactions were present and relatively severe in 5.5% of patients placed in the standardized extract-treatment group, characterized by asthma and urticaria, and adrenaline was required to cure the reaction. In the GOID-treatment group, 20% of patients had a systemic reaction (urticaria and/or mild asthma) requiring treatment (anti-H₁ histamine and prednisolone), but one patient receiving a dose of 100 PNU had an anaphylactic shock requiring adrenaline and oxygen therapy.

Symptom scores

Symptom and medication scores were analyzed for rhinitis, conjunctivitis, and asthma. All patients had had rhinitis in previous years, and all were analyzed. For nasal symptoms, patients in the placebo-treatment group had a significantly higher mean score of symp-

TABLE VI. Evolution of skin tests and serum-specific IgE and IgG after specific immunotherapy

	Placebo	GOID	HMW-GOID	Standardized allergen
Skin test end point				
Before immunotherapy	6.2 ± 1.6	6.6 ± 1.5	6.5 ± 1.1	6.1 ± 2.1
After immunotherapy	5.5 ± 1.6	4.8 ± 1.7	3.9 ± 1.7	4.0 ± 1.6
<i>p</i>	NS	<0.04	<0.005	<0.01
Before/after				
Specific IgE (% binding)				
Before immunotherapy	20.5 ± 11.1	22.6 ± 14.1	27.1 ± 13.1	27.6 ± 9.0
After immunotherapy	20.8 ± 10.7	26.5 ± 13.6	35.9 ± 6.6	26.8 ± 11.1
<i>p</i>	NS	NS	<0.01	NS
Before/after				
Specific IgG (% binding)				
Before immunotherapy	8.1 ± 3.7	6.9 ± 2.9	8.2 ± 4.8	10.0 ± 8.1
After immunotherapy	8.0 ± 3.2	16.5 ± 10.3	21.7 ± 15.3	29.6 ± 19.2
<i>p</i>	NS	<0.01	<0.005	<0.005
Before/after				

NS, Nonsignificant result.

Statistical analysis by Wilcoxon W test.

toms and medications than patients placed in the HMW-GOID-treatment or standardized allergen-treatment groups (Fig. 4; Table V). Patients of the GOID-treatment group had a significantly greater mean number of symptomatic days than patients placed in the other two treatment groups (HMW-GOID and standardized allergen).

Only a subset of patients were first observed with grass pollen-induced asthma in the preceding year(s), and data were calculated for these patients only. Only patients who had complained of asthma in previous years had bronchial symptoms during the period of evaluation. The results of symptom and medication scores for asthma demonstrate that patients placed in the three treatment groups had significantly lower mean symptom or medication scores than patients in the placebo-treatment group (Fig. 5; Table V). There was no significant difference between the three treatment groups.

From 66.7% to 92.3% of patients had conjunctivitis in the preceding year(s), and only these subjects were studied. Only patients who were first observed with ocular symptoms in previous years had conjunctivitis during the evaluation period. The results observed in conjunctivitis (Fig. 6; Table V) are similar to results observed in asthma, but patients in the HMW-GOID-treatment group had very few symptomatic days.

Quantitative skin tests

After immunotherapy, patients treated with placebo had no mean difference in skin test reactivity to allergen, whereas patients placed in the three treatment

groups had a significant reduction in the mean end point titer (Table VI).

Serum-mixed grass pollen-specific IgG and IgE

Mean serum-specific IgG level did not change after immunotherapy in the placebo-treatment group, whereas the level was significantly increased in all three treatment groups (Table VI). Mean serum-specific IgE level did not change in the placebo- the GOID-, and standardized allergen-treatment groups after immunotherapy, and it was significantly increased in the HMW-GOID-treatment group (Table VI).

There was no significant correlation between nasal symptom-medication scores and IgG or IgE levels.

DISCUSSION

The results of the present study indicate that specific immunotherapy is effective in decreasing symptoms of rhinitis, conjunctivitis, and asthma of grass pollen-allergic patients. The efficacy and the safety of the different extracts used were found to be different. HMW-GOID and the standardized extract were clinically more effective than GOID. The standardized orchard grass-pollen extract and GOID elicited severe systemic reactions, whereas HMW-GOID did not. The injections of all types of extracts induced a significant decrease in skin test reactivity and a significant increase in serum grass pollen-specific IgG.

