

Irrelevant IgE Binding to Cellulose Discs in RAST

A.W. van Toorenenbergen^a, R. Gerth van Wijk^b, P.H. Dieges^b, B. Leijnse^a

^a Department of Clinical Chemistry and ^b Department of Allergology, University Hospital Rotterdam-Dijkzigt, The Netherlands

Abstract. This study describes two sera that showed irrelevant IgE binding to all allergen-coated cellulose discs tested. This irrelevant IgE binding was not observed when allergen extracts were coupled to agarose beads. Such irrelevant IgE binding can be detected using human serum albumin-coated discs.

Introduction

Soon after the discovery of IgE as a separate class of immunoglobulins [1], radioimmunoassays (RIA) were developed for the estimation of total IgE and allergen-specific IgE in serum [2].

Results of specific IgE antibody measurements correlate positively with results of in vivo provocation tests like skin test, nasal and bronchial provocation test [3-5]. However, the radioallergosorbent test (RAST) is less sensitive than these in vivo assays: 10-25% of patients with positive challenge tests will have negative RAST results [5]. Technically, false positive RAST results can be observed in some food RASTs, when sera with high total IgE levels are tested [5]. Recently, Hamilton and Adkinson [6] described the occurrence of IgG anti-agarose and anti-cellulose antibodies and their interference in solid-phase immunoassays. In the present study we describe two sera in which IgE was present and became bound to the carrier (cellulose discs) of the covalently coupled allergen extracts. This irrelevant IgE binding was the cause of many false positive RAST results.

Patients and Methods

Patients

Patient A, a 26-year-old woman, had a history of atopic eczema since her infancy. At the age of 14, symptoms of perennial rhinitis and conjunctivitis started to occur, which were aggravated on expo-

sure to house dust, hay and on contact with cats, dogs and horses. Intradermal skin tests were positive with extracts of house dust mite, storage mites, grass pollen and animal danders (cat, dog, cow, horse). In order to exclude food allergy as a factor in her atopic eczema, serum from patient A was tested in various food RASTs. The results are shown in table I.

Patient B, a 30-year-old baker, developed asthma on inhalation of flour dust.

Patient C, a 44-year-old woman, had a history of perennial rhinitis and bronchial asthma which worsened in autumn. There were frequent attacks of facial edema. Intradermal skin tests were positive with house dust mite extract only. Because of the recurrent swellings of her face, serum from patient C was tested in various food RASTs. The results are shown in table II.

Experimental Procedure

Total IgE was determined by a competitive binding assay (IgE RIA, Pharmacia, Uppsala, Sweden), according to the manufacturer's instructions. Allergen-specific IgE was determined by RAST using agarose beads as allergen support [7] or, when indicated, using allergen discs from Pharmacia. ¹²⁵I-anti-IgE was obtained from Pharmacia. 5 mg human serum albumin (Behringwerke, Marburg, FRG) was coupled to 100 mg Sepharose 4B. Per test, 1 mg of the human serum albumin-agarose mixture was used. The RAST with agarose-coupled house dust mite, *Dactylis glomerata* pollen and egg-white extract was performed as described previously [8-10]. Penicilloyl agarose was prepared according to McGlashan and Lichtenstein [11]. Most RASTs with agarose-coupled food extracts were kindly performed by V. Koshte, as described previously [12].

Histamine Release from Whole Blood

Incubation of heparin-anticoagulated blood with allergen extracts and assay of histamine released into the supernatant were performed according to Siraganian and Hook [13]. House dust mite (*Dermatophagoides pteronyssinus*) and cat dander allergen were obtained from Pharmacia-Diephuis, Groningen, The Netherlands. Barley and wheat flour were obtained from a local grocery. Egg-

Table I. Measurement of allergen-specific IgE in serum of patient A by various food RASTs

Allergen	Percentage of ^{125}I -anti-IgE binding	
	disc	agarose
Egg-white	34	0
Cod	34	0
Rye	35	1
Wheat	35	1
Barley	35	1
Pea	27	1
Peanut	35	2
Hazelnut	31	0
Almond	26	1
Buckwheat	35	2
Penicillin G	30	0
Human serum albumin	29	0

50 μl serum A was tested in the RAST with allergen discs and agarose-coupled allergen extracts. The total IgE level of serum A was 3,000 IU/ml.

white and peanut allergen were prepared as described previously [10, 14]. For use in the histamine release test (HRT), 20 mg of these allergens were extracted for 1 h in PACM buffer (25 mM/l piperazine-N,N'-bis(2-ethanesulphonic acid), 110 mM/l NaCl, 5 mM/l KCl, 1 mM/l CaCl_2 , 0.5 mM/l MgCl_2 , 0.03% human serum albumin, pH 7.4). After centrifugation, the supernatant was used in the HRT.

