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A reverse enzyme immunoassay for detection of Vespula spp. sensitivity

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SUMMARY

The Reverse Enzyme Immunoassay (REIA) method for the detection of specific IgE recently described by us has been applied to the diagnosis of Vespula spp. sensitivity. To do so, 0.5 mg of Vespula venom of commercial origin was conjugated with peroxidase according to a previously described technique. The results obtained from the study of 50 nonallergic patients have demonstrated the specificity of the method in as much as no positive value was found. In addition, only one low positive value was obtained from a group of 47 atopic patients showing no evidence of sensitivity to insect stings. On the contrary, very high ELISA values from a group of 23 patients with suspected sensitivity to insect venom were obtained. With these results, we conclude that this method can constitute an aid in the diagnosis of these patients, besides being a low-cost method free from the interference of blocking antibodies. It is our opinion however, that this method is not as reliable as the RAST.

Key words: *REIA. RAST. IgE. Vespula venom sensitivity.*
Palabras clave: *REIA. RAST. IgE. Hipersensibilidad a veneno de avispa.*

INTRODUCTION

The study of insect sting sensitivity is gaining interest in allergy due to the frequency of presumed immunological reactions, which appear after the sting, as well as the peculiarities of the venom. This antigen has a relatively simple composition when compared, for example, to that of a pollen (3) and the concentration of antibodies in absolute weight amounts was also determined (1). Nonetheless, a controversy exists as to the validity of diagnostic tests in vivo and in vitro (5, 6).

Lichtenstein et al. also state that in individuals undergoing immunotherapy, the specific IgG levels can be 1,000 times greater than those of IgE. This situation can cause IgE values to be understimated by those diagnostic systems in which the antigen concentration is limited (3).

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We have recently described a system for the detection of specific IgE based on the isolation of the IgE using microtiter plates. In a second step, the specific IgE is revealed by means of a peroxidase labelled antigen. This method has proven its usefullness in the diagnosis of patients with D. pteronyssinus and Lolium perenne sensitivity as well as other allergens tested so far.

The purpose of this work is to study the sensitivity and specificity of this method when applied to the case of a highly purified antigen, such as the wasp venom in individuals with suspected sensitivity to insect venom.

MATERIALS AND METHODS

A) *Patients:* Three different population groups were studied: the first one was made up of 50 hospital patients without any clinical evidence of allergy. The second group contained 47 atopic patients without evidence of adverse reactions to insect stings. Finally, the third group was formed by 23 patients with suspected type I sensitivity to insect venom.

B) *Reverse enzyme immunoassay (REIA):* Dynatech M 24AR microplates were sensitized with monospecific anti-human IgE using the same concentrations as described elsewhere (2, 4). Once neutralized, duplicate serum samples (0.05 ml), diluted with 0.05 ml of Phosphate buffered saline 0.1% Tween 20 (PBS-Tw), were incubated while shaking at 4°C. After 18 h, the plates were washed 3 times with PBS-Tw, then filled with 0.2 ml of labelled antigen and left shaking at room temperature. The antigen had previously been diluted in PBS, 25% fetal calf serum, 0.5% Tw 20.

C) *Antigen:* The antigen used in this study, Vespula spp. (Venomenhal, Lot: 82470092), was a generous gift from Laboratorios Arístegui-Hal (Madrid). It was labelled with peroxidase, previously activated using the metaperiodate method described elsewere (2). Once activated, 0.5 mg of peroxidase was added to 0.5 mg of wasp venom, both in separate volumes of 0.2 ml of PBS, plus 0.1 ml of carbonate-bicarbonate buffer (1M pH 9.5). The reaction was

left to proceed at 4°C. After 24 h, 1 mg of sodium borohydride in 1 ml of H_2O was added and the solution was dialyzed against PBS for 24 h at 4°C. Then, 2 ml of PBS, 2% bovine serum albumin was added to stabilize the conjugate and aliquots of the conjugate were frozen at —20°C. No type of purification was performed to eliminate the free peroxidase or the unconjugate allergen.

D) *RAST:* The RAST was performed according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden) using Vespula spp. (i 3), Polistes spp. (i 4) and Honey Bee (i 1) discs.

RESULTS

Table I depicts the optical densities obtained with different positive and negative sera using different allergen concentration per well. As can be seen a decrease in the allergen concentration led progressively decreasing optical density values. For this reason, and to give good resolution in the low positive values, we chose an allergen concentration of 1 mcg per well.

