

Quantitative determination of total and specific human IgE with the use of monoclonal antibodies

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We have used two monoclonal antibodies (BS17 and Le27) that recognize two different epitopes of the constant region of human IgE in order to determine the total and specific IgE contained in human serum. Both types of assay are based on classic PRIST and RAST procedures and involve one or two monoclonal antibodies labeled with ^{125}I iodine. The performance of these two assays were compared systematically with those obtained with a commercially available polyclonal tracer. We first investigated the ^{125}I -labeling conditions for monoclonal antibodies. In our hands the best results were obtained by use of a tracer of specific radioactivity close to $10\ \mu\text{Ci}/\mu\text{g}$. We have been able to demonstrate that as a consequence of its limited affinity, the radioactive tracer is always incompletely bound to the solid-phase IgE. Nonetheless, the sensitivity of the assay is comparable to that obtained with the polyclonal antibody, since $<0.5\ \text{IU/ml}$ can be measured by the PRIST procedure. In contrast, the dilution curves obtained in the RAST with the monoclonal antibodies are very different from those observed with the polyclonal tracer. In fact, these curves are strictly parallel to the dilution curve and to the standard curve derived from the PRIST method, thus indicating that the monoclonal antibodies recognize all IgE equally well regardless of the way in which they are bound to the solid phase. On the basis of this observation, we propose a quantitative assay of specific IgE with the PRIST standard curve as reference. Our results demonstrate that this approach is indeed possible if high-capacity, solid-phase as well as long incubation times are used. (J ALLERGY CLIN IMMUNOL 77:808-22, 1986.)

Since the initial finding by Bennich et al.^{1,2} that IgE plays an essential role in mediating atopic allergy, many immunoassays for the determination of both total and specific IgE in serum have been proposed. Different procedures have been developed for the determination of total IgE. A first approach was based on competition between ^{125}I -iodinated myeloma IgE and unlabeled IgE for a limited concentration of anti-IgE antibodies. Two different separation methods involving a solid phase bearing a first antibody³⁻⁵ or second antibody immunoprecipitation⁶ have been proposed.

In a second approach, a two-step sandwich method

Abbreviations used

NSB: Nonspecific binding
BSA: Bovine serum albumin

(two-site immunoradiometric method) was used. In the first step of this method, a first anti-IgE antibody immobilized on a solid phase is reacted with sample or standard IgE. The amount of IgE bound to the solid phase is quantified in a second step with a second labeled antibody. As originally described, this method involved paper discs as the solid matrix and ^{125}I -iodinated antibodies⁷ and was named PRIST. A variety of solid-phase as well as enzymatic tracers have been developed since that time.⁸⁻¹¹

All these assays are relative analytic methods based on an adequate use of myeloma IgE as a standard. They are usually sensitive enough to allow the measurement of $<2\ \text{IU/ml}$ of IgE.

In 1967 an assay for the determination of specific IgE was proposed by Wide et al.¹² This method, named RAST, is based on the following principles: in

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a first step, an allergen immobilized on a solid matrix is reacted with the serum being studied, and in a second step, any IgE specifically bound to the solid phase is revealed by labeled anti-IgE antibodies.

Originally dextran (Sephadex) and ^{125}I -labeled anti-IgE were used.¹²

Subsequent developments involved the use of many different solid-phase matrices, including paper discs, microcrystalline cellulose or plastic surfaces, as well as different anti-IgE antibodies labeled with various enzymes for staining.^{8, 9, 13-17} Commercial kits for the determination of specific IgE for many allergens are now available (Pharmacia, Uppsala, Sweden; Allergenetics, Inc., Mountain View, Calif.; Abbott Laboratories, Irving, Texas).

The validity of the quantitative measurements allowed by these methods has been widely discussed in the literature.^{15, 16, 18-23} It is now currently admitted that these methods provide only qualitative or semiquantitative data. In fact, the measurement of absolute amounts of specific IgE is subject to various difficulties: (1) During preparation of allergen extracts and during their immobilization on a solid phase, allergenic determinants may be lost. (2) Allergen-specific IgE contained in a serum may exist in different subpopulations with varying affinities for the allergen. Thus, depending on their affinity and the quantity of allergen immobilized, part of the IgE may not be bound to the solid phase, thus leading to an unpredictable underestimation of specific IgE. (3) The presence of antibodies from another class of immunoglobulin (e.g., IgG) specific for the immobilized allergen may interfere with the assay by competing with IgE. Such interference has been clearly demonstrated for sera of patients under therapy.²⁴ (4) Quantification of solid-phase bound IgE is problematic because, with few exceptions,²⁵ there are no appropriate standards available. In addition, the assay may be influenced by an erroneous estimation of NSB caused by the presence of an excess of nonspecific IgE.²²