Results

When serum from patient A was analyzed in the RAST, a very high IgE binding was observed with all cellulose discs tested (table I). Serum A, heated for 2 h at 56°C, had lost most of its IgE binding to egg-white and peanut discs (fig. 1). Preincubation of serum A with a 10% peanut extract did not suppress the IgE binding to a peanut disc, whereas the 10% peanut extract was fully able to inhibit the specific IgE binding to peanut discs, when serum was used from a patient with a known allergy to peanuts (fig. 2).

The possibility was considered that the high IgE binding to all discs tested was caused by recognition by IgE from patient A of a cross-reactive carbohydrate determinant, common to grass pollen and many foods [12]. However, when serum A was incubated with agarose-coupled allergen extracts, all food RASTs were negative (table I). Whereas serum A showed a high IgE binding to penicilloyl G discs, no

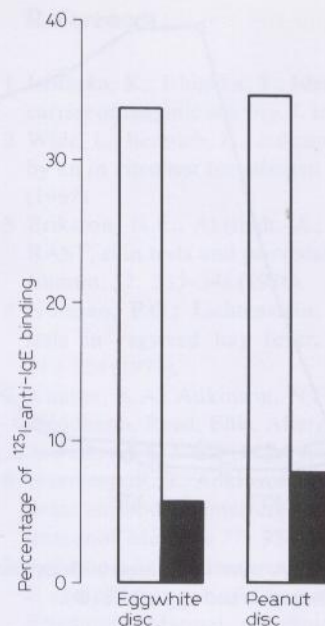


Fig. 1. 50 μl serum A was tested for IgE against egg-white and peanut. $\square$ = Unheated serum A; $\blacksquare$ = serum A, heated for 2 h at 56°C.

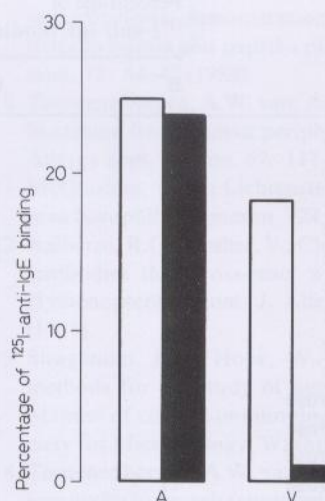


Fig. 2. 25 μl serum A and 50 μl serum V were incubated overnight with 100 μl of a 10% peanut extract. The residual IgE binding ($\blacksquare$) to peanut discs was compared with the uninhibited IgE binding ($\square$). Serum V was from a patient with an allergy to peanuts.

IgE binding was observed on incubation with penicilloyl G Sepharose (table I). Similarly, a high IgE binding was found on incubation of serum A with albumin discs, that are part of the Phadebas penicilloyl G test system; no IgE binding was observed on incubation of serum A with Sepharose-coupled human serum albumin (table I).

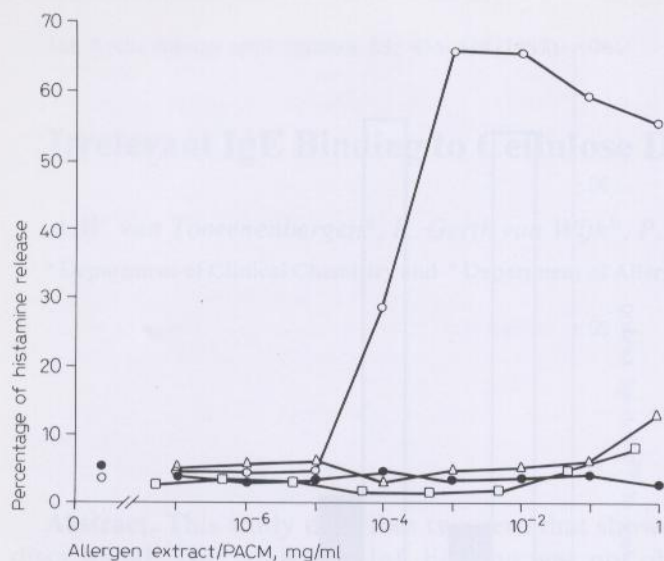


Fig. 3. Leukocytes from patient A were incubated with extracts of cat dander (○), peanut (△), wheat (□) and egg-white (●).

Table II. Measurement of allergen-specific IgE in serum of patients B and C using disc- and agarose-coupled allergen extracts

Allergen		Percentage of ¹²⁵ I-anti-IgE binding	
		B	C
Egg-white	disc		7
Milk	disc		6
Wheat	disc	9	9
Rye	disc	4	9
Barley	disc	8	10
Oat	disc	1	9
Pea	disc		7
Peanut	disc		6
Human serum albumin	disc		8
Human serum albumin	agarose	0	0
House dust mite	agarose	16	31
Grass pollen	agarose	0	1
Wheat	agarose	11	1

The total serum IgE levels of patients B and C were 80 and 1,150 IU/ml, respectively.

