



NON-SPECIFIC CROSS-REACTIVITY OF HYDROPHOBIC SERUM IgE TO HYDROPHOBIC DRUGS

J. L. GUÉANT,*† E. MATA,*‡ C. MASSON,* P. GÉRARD,*
D. A. MONERET-VAUTRIN,† C. MOUTON-FAIVRE* and M. C. LAXENAIRE§

*Laboratoire de Biochimie, LBCM, Faculté de Médecine, B.P. 184, 54505
Vandœuvre-lès-Nancy Cedex, France; ‡Service d'Allergologie-Immunologie, Hôpital de Brabois,
CHU de Nancy 54500 Vandœuvre-lès-Nancy, France; and §Département
d'Anesthésie-Réanimation, Hôpital Central, CHU de Nancy, 54000 Nancy, France

(First received 9 July 1994; accepted in revised form 10 October 1994)

Abstract—The purpose of this work was to study the involvement of serum hydrophobic IgE in non-specific cross-reactions with hydrophobic drugs such as cyclohexenyl derivatives. Hydrophobic IgE were detected by radioimmunoassay. The results were expressed as the percentage of labelled anti-IgE which were adsorbed to the drug solid phase via IgE of the patient serum. Phenyl-Sepharose IgE-RIA was at $4.6 \pm 0.7\%$, $125 \pm 6.5\%$ and $17.8 \pm 8.9\%$ in control subjects ($n = 24$), in atopic patients with positive Phadiatop™ ($n = 30$) and in patients with drug allergy ($n = 23$), respectively. We selected five patients who were allergic to either penicillin, propofol, glafenin or paracetamol and who had a Phenyl-Sepharose IgE-RIA greater than 20%. In these five cases, IgE-RIA were positive (percentage at least twice more than that obtained with control sera) with all the solid phases prepared with hydrophobic drugs such as penicillin, propofol, glafenin, paracetamol and mexiletine. Inhibition of IgE binding by monoethylene-glycol showed that the cross-reactivity was due to hydrophobic binding of IgE to the drug. Three of the five patients were allergic to penicillin and underwent an adverse reaction against another cyclohexenyl derivative, namely propofol and glafenin. In conclusion, we have observed the presence of 'hydrophobic IgE (with positive Phenyl-Sepharose RIA)' in 64% of patients allergic to a hydrophobic drug.

Key words: IgE, drug allergy, atopy, radioimmunoassay, penicillin, propofol.

INTRODUCTION

The high prevalence of allergic cross-reactions is generally explained by the presence of common allergens in different allergenic media, common epitopes present on different allergens and/or multiple sensitizations. Multi-specific recognition of allergens by IgE antibodies is another conceivable mechanism (Fischer and More, 1981).

Allergic IgE-mediated reactions represent an important proportion of adverse reactions induced by therapeutic doses of drugs. The diagnosis of IgE-mediated drug allergy can be established from skin testing, *in vitro* leukocyte histamine release and detection of specific IgE in serum by radioimmunoassay (Fischer and More, 1981; Watkins, 1987; Guéant *et al.*, 1992). Allergic cross-reactions have been frequently reported for several categories of drugs such as antibiotics and anesthetics (Guéant *et al.*, 1991, 1992; Baldo and Fischer, 1983). In these cases, cross-reactivity between the different drugs which bind to IgE has been demonstrated by radioimmunoassay (Guéant *et al.*, 1991, 1992; Baldo and

Fischer, 1983; Harle and Baldo, 1990). Cross-reactivity is usually explained by the presence of a common antigenic determinant in the cross-reacting drugs (Baldo and Fischer, 1983). Theoretically, it could also be the result of non-specific binding of the drugs to IgE (Guéant *et al.*, 1992).

The aim of the present study was to investigate whether hydrophobic non-specific binding of hydrophobic drugs to hydrophobic IgE could be responsible for cross-reactivity of hydrophobic drugs in drug allergy.

The antigen-antibody specific binding is the result of complementary molecular interactions, one of which is a hydrophobic interaction (Colman, 1988; Davies *et al.*, 1988). Among the drugs which have been found to be allergenic, most are hydrophobic compounds which contain a C₆H₅ phenyl ring (cyclohexenyl derivatives) (Harle *et al.*, 1989; Laxenaire *et al.*, 1988). These compounds can strongly bind to external hydrophobic areas of globular proteins and can therefore bind non-specifically to hydrophobic IgE.

The present work investigated: (1) the presence of seric hydrophobic IgE in atopy and drug allergy; and (2) the binding of a series of phenyl-containing drugs to these hydrophobic IgE in the serum of patients who underwent an adverse reaction to cyclohexenyl derivatives such as penicillin, propofol, paracetamol and glafenin. Hydro-

† Author to whom correspondence should be addressed at:
Laboratory of Biochemistry, Medical Faculty, University of
Nancy I, BP 184, 54505 Vandœuvre-Nancy, France.

phobicity of IgE from these patients was studied by an IgE-RIA using Octyl- and Phenyl-Sepharose as solid phase. IgE cross-reactivity against the corresponding solid drug phases was studied by direct binding radioimmunoassays and hapten inhibition methods.