Different modified procedures have been proposed to improve the described assays. Some of these involve an elution of specific IgE from the solid phase^{26, 27} before their quantitation. Guesdon and Avrameas⁹ have described an indirect method based on the measurement of total IgE after adsorption of specific IgE on the immobilized allergen. Zeiss et al.,^{24, 28, 29} with solid-phase bound anti-IgE and ^{125}I -labeled allergen, have developed a completely different procedure (polystyrene tubes radioimmunoassay), which is, in theory, unaffected by the presence of large amounts of blocking antibodies. Generalization of this method, however, is limited by poor availability of well-characterized labeled allergen.

We have demonstrated earlier that the two monoclonal antibodies, BS17 and Le27, recognizing, respectively, the De_1 and De_2 domains of human IgE, may be used in an enzyme immunoassay to detect low levels of human IgE in supernatants from cultures of mononuclear cells.³⁰ Both antibodies recognized different myeloma-derived IgE proteins as well as normal serum IgE. It has also been demonstrated that the highest sensitivity in a sandwich radioimmunoassay was obtained when Le27 was used as the solid-phase antibody and BS17 as the developing antibody.³¹ We intend to demonstrate here that these two monoclonal antibodies may be used for the determination of total and specific IgE with classic PRIST and RAST procedures. In the PRIST, a first antibody (Le27) is chemically coupled to a solid phase, and quantitation of solid-phase bound IgE is obtained with ^{125}I -labeled BS17. In the RAST, the presence of IgE bound to an immobilized allergen is revealed by one or both iodinated monoclonal antibodies. The performances of these assays are compared with those obtained with polyclonal antibodies. On the basis of the experimental results, we discuss the possibility of a quantitative determination of specific IgE.

MATERIAL AND METHODS

IgE

Two standard IgE solutions were used: (1) standard IgE from the PRIST kit of Pharmacia and (2) IgE from the serum of a patient having a high level of uncharacterized IgE (6400 IU/ml), which was standardized by use of the PRIST assay (Pharmacia). Pure human IgE used for the determination of affinity constants of BS17 and Le27 was purified from myeloma PS.

Preparation of monoclonal antibodies

Preparation of monoclonal antihuman IgE, BS17 (specific for the De_1 region) and Le27 (specific for the De_2 region), are described in detail elsewhere.³⁰ The antibodies used throughout this study were purified from ascitic fluid with ammonium sulfate precipitation (45%) and subsequent elution from DEAE Sephacel (10^{-2} mol/L Tris buffer, pH 8) with a linear NaCl gradient.³²

Radioiodination

Monoclonal antibodies (BS17 and Le27) and human IgE were labeled with ^{125}I by the chloramine-T method described by Hunter and Greenwood.³³ Four microliters of ^{125}I solution (CEA, Saclay, France) and 4 μL of chloramine T (3 mg/ml) (Sigma Chemical Co., St. Louis, Mo.) were added to 50 μL of antibody solution (variable concentration) in phosphate buffer 0.1 mol/L, pH 7.4. After a 1-minute incubation, 4 μL of sodium metabisulfite (6 mg/ml) (Sigma Chemical Co.) and 20 μL of sodium iodide, 1 mol/L, were added.

The bulk of unreacted iodine was separated from the

TABLE I. Binding of IgE to Le27-coated discs or *D. glomerata*-coated discs during successive incubations

Duration	First incubation period (variable duration)		Second incubation period (24 hours)	
	Le27-coated discs	<i>D. glomerata</i> -coated discs	Le27-coated discs	<i>D. glomerata</i> -coated discs
1 hr	21	20	91	65
2 hr	32	24	89	68
3 hr	43	33	91	71
5 hr	59	38	92	74
7 hr	69	44	94	77
24 hr	90	67	96	84

Time dependence of the reaction. Three successive incubations of a serum pool dilution were performed with either Le27- or *D. glomerata*-coated discs (see Methods). The duration of the first incubation period was variable, whereas the second and third incubations were of 24 hours. Bound IgE was detected with iodinated BS17 (12 Ci/gm, 5.10^4 cpm/disc, 18 hours). Results are expressed in terms of a cumulative percentage, taking the total amount of radioactivity bound during the three successive incubation periods as reference.