This concentration was therefore used to study the specificity of the method for IgE and the antigen. For this purpose, myeloma IgE (2 mcg) was added to the different positive sera which was subsequently processed with antigen. A second aliquot was processed in the normal way without the addition of myeloma IgE. This served as our control. At the same time, a third aliquot of the same serum was incubated just like the control except when adding the labelled allergen (1 mcg per well). At this point, 5 mcg of the same unlabelled antigen was also added in order to check the antigen specificity of the method. Fig. 1 shows that the O.D. obtained in 6 REIA positive sera could be inhibited by myeloma IgE and by the non-labelled antigen. This fact demonstrated that the assay was both antigen and IgE specific.

The specificity of the method for this particular antigen was further checked as follows: of the 50 controls performed using nonallergic patients, not one positive result was found. In addition, only one low positive value (0.42 O.D.) was found in the

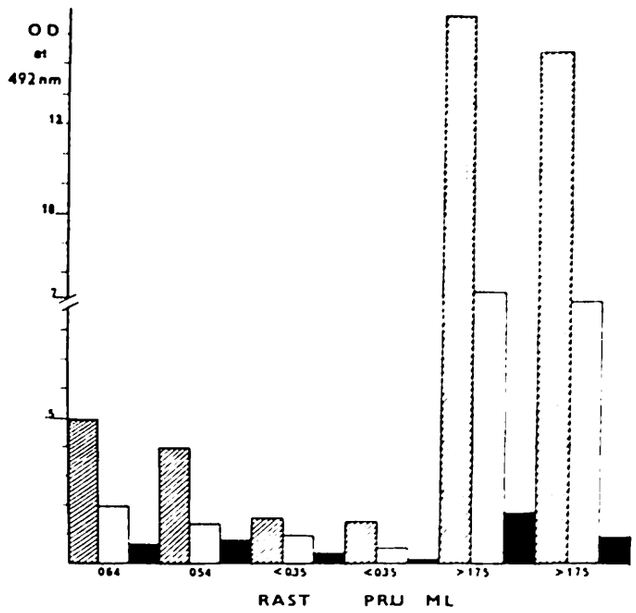

Fig. 1.—Inhibition induced in REIA positive sera by unlabelled antigen (white bars) or by myeloma IgE (shaded bars); striped bars: normal values.

second group of the atopic individuals (47) lacking evidence of insect sensitivity. The specificity of this result was confirmed by inhibiting the serum by IgE and antigen (data not shown for any of the above).

The results obtained with the group of 23 patients with suspected venom hypersensitivity are shown in table II together with the RAST values obtained. The 5 Vespula-RAST positive sera was also REIA positive. On the contrary, only 1 out of 6 Bee-RAST positive sera was positive on the REIA. The three Polister-RAST positive sera available also showed REIA positive values.

DISCUSSION

The method described here has proven to be highly sensitive and specific judging from the differences obtained between the patients with insect venom sensitivity and the control group. At the same

TABLE I

O.D. AT 492 nm USING DIFFERENT CONCENTRATIONS OF LABELLED VESPULA spp.

SERUM	1,000[a]	500	250	125	62.5	31.5
1	13.320	11.316	10.688	7.436	5.164	2.374
2	0.626	0.525	0.406	0.295	0.176	0.097
3	0.079	0.034	0.009	0.002	0.002	0.001
4	0.052	0.018	0.009	0.001	0.001	0.001
5	12.928	9.168	7.804	5.532	3.480	1.161
6	0.267	0.226	0.207	0.127	0.053	0.009
7	0.212	0.146	0.090	0.036	0.005	0.002
8	0.004	0.002	0.001	0.001	0.001	0.001

[a] Final amount of antigen (ng/well), mean of duplicates.

TABLE II

CORRELATION BETWEEN RAST AND REIA

Serum	RAST (P.R.U./ml[a])			REIA[b]
	Vespula spp.	Bee venom	Polistes spp.	Vespula spp.
1	>17.5	<0.35	3.5	24.205
2	2.4	<0.35	<0.35	1.218
3	<0.35	<0.35	<0.35	0.080
4	<0.35	<0.35	<0.35	0.056
5	<0.35	<0.35	<0.35	0.256
6	<0.35	<0.35	<0.35	0.025
7	0.38	3.2	<0.35	0.106
8	<0.35	<0.35	<0.35	0.035
9	<0.35	2	N.D.[c]	0.031
10	<0.35	0.36	N.D.	0.256
11	<0.35	1.5	<0.35	0.072
12	>17.5	<0.35	2.2	21.712
13	<0.35	>17.5	<0.35	0.027
14	<0.35	0.65	<0.35	0.075
15	<0.35	<0.35	<0.35	0.140
16	0.54	<0.35	<0.35	0.485
17	<0.35	>17.5	<0.35	0.068
18	<0.35	<0.35	0.64	0.850
19	<0.35	<0.35	<0.35	0.030
20	<0.35	<0.35	<0.35	0.050
21	<0.35	<0.35	<0.35	0.051
22	<0.35	<0.35	<0.35	0.048
23	<0.35	<0.35	<0.35	0.060
Blank				0.038

a) Phadebas RAST units/ml.
b) Mean of duplicates, O.D. 492 nm.
c) N.D.: not determined.

time, it has proven to be antigen and IgE specific. We attribute this fact to the fine quality of the antigen used, a pure wasp venom, in comparison to crude extracts of the other allergens which are complex mixtures of proteins.