MATERIALS AND METHODS

Patients and control subjects

The patients included had undergone an adverse reaction, i.e. giant urticaria, bronchospasm, angioedema or anaphylactoid shock, against either penicillin ($n = 7$), propofol ($n = 10$), paracetamol ($n = 1$) or glafenin ($n = 6$). All the patients had a positive IgE-RIA to the drug incriminated in the reaction.

The cross-reactivity of IgE for phenyl-containing drugs was extensively studied in five of these patients. The clinical data for these is summarized as follows. Patient RA was a 40 year-old female who suffered from facial angioedema and bronchospasm after an injection of Diprivan® (propofol in Intralipid emulsion). The intradermal reaction to Diprivan® and a challenge test with Intralipid (with determination of histamine blood level) were negative. Patient PA was a 43 year-old male who had an anaphylactoid reaction after the simultaneous injection of Diprivan® and alfentanil. Intradermal reactions to the two drugs were negative. This patient was allergic to penicillin, with positive skin tests and positive RAST-IgE. Patient HA was a 30 year-old male who had giant urticaria after ingesting glafenin (positive prick test). He was also allergic to penicillin (positive skin tests and positive RAST-IgE). Patient DO was a 46 year-old female who had an anaphylactoid reaction after ingesting 500 mg of paracetamol. This case has been reported elsewhere. The diagnosis was established by a positive oral challenge test with pruritus, hypotension and increased histamine blood level (Diem and Grilliat, 1990). Patient HA was a 59 year-old male chemist working in the hospital. He had suffered from asthma for 20 years, and had had an anaphylactic shock after an injection of penicillin V, as well as two successive episodes of bronchospasm after inhaling phenol and amoxycillin, respectively. Skin tests and RAST-IgE were positive to penicillin V (phenoxymethyl penicilloyl) and penicillin G (benzyl penicilloyl).

Control subjects were healthy persons from the medical staff. They had never presented any allergic reaction and had a normal seric levels of IgE and a negative Phadiatop™.

Methods

Drugs tested in IgE-RIA. Propofol (2,6 di-isopropyl-phenol) was obtained from ICI-Pharma, Cergy, France; mexiletine (2,6-dimethylphenoxy-2-propanamine) was from Boehringer Ingelheim, Paris, France; paracetamol (N-4-hydroxyphenyl acetamide) was from Laboratoires UPSA, Rueil-Malmaison, France; penicillin G (benzyl

penicillin), penicillin V (phenoxymethyl penicillin) and pentagastrin (1,1-dimethylethoxycarbonyl- β -alaninyl-L-tryptophyl-L-methionyl-L- α -aspartyl-L-phenyl-alaninamide) were obtained from Sigma Chemical Co., St Louis, MO, U.S.A.; tiemonium iodide (4-[3-hydroxy-3-phenyl-3-(2-thienyl)propyl]-4-methylmorpholinium iodide) was from Laboratoires CERM, Riom, France and glafenine (2-[7-chloro-4-quinolinylamino] benzoic acid 2,3-dihydroxypropylester) from Laboratoires Roussel, Paris, France.

Preparation of drug Sepharose gels. Octyl-Sepharose and Phenyl-Sepharose were obtained from Pharmacia (Saint-Quentin en Yvelines, France). Drug solid phases were prepared by covalently coupling the drugs to epoxy-Sepharose as described previously (Baldo and Fischer, 1983; Guéant *et al.*, 1991). Epoxirane groups are known to react with amines and hydroxyles by forming covalent linkage (Dean *et al.*, 1985). Mexiletine and glafenin were dissolved in 50% (v/v) dioxane in water at a concentration of 70 μ moles/ml. Propofol was dissolved in 70% (v/v) dimethylformamide in water at a concentration of 60 μ moles/ml. The pH was raised to 12 by drop addition of 1 mol/l NaOH and the drug solution (4 ml) was incubated overnight with 4 ml of swollen epoxy-activated Sepharose. The yield for coupling was determined by the difference between the concentration of the drug solution before and after incubation of the solution with epoxy-Sepharose. The concentration was determined by spectrophotometry. The gel was then successively washed with 400 ml of the dissolution solution (either dioxane/water or dimethylformamide/water), with 400 ml of water and finally with 400 ml of 0.02 M Tris-HCl buffer pH 8.0. They were incubated in Tris-HCl buffer for 18 hr in order to block the remaining epoxirane groups. The gels were stored in 0.5 mol/l sodium chloride containing 0.02% sodium azide and washed in 0.1 mol/l sodium phosphate buffer pH 8.0 containing 0.15 mol/l sodium chloride prior use.