TABLE II. Increase in IgE binding as a function of the number of allergen-coated discs used

No. of house dust-coated discs	Bound radioactivity (cpm)
1	2720
2	3890
3	4300
4	4800
5	5580

A constant amount of a serum pool from atopic subjects was incubated with an increasing number of house dust-coated discs for 24 hours. Bound IgE was detected as described in Table I. House dust-coated discs were selected for this experiment precisely because they appeared especially ineffective in immobilizing specific IgE.

proteins by gel filtration in a Sephadex G-25 column, equilibrated in a 0.1 mol/L of phosphate buffer, pH 7.4, containing 0.4 mol/L NaCl, 0.1% BSA, 0.03% EDTA, and 0.01% sodium azide. In order to obtain tracers with various specific radioactivities, different concentrations of antibody were used during the iodination reaction. Correspondence between specific radioactivity and protein concentration was:

BS17 6 Ci/gm, 2 mg/ml Le27 16 Ci/gm, 0.7 mg/ml
BS17 12 Ci/gm, 1 mg/ml Le27 50 Ci/gm, 0.2 mg/ml
BS17 47 Ci/gm, 0.2 mg/ml IgE 0.7 Ci/gm, 10 mg/ml

When chromatographed on a 1.5×90 cm biogel A 0.5 m column (Bio-Rad, Richmond, Calif.), the iodinated material was eluted as a single homogeneous peak. Furthermore, immunoprecipitation experiments performed on fresh preparations of labeled antibodies revealed that >95% of the radioactivity could be immunoprecipitated by use of

appropriate dilution of a rabbit antimouse IgG antiserum (data not presented). Both these results provided a good control for the purity of the antibody preparation used for radiolabeling.

Determination of affinity constants

The values of the affinity constants for BS17 and Le27 were determined with a conventional Scatchard analysis.³⁴ Briefly, 100 μ l of serial dilutions of iodinated IgE (PS) (10^3 to 5×10^6 cpm) were incubated with 100 μ l of anti-IgE antibodies (100 ng/ml) for 18 hours at room temperature; IgE/anti-IgE complexes were precipitated by rabbit antimouse IgG antibodies as described elsewhere.³⁵ After centrifugation, the radioactivity of the pellet was measured in a scintillation counter (LKB, Turku, Finland), and the results were plotted in terms of $B/F = f(B)$, where B and F represent the bound and free fractions, respectively. Dissociation constants were determined from the reciprocal of the slope of the curves. Values of 1×10^{-9} mol/L and 2×10^{-9} mol/L were found for BS17 and Le27, respectively.

Allergen extracts

All the allergen extracts were prepared by the Laboratoire des Stallerènes, Fresnes, France. The crude allergenic materials were extracted in aqueous solution. After filtration the extract was purified by three methods depending on the nature of material: (1) dialysis or ultrafiltration, (2) gel filtration (10,000 to 80,000 daltons), and (3) ammonium sulfate or acetone precipitation. In the first two cases the purified solution was sterilized by filtration and lyophilized, whereas in the third case the precipitate was dried or lyophilized. As an example, the preparation of cat-allergen extract is described by Guerin.³⁶

Solid-phase preparation

Insolubilized allergens were prepared by coupling allergen extracts to paper discs (reference 589-3, Schleicher &