Another case of elevated IgE binding to all cellulose discs tested was seen with serum C. Table II shows that serum C was found to contain significant amounts of IgE against wheat and barley when cellulose discs were used in the RAST. However, agarose-coupled wheat extract showed no IgE binding to this allergen (table II). Furthermore, substantial IgE binding to albumin discs was observed with serum C,

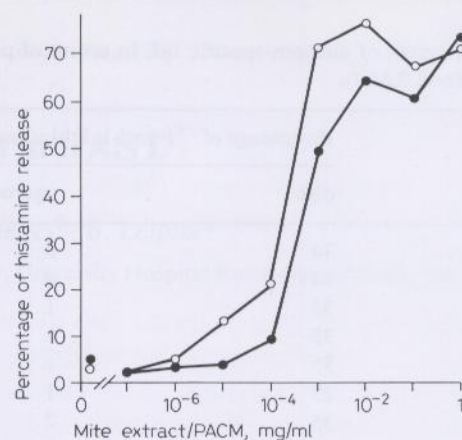


Fig. 4. Leukocytes from patients B (●) and C (○) were incubated with house dust mite extract.

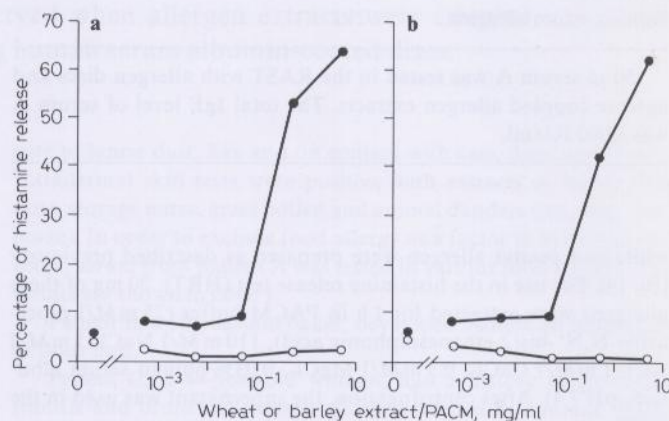


Fig. 5. Leukocytes from patients B (●) and C (○) were incubated with wheat (a) and barley (b) extracts.

whereas the IgE-binding to agarose-coupled albumin was negative (table II).

Leukocyte Histamine Release

Leukocytes from patient A, on incubation with extracts of cat dander, peanut, wheat and egg-white, released histamine with cat dander only (fig. 3), despite a high IgE binding to peanut, wheat, and egg-white discs (table I). Whereas both patients B and C were positive in the HRT on challenge with house dust mite (fig. 4), only leukocytes from patient B released histamine on incubation with extracts of wheat and barley (fig. 5).

Discussion

In the RAST, nonspecific IgE binding is directly related to the total IgE level of the serum tested [15].

Certain food allergens, like pea, have more non specific IgE binding than other allergens [5]. However, with a total serum IgE level of 2,000–3,000 IU/ml, this nonspecific IgE binding is less than 2–3% of ^{125}I -anti-IgE binding [16]. The high irrelevant IgE binding to various food discs, that was seen with the two sera described above, was probably not caused by IgE binding to the disc-coupled allergen extracts, because coupling of the same allergen to agarose beads did not result in IgE binding. The fact that control discs, to which human serum albumin was coupled, showed as much IgE binding as the allergen discs on incubation with sera A and C strongly suggests that a yet unknown structure on the cellulose discs was responsible for the irrelevant IgE binding. The conclusion that IgE was not bound to the food allergens but to the discs themselves is supported by the negative results of the HRT after challenge with food extracts (fig. 3, 5).

Figure 3 shows that leukocytes from patient A released a substantial amount of histamine on incubation with cat dander extract. This demonstrates that the lack of histamine release after challenge with peanut, wheat or egg-white extract was not caused by a poor releasability of the leukocytes from patient A. Similarly, figure 4 shows that the lack of histamine release from leukocytes of patient C on challenge with wheat and barley (fig. 5) was not caused by a poor releasability of her leukocytes. We have shown before that the peanut and egg-white preparations used are able to induce histamine release from leukocytes of patients with an IgE-mediated allergy towards these two allergens [10, 14]. Figure 5 shows that the wheat and barley extracts used had the capacity to stimulate leukocytes from correspondingly allergic patients to the release of histamine. In summary, this paper demonstrates the existence of irrelevant IgE binding to cellulose discs with sera from two patients. In cases where all RASTs with cellulose discs are positive, a test with albumin discs should be performed when the clinical relevance of some of the RAST results is questionable.

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Correspondence to: Dr. A.W. van Toorenenbergen, Department of Clinical Chemistry, University Hospital Rotterdam-Dijkzigt, Molewaterplein 40, NL-3015 GD Rotterdam (The Netherlands)