IgE-radioimmunoassays. IgE-radioimmunoassays were performed by incubating the patients' serum with the drug covalently bound to a solid phase such as a paper disc (RAST paper disc from Pharmacia, Uppsala, Sweden) or Sepharose gel. The results were expressed as the percentage of labelled anti-IgE which was adsorbed to the drug solid phase via IgE of the patients serum (Baldo and Fischer, 1983). The specificity of IgE binding to the drug solid phase was established by inhibition in the presence of the soluble drug or related compounds (Guéant *et al.*, 1992; Baldo and Fischer, 1983). The IgE-radioimmunoassay was performed as follows: 50 μ l of serum were incubated with 50 μ l of drug-Sepharose gel for 3 hr at room temperature. The gel was washed three times with 2.0 ml of 0.1 mol/l sodium phosphate buffer containing 0.15 mol/l sodium chloride. ^{125} I-labelled anti-IgE (Pharmacia) was added (40 000 cpm per tube). The tubes were incubated overnight at room temperature and the gels were washed again three times before γ -counting. The detection of specific IgE corresponded to the percentage of uptake of labelled anti-IgE onto the

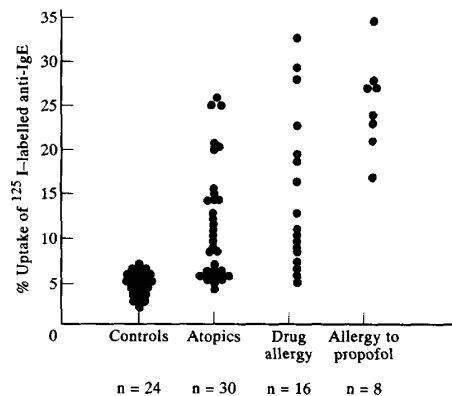


Fig. 1. Seric level of hydrophobic IgE measured by Phenyl-Sepharose IgE-RIA in controls ($n = 24$), atopic patients ($n = 30$), in patients who reacted against either penicillin, paracetamol and glafenin ($n = 16$), and patients who reacted against propofol ($n = 8$).

drug solid phase. In each series, control tubes were prepared by the same procedure except that 50 μ l of inactivated epoxy-Sepharose was added instead of the drug solid phase. In all cases, the result obtained with these control tubes was lower than 0.6%. The radioimmunoassays of total IgE (Immunotech, Luminy, France), and IgE specific to penicillin V, penicillin G and pneumallergens (RAST PhadiatopTM, Pharmacia, Uppsala, Sweden) were performed according to the manufacturer's instructions.

Phenyl-Sepharose chromatography

One milliliter of serum was poured onto a Phenyl-Sepharose column (1.5 $\times$ 5 cm). The column was successively filtered with 0.1 mol/l sodium phosphate buffer pH 7.4 in a linear gradient of sodium chloride decreasing from 2 mol/l to 0 mol/l, with water and finally with 20% (v/v) ethylene-glycol. Flow rate was 1 ml/min. Radioimmunoassay of IgE and RAST-IgE to penicillin were performed in collected fractions.

RESULTS

Octyl-Sepharose and Phenyl-Sepharose radio-immunoassays (RIA) were performed to detect the hydrophobic IgE of the patients. The result was considered weakly positive ($\pm$) and positive (+) when it represented, respectively, twice and three times the result obtained with 24 control sera. No correlation was found between the binding of IgE to Phenyl-Sepharose and the seric total IgE level in 70 sera including 16 patients with drug adverse reaction, 24 non-atopic cases and 30 atopic patients ($r = 0.09$). A poor correlation was observed when the PhadiatopTM-RIA was compared to the Phenyl-Sepharose RIA ($r = 0.31$, $n = 27$, $p < 0.1$). Phenyl-Sepharose IgE-RIA was deemed positive in 27% of atopic patients and 64% of patients with drug allergy (Fig. 1). It was positive in 100% of patients who reacted against propofol (Fig. 1).

Among the seven patients allergic to penicillin, only four had a positive Phenyl-Sepharose RIA (Table 1). No correlation was observed between RAST-IgE to penicillins and Phenyl-Sepharose RIA. However, patient HA had the highest results for both RAST-IgE to penicillin V and Phenyl-Sepharose RIA (Table 1). From the six patients who underwent an adverse reaction to glafenin, four had a positive Phenyl-Sepharose RIA. Again, no correlation was observed between Phenyl-Sepharose and glafenin-Sepharose RIA (Table 2). All nine patients who had had an allergic reaction to propofol, and one who had reacted against paracetamol had a positive Octyl- or Phenyl-Sepharose RIA (Table 3).