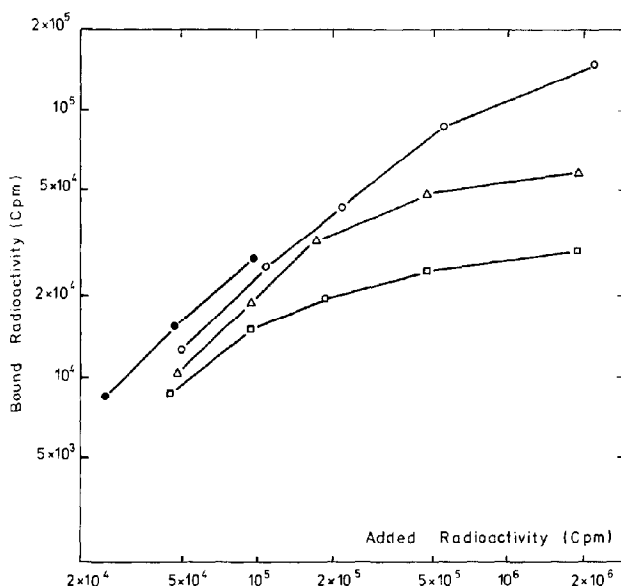


FIG. 1. Effect of variation in the quantity of ^{125}I -anti-IgE introduced in the second step of the RAST procedure. During the first step of the RAST, *D. glomerata*-coated discs were reacted (3 hours at room temperature) with a $\frac{1}{2}$ dilution of a pool of serum. During the second step, serial dilutions of different tracers were incubated (18 hours at room temperature) with the discs. Tracers $\circ$ — $\circ$, BS17, 47 Ci/gm ($2.4\% < \text{NSB} < 2.9\%$); $\triangle$ — $\triangle$, 12 Ci/gm ($0.8\% < \text{NSB} < 1.1\%$); $\square$ — $\square$, BS17, 6 Ci/gm ($0.6\% < \text{NSB} < 0.8\%$); and $\bullet$ — $\bullet$, tracer from Pharmacia ($0.2\% < \text{NSB} < 1.8\%$).

Schuell, Dassel, W. Germany) activated with CNBr according to the technique of Brighton et al.³⁷ Le27-coupled discs were prepared by use of 5 μg of the anti-IgE antibody per disc.

Allergens and Le27 were also covalently bound to CNBr-activated Sepharose 4B (Pharmacia) according to the technique prescribed by the manufacturer. For Le27, 1.5 mg of the antibody was coupled with 1 gm of gel; for allergens, from 0.7 to 3 mg of proteins was coupled with 1 gm of gel.

PRIST and RAST assays

Paper disc assay. Fifty microliters of serum (diluted in phosphate buffer containing 1% BSA if it were needed) was incubated (3 hours at room temperature, unless it was otherwise stated) with discs coated with allergen (RAST) or Le27 (PRIST). At the end of this first incubation period, the discs were washed with a 0.1 mol/L of phosphate buffer containing 1% Tween 20. The discs were then incubated for 18 hours at room temperature with labeled anti-IgE (BS17, Le27, or a 1/1 mixture of the two monoclonal antibodies). After a second washing step, the radioactivity in the discs was counted in a scintillation counter (LKB).

Gel assay. Fifty microliters of serum (diluted in phosphate buffer containing 1% BSA if it were needed) was

added to 100 μl of a gel suspension containing either allergen or Le27 covalently coupled to Sepharose 4B. The mixture was then incubated for 24 hours at room temperature. At the end of this first incubation period, the gel was washed (four times) by adding 1.5 ml of washing buffer (see above), spun at low speed (5 minutes, $2000 \times g$), and the supernatant was decanted. The gel was then incubated for 18 hours at room temperature with labeled anti-IgE. After a second washing procedure, the radioactivity in the final pellet was counted as for the discs.

Reincubation of labeled anti-IgE contained in the supernatant

In order to test the reactivity of the labeled anti-IgE contained in the supernatant of the second step of a RAST assay (Fig. 2), we proceeded as follows: At the end of the anti-IgE incubation period and before the discs were washed, 50 μl of phosphate buffer was added to the supernatant above the discs; 50 μl of this mixture was transferred onto fresh discs that had previously been incubated with the same serum in the same conditions. After a new incubation of 18 hours at room temperature, this second set of discs was washed and counted as previously described. The results of these two series of measurements were plotted as a function of the total radioactivity added (Fig. 2).