A single grass-pollen extract was used for the rush protocol, whereas allergoids were prepared from a

CONJUNCTIVITIS

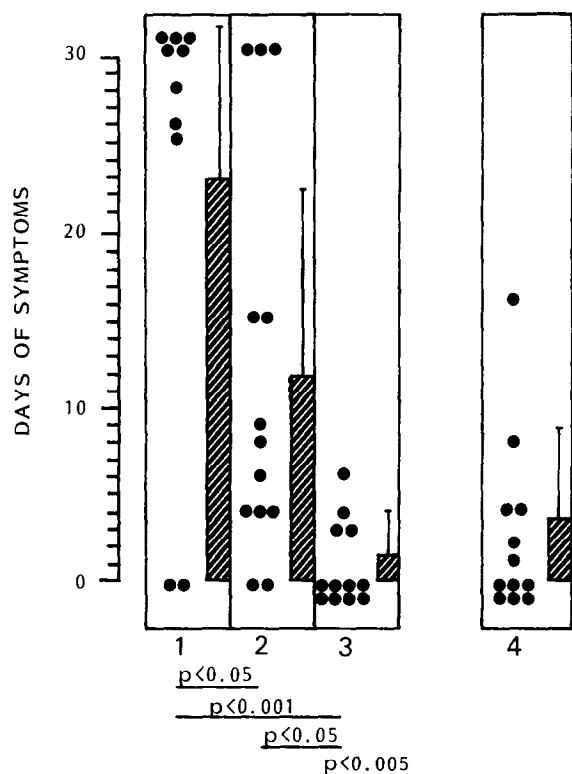


FIG. 6. Number of days of ocular symptoms of patients observed during the month of June. (Only patients who had been suffering from conjunctivitis the previous years were considered.) Group 1, placebo; group 2, GOID; group 3, HMW-GOID; and group 4, standardized orchard grass-pollen extract. Statistical analysis by Mann-Whitney U test.

mixture of six grasses. We had previously investigated (unpublished data) possible differences between the results of immunotherapy with orchard-grass pollen or mixtures in grass-pollen allergy and found no difference. These results were consistent with other studies,¹⁸ and since single allergens are easier to standardize than mixtures, we decided to use a single orchard grass-pollen extract in subsequent trials.^{5, 14} Moreover, this group was only taken as a positive control group.

The comparison between the native mixed grass-pollen allergen and both allergoid preparations demonstrated that HMW-GOID was almost as active as GOID in antigen-binding inhibitory activity in RAST and 1000-fold less active than the native allergen, confirming therefore previous experiments with formalinized allergoids.²⁹ The skin test sensitivity was decreased by 130-fold with GOID and 8500-fold with HMW-GOID. Since this parameter was related to the safety of the extracts,^{30, 31} it was expected that HMW-GOID treatment should be safer than GOID or the native allergen treatment. It may be difficult to explain

the very different estimates of allergenicity of GOID and HMW-GOID obtained by RAST inhibition and skin tests. In vivo mediator release requires IgE combination with two sites on the molecule at a spacing that fulfills the requirement for bridging between two receptors, whereas RAST inhibition requires combination but not bridging; therefore, both tests measure two quite different things. Ordinarily, with unmodified extracts, the two methods agree, but allergen molecules in nature do not approach the size of HMW-GOID molecules.

The study was to compare two allergoid preparations differing by their MW on a similar PNU content. The protocol of immunotherapy used with allergoids was different from the rush schedule used the previous year⁵ because we have observed a rather high rate of systemic reactions (52.6%). With a slower step schedule, we noticed no systemic reaction with HMW-GOID and less systemic side reactions (20%) with GOID; however, one reaction was very severe for a low dose (100 PNU). This finding suggests that GOIDs are not totally safe and that the high MW preparation was safer, confirming therefore that polymerized extracts are well tolerated.^{11, 12} With consideration of the skin test results, it was observed that GOID was 65 times more reactive than HMW-GOID; thus, it is reasonable to observe a greater number of systemic reactions when similar doses are administered. The long-term safety of formalinized allergoids may be questioned, since formaldehyde was demonstrated to be carcinogenic, but it appears that this effect is dose dependent. The European Pharmacopea allows a level of 200 ppm.¹⁹ In the present extracts, formaldehyde was under this threshold level (130 ppm).