In our series of patients with drug allergy, we selected five patients who had highly positive Phenyl-Sepharose and Octyl-Sepharose radio-immunoassays (results greater than 20% and 5%, respectively). Two of them had reacted against propofol (patients RA and PA) and the other three, after receiving either penicillin, paracetamol or glafenin (patients HA, DO and RA, respectively). The sera of these five patients gave a positive RIA with most of the drug solid phases tested (Table 4). All the drugs tested for IgE-RIA (propofol, glafenin, paracetamol, mexiletine and penicillin) contained a phenyl ring. In order to determine whether the IgE of the patients were

Table 1. Adsorption to Phenyl- and Octyl-Sepharose of seric IgE from patients who had undergone an allergic reaction to penicillin

Patient	RAST paper disc			Sepharose coupled to:			Total IgE (KU/l)
	Penicillin V	Penicillin G % (+/-)	HSA	Phenyl % (+/-)	Octyl % (+/-)	Phadiatop (+/-)	
BO	4.7(+)	4.2(+)	0.2	6.4(-)		nd	20
TO	5.0(+)	6.1(+)	0.2	7.6(-)		-	70
ZA	3.2(+)	5.8(+)	0.2	9.0(-)	2.6(-)	-	72
CH	1.9(+)	0.9($\pm$)	0.3	10.3($\pm$)	-	-	279
TH	0.9($\pm$)	0.8($\pm$)	0.2	15.8(+)	4.7($\pm$)	+	-
HE	1.0(+)	1.1(+)	0.4	18.0(+)		+	1247
HA	8.1(+)	1.7(+)	0.2	34.2(+)	6.1(+)	+	386
Controls ($n = 24$)	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1	4.6 $\pm$ 0.7	1.6 $\pm$ 0.2	-	66 $\pm$ 39

nd, non-determined; HSA, human serum albumin.

Table 2. Adsorption to Phenyl- and Octyl-Sepharose of seric IgE from patients who had undergone an allergic reaction to glafenin

Patient	Sepharose coupled to:					Total IgE (KU/l)
	Glafenin	Hydroxyglafenin % (+/-)	Phenyl	Octyl	Phadiatop (+/-)	
CO	5.6(±)	9.7(+)	8.0(-)	1.7(-)	+	ND
OD	5.8(±)	14.5(+)	6.9(-)	2.2(-)	-	ND
MI	3.7(-)	17.2(+)	10.2(±)	3.4(-)	-	245
CA	3.6(-)	11.2(+)	12.4(±)	2.6(-)	-	149
BA	3.9(-)	8.1(+)	9.8(±)	2.6(-)	-	228
KA	5.1(±)	14.4(+)	18.9(+)	5.2(+)	-	80
Controls	2.5 ± 1.3	2.5 ± 1.2	4.6 ± 0.7	1.6 ± 0.2	-	66 ± 39

Abbreviations as in Table 1.

adsorbed to the solid drug phase via binding to the phenyl group, inhibition studies were performed with a number of cyclohexenyl derivatives. These inhibition studies were performed with the sera of patients HA, DO and RA (Table 5). In patient HA, phenyl compounds produced a significant inhibition of the penicillin G-RAST, with an inhibition percentage ranging from 18.4 to 57.2. However, the binding of IgE from patient HA to mexiletine-Sepharose was significantly inhibited by penicillin. The greater quantity of penicillin used, the higher was the degree of inhibition (Fig. 2). The inhibition of penicillin RAST with phenyl compounds was also observed with the sera from patients DO and RA. In addition, the adsorption of IgE to Sepharose-propofol and to Sepharose-paracetamol was also inhibited in the presence of other phenyl compounds such as tiemonium, mexiletine and pentagastrin (Table 5). Ethylene-glycol is a polar organic solvent used to prevent hydrophobic interactions. In the present experiment, it inhibited the binding of IgE to Sepharose coupled with phenyl compounds such as paracetamol and propofol, but not the binding of IgE to penicillin-RAST.

Phenyl-Sepharose chromatography was used to separate the IgE of patient HA according to their hydrophobicity (Dean *et al.*, 1985). Two peaks of IgE were identified. The first peak was eluted in the presence of 1 mol/l sodium chloride and therefore corresponded to non-hydrophobic IgE which were not retained by Phenyl-

Sepharose. The second peak corresponded to hydrophobic IgE which were eluted in the presence of 20% monoethylene-glycol (Fig. 3). Similar data were obtained when the same experiment was performed with the serum of patient HE (Table 1). The RAST to penicillin was positive in the pooled fractions corresponding to the second peak and it was negative in the pooled fractions of the first peak (Fig. 3). A very weak hydrophobic peak of IgE was also observed in serum of one patient allergic to penicillin and which had a negative RIA at 6.4% (patient BO, Table 1); the IgE specific to penicillin were again detected in the hydrophobic peak. Phenyl-Sepharose chromatography was also performed with a serum sample of a control subject, which gave a negative Phenyl-Sepharose RIA (lower than 4%). In that case, only the first peak was observed (data not shown).