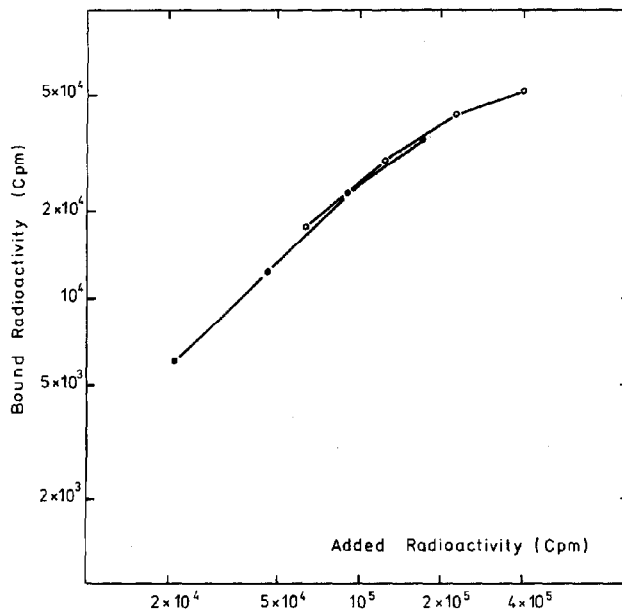


FIG. 2. Reincubation of iodinated BS17 from supernatants of a first binding equilibrium with fresh discs. In a first RAST experiment, different quantities of iodinated BS17 (12 Ci/gm) were incubated as described for Fig. 1. In a second experiment supernatants of this first binding equilibrium were reacted with fresh discs (for details, see Methods). Results are expressed as a function of added radioactivity. ○—○, Bound radioactivity during the first RAST; and ●—●, bound radioactivity during the second RAST (1.4% < NSB < 2.6%).

Reincubation of serum IgE contained in the supernatant

In order to test the reactivity of the IgE contained in the supernatant of the first step of a PRIST or a RAST assay (Tables I and II), we proceeded as follows: At the end of a first serum incubation (variable duration at room temperature) and before the discs were washed, all the supernatant was removed and incubated with a fresh, identical disc for 24 hours at room temperature. This procedure could be repeated once (Table I). The amount of IgE bound to the solid phase during these two or three successive incubations was quantified with labeled BS17, as in a standard test (18 hours at room temperature).

Unless it is otherwise stated, all the measurements performed during this study were made in duplicate. All the results presented here are those from representative single experiments.

In each experiment NSB of the radioactive tracer was evaluated by incubating discs or gels with a 5% solution of human serum albumin. This NSB was systematically subtracted from other experimental measurements. NSB values expressed as a percentage of total radioactivity are listed in the legends of the corresponding figures (data in parentheses).

RESULTS

Radiolabeling of BS17 and Le27

Three different tracers of specific radioactivities 6, 12, and 47, Ci/gm, have been obtained with BS17 (see Methods). The properties of these different preparations have been tested in the RAST procedure (allergen, *Dactylis glomerata*) by use of a constant dilution of reaginic serum and different concentrations of labeled monoclonal antibody. A comparison was made under the same conditions with iodinated polyclonal anti-IgE antibodies obtained from Pharmacia. The corresponding data are presented in Fig. 1.

The results obtained with monoclonal antibodies demonstrated that with increasing concentrations of tracer, the radioactivity bound to the disc also increased until a plateau proportional to the specific radioactivity was reached. At low concentrations of tracer, only 30% to 40% of the added radioactivity was bound, although there were still free IgE binding sites available as suggested by the fact that at higher tracer concentrations more radioactivity was bound to the discs. The incomplete binding at lower concen-

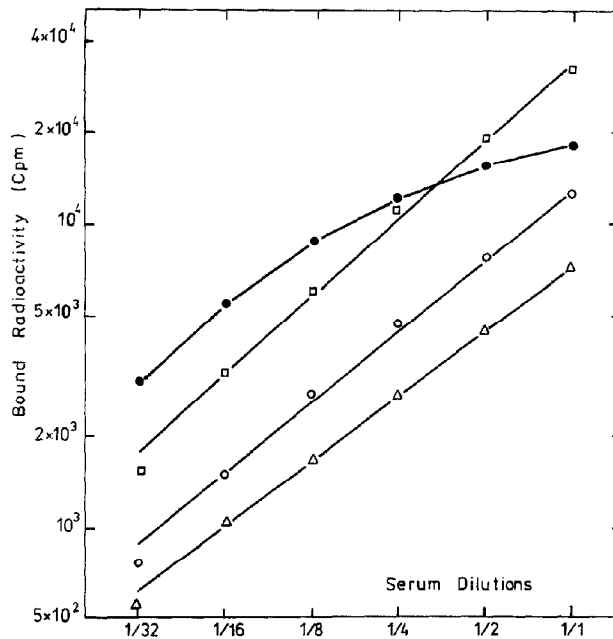


FIG. 3. Comparison of RAST dilution curves obtained with monoclonal and polyclonal tracers. $\circ$ — $\circ$, BS17 (12 Ci/gm, 5×10^4 cpm) (NSB = 1.4%); $\square$ — $\square$, BS17 (12 Ci/gm, 1.9×10^5 cpm) (NSB = 1%); $\triangle$ — $\triangle$, Le27 (16 Ci/gm, 5×10^4 cpm) (NSB = 3.7%); and $\bullet$ — $\bullet$, tracer from Pharmacia (NSB = 2.2%). *D. glomerata* was used as disc-immobilized allergen. The durations of the first and second steps of the assay were 3 hours and 18 hours, respectively.