The efficacy of HMW-GOID and the standardized orchard grass-pollen extract was found to be similar, the former being more effective in alleviating ocular symptoms and the latter protecting patients better from nasal and bronchial symptoms. However, the differences are small and nonsignificant. This study confirms the value of standardized extracts in grass-pollen allergy already demonstrated in other investigations^{5-10, 18} and demonstrates that HMW-GOID is as effective as the best extracts currently proposed in Europe. In contrast, GOID was less effective than the other two preparations tested, although the mean total doses of the two allergoid-treatment groups were not significantly different. With consideration of RAST-inhibition results, it was observed that GOID was slightly less reactive than HMW-GOID, and it could be postulated that the efficacy of HMW-GOID should be at least equivalent and possibly better than that of GOID. Our results confirm this hypothesis; RAST-inhibition studies may be used for the efficacy of allergoids, whereas skin test studies may be taken into account for their safety.

Nasal, ocular, and bronchial symptoms were significantly reduced by immunotherapy, but it appears from this study and other studies^{6, 14, 15, 32} that in grass-pollen allergy, the protection is better for asthma than for rhinitis. These results do not agree with other results published for grass pollen¹⁶ or ragweed pollen.³¹ However, in the study of Hill et al.,¹⁶ the type of extract and the doses administered are not clear and may not be sufficient to protect patients exposed in Australia to very high numbers of pollen grains. The differences between ragweed and grass-pollen allergy may explain the discrepant results found.³³ Ocular symptoms of grass-pollen allergy were significantly improved by all these extracts, and this result agrees with other results published in grass-^{6, 15, 32} or ragweed-pollen allergy.¹⁷

The clinical efficacy of immunotherapy with a standardized allergen, GOID, or HMW-GOID was confirmed by a reduced skin test reactivity and increased IgG levels. Other studies have already demonstrated the reduction of the immediate-type reaction of skin tests induced by allergens during immunotherapy,^{5, 6, 9, 10, 14, 32, 34} and it has been suggested that this parameter might be used to determine whether doses of allergen are administered in sufficient amount. Many studies have also observed that allergen-specific IgG was increased after immunotherapy,^{5, 7, 8, 10, 14, 16, 21, 34-36} but there were only two studies that found a correlation between symptoms and IgG levels.^{7, 34} In the present study, we did not observe such a correlation.

We thank Dr. P. S. Norman for his help in reviewing the manuscript.