DISCUSSION

Our study is the first to describe the presence of hydrophobic IgE in serum. These hydrophobic IgE were found in 27% of atopic patients with a positive Phadiatop and in 64% of these patients who had had an allergic reaction to a hydrophobic drug and in all the patients who reacted against propofol (Fig. 1). They were not found in the control group. Phenyl-Sepharose chromatography helped to determine the meaning of a positive IgE Phenyl-Sepharose RIA as seric IgE could be cleared in a

Table 3. Adsorption to Phenyl- and Octyl-Sepharose of seric IgE from patients who had had an adverse reaction to propofol

Patient	Sepharose coupled to:			Total IgE (KU/l)
	Propofol	Phenyl % (+/-)	Octyl	
DA	9.2(+)	27.8	4.3(+)	260
HU	9.3(+)	23.6(+)	3.3(±)	46
SE	22.4(+)	16.6(+)	9.0(+)	154
HE	5.8(±)	34.6(+)	28.9(+)	nd
DO	7.3(+)	23.6(+)	1.8(-)	nd
BE	3.7(±)	20.9(+)	3.4(±)	25
PO	5.0(+)	26.9(+)	2.9(-)	112
RA	7.9(+)	nd	5.5(+)	1721
PA	nd	23.1	5.0(+)	ND
Controls (n = 24)	1.7 ± 0.4	4.6 ± 0.7	1.6 ± 0.2	66 ± 39

Abbreviations as in Table 1.

Table 4. Adsorption to hydrophobic drug solid phases of seric IgE from patients who had hydrophobic IgE detected by Phenyl-Sepharose and Octyl-Sepharose IgE-RIA

Patient	Adverse reaction to:	Phenyl-Sepharose adsorbed to		Epoxy-Sepharose coupled to:				RAST paper disc				Total IgE (KU/l)	
		Octyl-Sepharose	Phenyl-Sepharose	Diprivan	Propofol	Hydroxyglafenin (%) (+/-)	Paracetamol	Mexiletine	Penicillin V	Penicillin G	HSA		Phadiatop (+/-)
RA PA	Diprivan	5.5(+)	nd	24.2(+)	7.9(+)	13.2(+)	3.2(±)	24.6(+)	2.5(+)	2.1(+)	1.0(-)	-	1721
	Diprivan	5.0(+)	23.1(+)	28.2(+)	nd	nd	nd	29.4(+)	1.2(+)	4.8(+)	0.6(-)	nd	nd
KA DO HA	Allentanyl Glafenin Paracetamol Penicillin	5.2(+)	27.7(+)	22.0(+)	3.4(+)	14.4(+)	nd	4.5(+)	1.3(+)	0.3(-)	0.2(-)	-	80
		17.6(+)	29.0(+)	25.0(+)	11.1(+)	10.1(+)	17.5(+)	17.3(+)	1.0(+)	2.0(+)	0.5(-)	+	392
		6.1(+)	34.2(+)	34.7(+)	9.4(+)	5.4(±)	2.9(-)	9.7(+)	8.1(+)	1.7(+)	1.2(-)	+	386
		1.6 ± 0.2	4.6 ± 0.7	3.5 ± 0.5	1.7 ± 0.4	2.5 ± 1.2	1.5 ± 0.4	3.0 ± 0.7	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	66 ± 39	
Controls													(n = 24)

Abbreviations as in Table 1.

Table 5. Inhibition percentage of the uptake of seric IgE on solid drug phases in the presence of soluble drugs

Patient	Solid drug phase	% Uptake	% Inhibition*							
			Penicillin G	Diprivan	Mexiletine	Paracetamol	Tiemonium	Pentagastrin	Ethylene-glycol	
RA	RAST Penicillin G	2.1	68.1	5.6	nd	53.0	33.4	35.1	nd	
	Sepharose-propofol	7.9	36.4	68.5	48.2	78.7	nd	nd	44.2	
DO	RAST Penicillin V	2.0	48.3	nd	nd	nd	19.1	11.9	nd	
	Sepharose-paracetamol	17.5	nd	nd	nd	85.1	nd	nd	88.6	
HA	Sepharose-propofol	11.1	42.8	50.8	nd	79.7	39.6	24.0	nd	
	RAST Penicillin G	1.7	100	18.4	19.8	nd	57.2	50.0	0.0	
HA	RAST Penicillin V	8.1	98.0	nd	nd	nd	16.8	nd	0.0	
	Sepharose-mexiletine	29.4	62.5	47.7	47.0	nd	nd	nd	nd	

*Penicillin G, diprivan, mexiletine, paracetamol, tiemonium and pentagastrine were tested at the concentration of 0.5 µmol/l and ethylene-glycol at the concentration of 3.6 moles/l (corresponding to 20% (v/v) of the incubation mixture). The results are the mean of duplicated assays.