trations of BS17 could be due to heterogeneity of the iodinated preparation linked either to the presence of antibodies nonspecific for IgE or to a partial inactivation of BS17 during the iodination procedure as previously suggested by other authors.¹⁰ Alternatively, partial binding of tracer might be related to a limited affinity of BS17 for IgE. In order to decide between these two possibilities, supernatants from a first binding equilibrium were reincubated under the same conditions with fresh discs (see Methods). The corresponding results (Fig. 2) clearly demonstrated that the tracer in supernatants has binding properties identical to those of the whole preparation. This result argued strongly in favor of the second hypothesis. Similar experiments were performed with iodinated Le27 and led to the same conclusions (results not presented), as was expected, since the two monoclonal antibodies have similar affinities for IgE (see Methods). This limited binding reactivity, observed with the monoclonal antibodies, probably also occurs in the case of polyclonal antibodies, which behaved similarly to the monoclonal antibodies (Fig. 1). However, it appears

that the antiserum from Pharmacia possesses a greater affinity, since a higher percentage of the tracer can be bound to the solid phase. Although optimal results were obtained with fresh preparations of iodinated BS17 of specific radioactivity, 50 Ci/gm, we preferred to use a tracer with a specific radioactivity close to 10 Ci/gm, because antibody preparations of greater specific radioactivity were only stable during a period of 2 or 3 weeks (data not presented). Similar findings were obtained for the Le27 antibody.

Determination of specific IgE

Iodinated BS17 (12 Ci/gm) and Le27 (16 Ci/gm) were compared with the commercially available polyclonal anti-IgE from Pharmacia in a conventional RAST procedure with *D. glomerata* antigen immobilized on a paper disc as the solid phase. In this experiment, allergen-coated discs were incubated with various dilutions of pooled sera from pollen-allergic patients (total IgE = 360 IU/ml). The results obtained with the four different tracers are presented in Fig. 3.

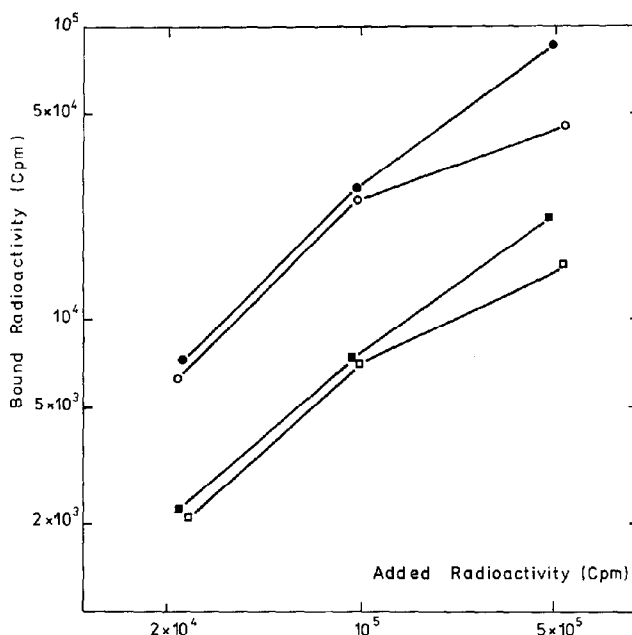


FIG. 4. Additive binding of monoclonal antibodies BS17 and Le27 in the RAST procedure. RAST experiments (*D. glomerata*) were performed with either a mixture (1/1) of iodinated BS17 (12 Ci/gm) and Le27 (16 Ci/gm) or iodinated BS17 alone. The results obtained for two dilutions of a serum pool are expressed as a function of the total radioactivity added. $\circ$ — $\circ$, BS17 (12 Ci/gm), serum dilution $1/2$; $\square$ — $\square$, BS17 (12 Ci/gm), serum dilution $1/4$ ($1.8\% < \text{NSB} < 2\%$); $\bullet$ — $\bullet$, mixture, serum dilution $1/2$; and $\blacksquare$ — $\blacksquare$, mixture, serum dilution $1/4$ ($2.7\% < \text{NSB} < 2.8\%$).