REFERENCES

- Marsh DG, Lichtenstein LM, Campbell DH. Studies on "allergoids" prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology* 1970;18:705-22.
- Marsh DG, Norman PS, Roebber M, Lichtenstein LM. Studies on allergoids from naturally occurring allergens. III. Preparation of ragweed-pollen allergoids by aldehyde modification in two steps. *J ALLERGY CLIN IMMUNOL* 1981;68:449-59.
- Norman PS, Lichtenstein LM, Kagey-Sobotka A, Marsh DG. Controlled evaluation of allergoid in the immunotherapy of ragweed hay fever. *J ALLERGY CLIN IMMUNOL* 1982;70:248-60.
- Merirey DK, Kothari H, Chinoy P, Grieco MH. The clinical and immunologic efficacy of immunotherapy with modified ragweed extract (allergoid) for ragweed hay fever. *Ann Allergy* 1986;56:34-8.
- Bousquet J, Hejjajoui A, Skassa-Brocick W, et al. Double-blind, placebo-controlled immunotherapy with mixed grass-pollen allergoids. I. Rush immunotherapy with allergoids and standardized orchard grass-pollen extract. *J ALLERGY CLIN IMMUNOL* 1987;80:591-8.
- Østerballe Ø. Immunotherapy in hay fever with two major allergens 19, 25 and partially purified extract of timothy grass pollen. A controlled double blind study. In vivo variables, season I. *Allergy* 1980;35:473-89.
- Nordvall SL, Berg T, Johansson SGO, Lanner A. Clinical studies of a purified timothy pollen extract: desensitization therapy with a purified timothy pollen preparation compared to a crude timothy pollen extract. II. Results of the tests in vitro and their relation to symptoms and tests in vivo. *Int Arch Allergy Appl Immunol* 1978;67:132-8.
- Ortolani C, Pastorello E, Moss RB, et al. Grass pollen immunotherapy: a single year double-blind, placebo-controlled study in patients with grass pollen induced asthma and rhinitis. *J ALLERGY CLIN IMMUNOL* 1984;73:283-90.
- Berg T, Nordvall SL, Lanner A. Clinical studies of a purified timothy pollen extract: desensitization therapy with a purified timothy pollen preparation compared to a crude timothy pollen extract. I. Results of tests in vivo. *Int Arch Allergy Appl Immunol* 1980;63:266-74.
- Kjellman NIM, Lanner R. Hyposensitization in childhood hay fever: A comparison of refined and whole timothy extracts. *Allergy* 1980;35:323-34.
- Metzger WJ, Patterson R, Zeiss CR, et al. Comparison of polymerized and unpolymerized antigen E for immunotherapy of ragweed allergy. *N Engl J med* 1976;295:1160-5.
- Kelly JF, Zeiss CR, Suszko JM, Levitz D, Patterson R. Polymerized whole ragweed: human safety and immune response. *J ALLERGY CLIN IMMUNOL* 1980;65:50-6.
- Bousquet J, Cour P, Guérin B, Michel F-B. Allergy in the Mediterranean area. I. Pollen counts and pollinosis of Montpellier. *Clin Allergy* 1984;14:249-59.
- Bousquet J, Guérin B, Dote A, et al. Comparison of rush immunotherapy with a standardized grass pollen extract and classical immunotherapy with a pyridine extracted alum adjuvanted extract. *Clin Allergy* 1985;15:179-94.
- Frankland AW, Augustin R. Prophylaxis of summer hay fever and asthma: controlled trial comparing crude grass pollen extract with isolated main protein component. *Lancet* 1954;1:1055-7.
- Hill DJ, Hosking CS, Shelton MJ, Turner MW. Failure of hyposensitization in treatment of children with grass-pollen asthma. *Br Med J* 1982;284:306-8.
- Juniper EF, Roberts RS, Teck M, et al. Polyethylene glycol-modified ragweed pollen extract in rhinoconjunctivitis. *J ALLERGY CLIN IMMUNOL* 1985;75:578-85.
- Frostad AB, Grimmer O, Sandvik L, Moxnes A, Aas K. Clinical effects of hyposensitization using a purified allergen preparation from Timothy pollen as compared to crude aqueous extracts from Timothy pollen and a four-grass pollen mixture respectively. *Clin Allergy* 1983;13:337-58.
- Europäisches Arzneibuch II. Stuttgart: Deutscher Apotheker-Verlag, Frankfurt: Govi-Verlag GMBH, 1975:37.
- Wahl R, Maasch HJ, Geissler W. High-performance liquid chromatographic molecular weight determination of allergen extracts: examination of the influence of the column material on allergenic activity and allergen patterns. *J Chromatogr* 1986;351:39-46.
- Puttonen E, Pilsström L. Purification of birch pollen allergen extract by gel filtration. *Int Arch Allergy Appl Immunol* 1980;61:299-307.
- Ceska M, Eriksson R, Varga JM. Radioimmunosorbent assay of allergens. *J ALLERGY CLIN IMMUNOL* 1972;49:1-7.
- Dreborg S, Grimmer Ø. Biological standardization of allergen extracts/preparations. In: Brede H, Goings H, Schaeffer M, eds. Proceedings of the Second Paul Ehrlich Symposium on Allergen Standardization and Control. Stuttgart: Gustav Fischer Verlag, 1983:77-82.
- Pepys J. Skin testing. *Br J Hosp Med* 1976;14:412-7.
- Bousquet J, Calvayrac P, Guérin B, et al. Immunotherapy with a standardized *Dermatophagoides pteronyssinus* extract. I. In vivo and in vitro parameters after a short course of treatment. *J ALLERGY CLIN IMMUNOL* 1985;76:734-44.