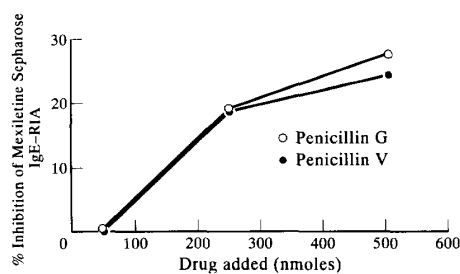


Fig. 2. Inhibition of mexiletine-Sepharose IgE-RIA (patient HA) by penicillin V (●) and penicillin G (○).

hydrophobic and a hydrophilic peak. The serum of a control subject with negative Phenyl-Sepharose RIA was only cleared in a hydrophilic peak. The meaning of this heterogeneity of IgE (in regard to their hydrophobicity) must be studied further. The high level of hydrophobic IgE could not be related to a higher level of serum IgE in patient sera than in control sera. In fact, no correlation was observed between phenyl IgE-RIA and the total serum IgE level in 70 sera including atopic patients. The fact that no correlation was observed between total serum IgE and the level of binding to Phenyl-Sepharose speaks rather to the interactions being more related to the V-region. Hydrophobic IgE may represent that fraction of IgE which recognizes hydrophobic epitopes. If that were the case, hydrophobic IgE should be multi-specific for hydrophobic chemical compounds. It is well established that antibodies of the A, G and M classes can be multi-specific, since they can bind structurally dissimilar epitopes (Varga *et al.*, 1974; Czaja *et al.*, 1977). Since the same repertoire of V-region genes are involved in the synthesis of the various classes of immunoglobulins (Esser and Radbuch, 1990), it may be anticipated that IgE are also multi-specific. It has already been shown that a mouse monoclonal anti-dinitrophenol IgE can bind to structurally unrelated chemical compounds such as aspirin, naphthoquinone and dehydrocholic acid (Varga *et al.*, 1991). These chemical compounds may also induce a cross-stimulation of rat basophils sensitized with the anti-DNP IgE monoclonal antibody (Varga *et al.*, 1991). Another hypothesis is that the hydrophobic interaction is produced by hydrophobic amino acids located near but not in the binding site of the V-region as the competitive inhibition of the IgE binding to drug solid phases by hydrophobic drugs was lower than 80% in most cases (Table 5).

The serum of five cases with positive Phenyl-Sepharose IgE-RIA also gave positive results with most of the IgE-RIA performed with solid drug phases prepared with hydrophobic drugs which contained a phenyl ring in their structure. This phenyl ring can be responsible for strong π - π hydrophobic non-specific binding with aromatic amino acids of proteins and peptides (Dean *et al.*, 1985). In the present case, the positive IgE-RIA could be explained by a non-specific hydrophobic binding of IgE to the hydrophobic drug solid phase, rather than by a true specific cross-reactivity of anti-drug IgE due to the presence of the cyclohexenyl ring in the drugs tested.