On the other hand, we do not wish to emphasize the differences observed between the RAST results and those of our method for various reasons. In the first place, we have not used the same source of antigen which can most likely lead to differences in the representation of the allergenic fractions. It is also necessary to point out that the two methods detect IgE in a completely opposite way which would make a correlation difficult to interpret. Besides, the RAST is a highly reliable method, while work carried out with microtiter plates does not offer the same degree of repetitivity. This is especially true when high peroxidase concentrations per well, (as in our case), must be used.

It is interesting to note however, that the use of U-shaped plates has provided us with better results at low sensitivity levels, than those obtained when using flat-bottomed plates as we have previously employed (data not shown). Perhaps this reason is true due to the lack of corners in the well and for this reason the washing steps are more effective, at least when the plates are washed by hand as in our case.

In spite of all the problems, this method appears to be of interest in the field of insect venom sensitivity due to the contradiction between other diagnostic methods currently used (7). On the other hand, it can constitute an alternative for the follow-up of those patients undergoing immunotherapy. In this case, it is of vital interest to dispose of a method described here which is not affected by the presence of blocking antibodies. This method permits the measurement of specific IgE for insect venom throughout the patient's treatment.

Finally, we hope that the results obtained with this wasp venom can be repeated with that of other insects, which would perhaps offer a new way to study this type of patient's sensitivity.

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RESUMEN

La sensibilización a veneno de Himenópteros se reconoce cada día con más frecuencia. Se ha estimado que la incidencia de reacciones sistémicas tras picadura de Himenópteros (avispa o abeja) en población general es de un 0,4%, aumentando este porcentaje de manera significativa si se tienen en cuenta las reacciones locales.

El diagnóstico etiológico de estos pacientes se afectúa en la práctica diaria a través de la realización de pruebas cutáneas (tanto en prick-test como pruebas intradérmicas), así como por métodos serológicos encaminados a detectar la presencia de IgE específica circulante (RAST).

En el presente trabajo describimos un método original de Enzimoinmunoensayo Reverso (REIA) para la detección de IgE específica circulante a veneno de avispa en un grupo de pacientes con sospecha de sensibilización a dicho antígeno, con el propósito de ofrecer un complemento útil a las técnicas ya existentes (test cutáneos y RAST).

Para ello incluimos en este estudio a 3 grupos de pacientes: un primer grupo constituido por 50 sujetos (grupo control) sin antecedentes de enfermedad alérgica conocida; un segundo grupo compuesto por 47 pacientes atópicos sin historia de reacciones adversas tras picadura de insectos; y un último grupo formado por 23 enfermos con sospecha de sensibilización de tipo inmediata a veneno de avispa.

Conjugamos 0,5 mg de veneno de avispa véspula comecial con peroxidasa, siguiendo la metodología descrita para el Enzimoinmunoensayo Reverso (REIA) por nuestro grupo en anteriores publicaciones.

Los resultados obtenidos en este trabajo en los 50 pacientes no alérgicos demuestran la especificidad del método, dado que no obtuvimos valor positivo en ninguno de ellos. Dentro del grupo de los 47 pacientes atópicos, solamente en uno obtuvimos un valor positivo límite. Por el contrario, en los 23 enfermos con sospecha de sensibilización a veneno de avispa detectamos valores muy altos de IgE específica a través del Enzimoinmunoensayo Reverso (REIA).

Basándonos en estos resultados podemos concluir que este método puede constituir una ayuda en el diagnóstico de los pacientes con sospecha de sensibilización a veneno de avispa, dado que es una técnica rápida, reproductible, específica, de bajo costo y que permite obviar la existencia de anticuerpos bloqueantes.

Asimismo consideramos que este método puede constituir una alternativa válida para el seguimiento de la eficacia de la inmunoterapia en aquellos pacientes sometidos a la misma. Resultaría, además de gran utilidad en la valoración de los niveles de IgE e IgG específica durante y tras el tratamiento hiposensibilizante.

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