26. Turkeltaub P, Rastogi SC, Baer H, Anderson MC, Norman PS. A standardized quantitative skin test assay of allergen potency and stability: studies on the allergen dose-response curve and effect of wheal, erythema, and patient selection on assay results. *J ALLERGY CLIN IMMUNOL* 1982;70:343-52.
27. Puttonen F, Maasch HJ. Measurement of "blocking antibodies" to pollen antigens by radiolabelled protein A from *Staphylococcus aureus*. *Int Arch Allergy Appl Immunol* 1981;64:157-66.
28. Armitage P. Statistical methods in medical research. Oxford: Blackwell Scientific, 1977.
29. Puttonen E, Maasch HJ, Pilström L. Studies on allergen and allergoid preparations from purified timothy (*Phleum pratense*) pollen extracts. I. Physicochemical characteristics and binding to allergen-specific human IgE. *Int Arch Allergy Appl Immunol* 1982;68:1-6.
30. Patterson R, Suszko IM, Bacal E, Zeiss CR, Kelly JF, Pruzansky JJ. Reduced allergenicity of high-molecular-weight ragweed polymers. *J ALLERGY CLIN IMMUNOL* 1979;63:47-50.
31. Grammer LC, Silvestri L, Suszko IM, Shaughnessy MA, Patterson R. Evaluation and standardization of polymerized ragweed extracts by chromatography, radioimmunoassay inhibition, and end point cutaneous titration. *J ALLERGY CLIN IMMUNOL* 1983;72:160-7.
32. Østerballe O. Immunotherapy with grass pollen major allergens: clinical results from a prospective 3-year double-blind study. *Allergy* 1982;37:379-88.
33. Bruce CA, Norman PS, Rosenthal RR, Lichtenstein LM. The role of ragweed pollen in autumnal asthma. *J ALLERGY CLIN IMMUNOL* 1977;59:449-59.
34. Tipton WR, Nelson HS. Experience with daily immunotherapy in 59 adult allergic patients. *J ALLERGY CLIN IMMUNOL* 1982;69:194-9.
35. Østerballe O, Løwenstein H, Norn S, et al. Immunotherapy with grass pollen major allergens: immunological results from a prospective 3-year double-blind study. *Allergy* 1982;37:491-501.
36. Østerballe O. Nasal and skin sensitivity during specific immunotherapy with two major allergens 19, 25 and partially purified extract of timothy grass pollen. *Allergy* 1982;37:169-77.

Immunoglobulin composition of three commercially available intravenous immunoglobulin preparations

Alma M. Herrera, MD, MAJ, MC,* Nancy B. Saunders, MS,** and James R. Baker, Jr., MD, MAJ, MC** Washington, D.C.

*To evaluate whether significant differences in immunoglobulin composition exist among the three commercially available intravenous immunoglobulin (IVIG) preparations, we compared three of these products in terms of their quantitative immunoglobulin concentration, IgG subclass concentration, and the presence of IgG aggregates. Three different lots were tested for each of the IVIG formulations, and the IgG subclass assays were performed by three different laboratories. Differences were found among the three formulations in subclass concentration and aggregate content. Sandoglobulin (Sandoz Pharmaceuticals, East Hanover, N.J.) contained a significantly higher amount of IgG2 ($p < 0.002$) compared to the other formulations. All the formulations tested were found to be deficient in IgG4 relative to the World Health Organization standards, with Gammagard (Hyland Therapeutics, Glendale, Calif.) demonstrating only negligible amounts. Immunoglobulin aggregate content was different among manufacturers with Gamimune N (Cutter Biological, Berkeley, Calif.) containing the highest amount of monomer IgG (99.8%). Significant differences were found in the subclass results obtained by the ICN ImmunoBiologicals assay (Lisle, Ill.), compared to the two reference laboratories. This difference among the subclass assays raises the question as to the use of these assays in the evaluation of patients with suspected subclass deficiencies. The differences in subclass concentration and aggregate content in IVIG preparations were great, and future clinical trials with these formulations would be indicated to determine the clinical significance of these findings. (*J ALLERGY CLIN IMMUNOL* 1989;84:556-61.)*

From the *Department of Allergy and Immunology, and **Department of Clinical Investigation, Walter Reed Army Medical Center, Washington, D.C.

Received for publication Nov. 28, 1988.

Revised March 1, 1989.

Accepted for publication June 22, 1989.

Reprint requests: Alma M. Herrera, MD, Dept. of Allergy and Immunology, Walter Reed Army Medical Center, Washington, DC 20307-5001.

1/1/14800