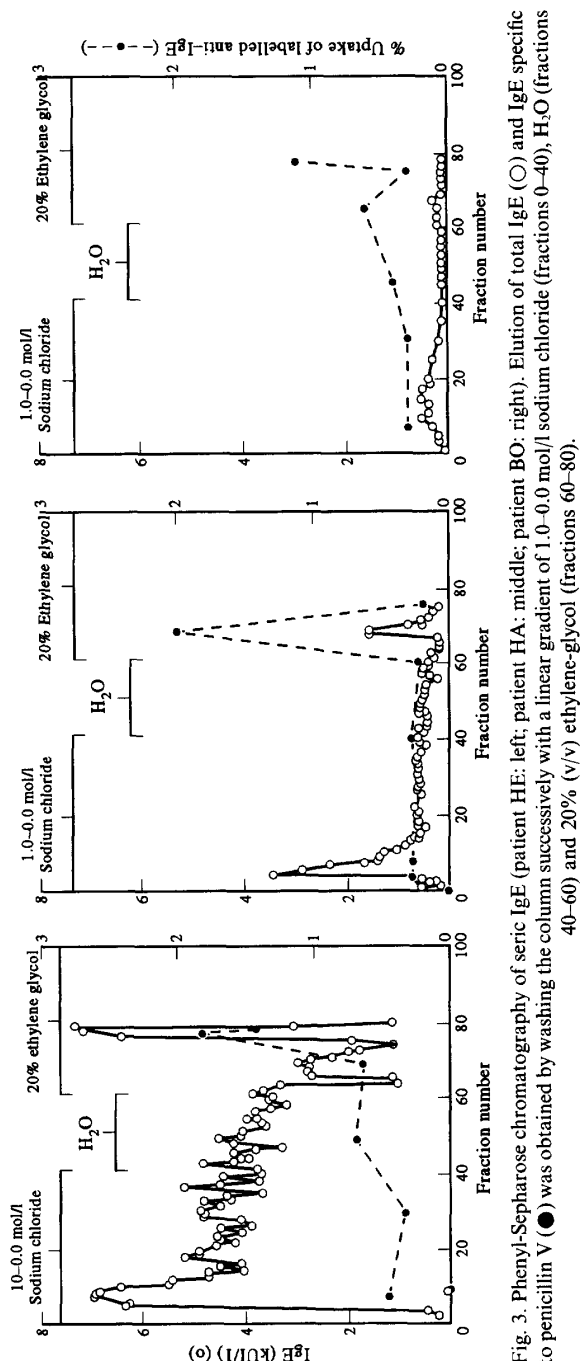


Fig. 3. Phenyl-Sepharose chromatography of serum IgE (patient HE: left, patient HA: middle; patient BO: right). Elution of total IgE (○) and IgE specific to penicillin V (●) was obtained by washing the column successively with a linear gradient of 1.0-0.0 mol/l sodium chloride (fractions 0-40), H₂O (fractions 40-60) and 20% (v/v) ethylene-glycol (fractions 60-80).

Hydrophobic IgE reacted not only with Phenyl-Sepharose but also with a non-aromatic hydrophobic compound such as Octyl-Sepharose. Table 5 showed the inhibition of anti-drug IgE-RIA by soluble drugs and by monoethylene-glycol. The RAST to penicillin and the RIA to Sepharose-paracetamol and to Sepharose-propofol were inhibited by preincubation of the serum with drugs such as penicillin, diprivan, mexiletine, paracetamol, tiemonium and pentagastrin. The single common molecular characteristic of these drugs is the presence of a C₆H₅ phenyl ring. The hydrophobic binding of seric IgE to the solid phase was not specific in the case of Sepharose-paracetamol RIA and Sepharose-propofol RIA, since it was abolished when the incubation of the solid drug phase with the serum was performed in the presence of 20% monoethylene-glycol (Table 5).

This effect of monoethylene-glycol was not observed with penicillin RAST. Penicillins can be highly allergenic substances since they can bind covalently with serum proteins and share several epitopes (de Weck, 1983). Recently, Harle *et al.* proposed the benzyl side chain as a possible epitope involved in allergic reactions to penicillin (Harle and Baldo, 1990). The inhibition results of RAST to penicillin observed with the serum of patient HA showed that the phenyl ring takes part in the epitope recognized by the anti-penicillin IgE (Table 5). However, hydrophobicity was not the single interaction involved in the binding of penicillin to IgE since no inhibiting effect on the penicillin-RAST was observed with 20% monoethylene-glycol. Phenyl-Sepharose chromatography confirmed this result. Anti-penicillin specific IgE which were cleared in the hydrophobic IgE peak still reacted with penicillin paper discs in the presence of 20% monoethylene-glycol (Fig. 3).

Our results led to the following question: can we consider that a high level of hydrophobic IgE (with Phenyl-Sepharose IgE-RIA higher than 20%) in the serum of a patient allergic to a hydrophobic drug such as penicillin is a risk factor for IgE cross-reactivity to another drug? The case reports of patients KA and PA are in favour of such a hypothesis. Both patients were allergic to penicillin and had a highly positive Phenyl-Sepharose IgE-RIA. These patients later had either a giant urticaria or an anaphylactoid reaction to other phenyl-containing drugs, namely propofol and glafenin. These two compounds are small molecules which could act as haptens to provoke an IgE-mediated reaction (Laxenaire *et al.*, 1992). Such a reaction could be the result of non-specific drug-binding with IgE, rather than a classical allergic reaction via a mono-specific anti-drug. In addition, our results demonstrate that the specificity of a IgE for a drug structure cannot be demonstrated by drug solid phase IgE-RIA since the presence of hydrophobic IgE in serum can produce false positive results. In addition, false positive results cannot be detected by the classical combination of the drug solid phase IgE-RIA with the hapten inhibition method (Guéant *et al.*, 1991, 1992; Baldo and Fischer, 1983; Harle *et al.*, 1989, 1990; Colman, 1988; Davies *et al.*, 1988). Most of the recent progress in which epitopes of IgE-mediated drug allergy

were identified by way of solid drug phase IgE-RIA have to be reconsidered in function of a possible binding of hydrophobic seric IgE to the solid phase. A good way to check false positive results could be to perform the assay in the presence of monoethylene-glycol.

In conclusion, we have found hydrophobic IgE in serum of patients who have had an allergic reaction to hydrophobic drugs. The presence of hydrophobic IgE in serum, detected by highly positive Phenyl-Sepharose IgE-RIA could therefore be considered as a risk factor for cross-reactions to hydrophobic drugs and further epidemiological studies will be necessary to establish this correlation.

Acknowledgements—The authors thank Drs Marie-Caroline Bonnet (Montpellier), Francisque Leynadier (Paris), Amédéo (Nice), Ocelli (Nice), Jean Motin (Lyon) and Alain Rossi (Pharmacovigilance ICI, France), for allowing us to investigate their patients and Dr Nicolas Drouet for his help with translating the text.

REFERENCES

- Baldo B. A. and Fischer M. M. (1983a) Substituted ammonium ions as allergenic determinants in drug allergy. *Nature* **306**, 262–264.
- Baldo B. A. and Fischer M. M. (1983b) Anaphylaxis to muscle relaxant drugs. Cross-reactivity and molecular basis of binding of IgE antibodies detected by radioimmunoassay. *Molec. Immun.* **20**, 1393–1400.
- Colman P. M. (1988) Structure of antibody–antigen complexes: implications for immune recognition. *Adv. Immun.* **43**, 99–112.
- Czaja M. J., Richards F. F. and Varga J. M. (1977) Multispecific lymphoid cell surface receptors. *Proc. natn. Acad. Sci. U.S.A.* **74**, 1224–1228.
- Davies D. R., Sheriff S. and Padlan E. A. (1988) Antibody–antigen complexes. *J. biol. Chem.* **263**, 10541–10544.
- Dean P. D. G., Johnson W. S. and Middle F. A. (1985) Affinity chromatography: a practical approach. IRL Press (Oxford, Washington DC), pp. 138–144.
- Diem L. V. and Grilliat J. P. (1990) Anaphylactic shock induced by paracetamol. *Eur. J. clin. Pharmacol.* **38**, 389–390.
- Esser C. and Radbuch A. (1990) Immunoglobulin class switching: Molecular and cellular analysis. *A. Rev. Immun.* **8**, 717–735.
- Fischer M. M. and More D. G. (1981) The epidemiology and clinical features of anaphylactic reactions in anaesthesia. *Anaesth. Intensive Care* **9**, 226–234.
- Guéant I. L., Mata E., Monin B., Moneret-Vautrin D. A., Kamel L., Nicolas J. P. and Laxenaire M. C. (1991) Evaluation of a new reactive solid phase for radioimmunoassay of serum specific IgE against muscle relaxant drugs. *Allergy* **46**, 452–458.
- Guéant I. L., Masson C. and Laxenaire M. C. (1992) Biological tests for diagnosing the IgE-mediated allergy to anesthetic drugs. *Monogr. Allergy* **30**, 94–107.
- Harle D. G., Baldo B. A., Coroneos N. J. and Fischer M. M. (1989) Anaphylaxis following administration of papaveretum. Case report: implication of IgE antibodies that react with morphine and codeine, and identification of an allergenic determinant. *Anesthesiology* **71**, 489–494.

- Harle D. G. and Baldo B. A. (1990) Identification of penicillin allergenic determinants that bind IgE antibodies in the sera of subjects with penicillin allergy. *Molec. Immun.* **27**, 1063–1071.
- Harle D. G., Baldo B. A. and Fischer M. M. (1990) The molecular basis of IgE antibody binding to thiopentone binding of IgE from thiopentone-allergic and non-allergic subjects. *Molec. Immun.* **27**, 853–858.
- Laxenaire M. C., Guéant I. L., Bermejo E., Mouton C. and Navez M. T. (1988) Anaphylactic shock due to propofol. *Lancet* **i**, 739–740.
- Laxenaire M. C., Mata E., Moneret-Vautrin D. A. and Guéant I. L. (1992) Life-threatening anaphylactoid reactions to propofol (Diprivan®). *Anesthesiology* **77**, 275–280.
- Varga J. M., Laude S. and Richards F. F. (1974) Immunoglobulins with multiple binding functions. II—The use of nylon-polyserine whisker disks in screening myeloma immunoglobulins for binding activity. *J. Immun.* **112**, 1565–1570.
- Varga J. M., Kalchschmid G., Klein G. F. and Fritsch P. (1991) Mechanism of allergic cross-reaction. I. Multispecific binding of ligands to a mouse monoclonal anti DNP IgE-antibody. *Molec. Immun.* **28**, 641–654.
- Varga J. M., Kalchschmid G., Klein G. F. and Fritsch P. (1991) Mechanism of allergic cross-reaction. II. Cross-stimulation by chemically unrelated ligands of rat basophilic leukemia cells sensitized with an anti DNP antibody. *Molec. Immun.* **28**, 655–659.
- Watkins J. (1987) Investigation of allergic and hypersensitivity reactions to anaesthetic agents. *Br. J. Anaesth.* **59**, 104–111.
- de Weck A. L. (1983) Penicillins and cephalosporins. In *Allergic Reactions to Drugs* (Edited by de Weck A. L. and Bundgaard H.), pp. 423–482. Springer, Berlin.