As already observed (Fig. 1), the sensitivity of the assay was increased when greater concentrations of labeled BS17 were used. In contrast to the dilution curve obtained with antibodies from Pharmacia, the dilution curves obtained with BS17 or Le27 were linearly related to the concentration of specific IgE in serum. Although it is not possible to explain precisely the shape of the curves produced by polyclonal antibodies, we may assume that nonlinear curve shape might be due to a possible heterogeneity of the polyclonal tracer. In contrast, the tracer from Pharmacia appears more effective in "detecting" low concentrations of specific IgE.

In order to improve the detection of specific IgE, we used a 1/1 mixture of the two labeled monoclonal antibodies. The results in Fig. 4 illustrate that whatever the quantity of immobilized IgE, an additional binding is observed only when tracer quantities $>10^5$ cpm are used. This result is in agreement with our previous findings that antibodies have a similar and limited affinity for IgE. Thus, under these conditions, additive binding of the two different antibodies can

only be observed when saturation conditions are achieved.

Determination of total IgE and quantification of specific IgE

In order to evaluate the performance of iodinated BS17 (12 Ci/gm) for the determination of total IgE in a classic PRIST procedure, a standard curve with Le27-coated discs and standard myeloma IgE was established. The results were compared with those obtained with the commercially available PRIST assay (Pharmacia), which was performed under the conditions prescribed by the manufacturer. The results in Fig. 5 demonstrate that in these conditions both assays provided very similar standard curves. For a given concentration of IgE the Pharmacia system produced a slightly larger amount of bound radioactivity (≈ 100 cpm), but a linear relationship was observed over a wider range with the monoclonal tandem system. As previously observed for the RAST (Fig. 3), increased binding of BS17 was obtained when greater quantities of tracer were used.

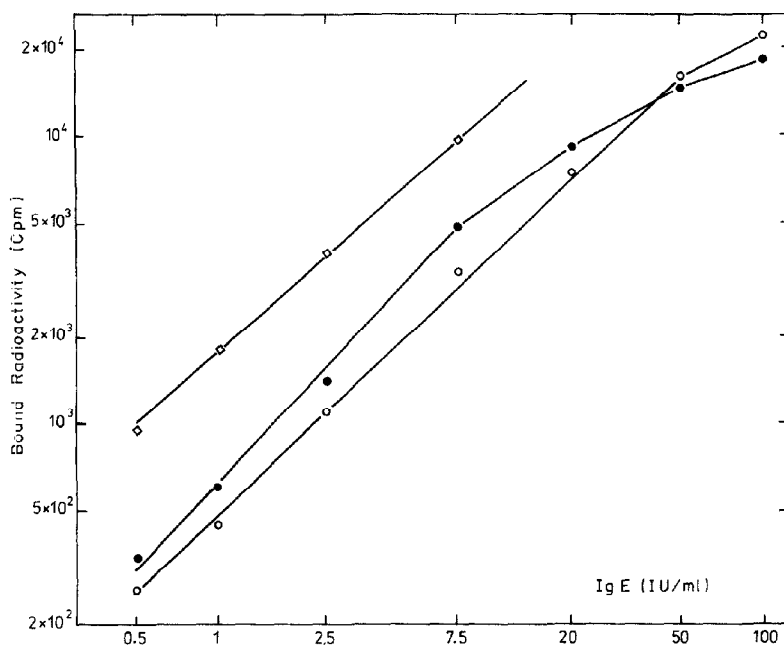


FIG. 5. PRIST standard curves obtained with iodinated BS17 or polyclonal tracer. All the curves were established with standard IgE from Pharmacia and 3-hour plus 18-hour incubation periods. Experiments involving polyclonal tracer from Pharmacia were performed with anti-IgE-coated discs from Pharmacia; those involving iodinated BS17 were performed with Le27-coated discs (see Methods). ●—●, Polyclonal tracer from Pharmacia ($\approx 5.10^4$ cpm/disc) (NSB = 0.7%); ○—○, BS17 (12 Ci/gm, 5.10^4 cpm/disc) (NSB = 0.9%); and □—□, BS17 (12 Ci/gm, 2×10^6 cpm/disc) (NSB = 0.7%).

When iodinated polyclonal antibody was used together with Le27-coated discs or, conversely, when BS17 tracer was used with discs from Pharmacia, the amount of bound radioactivity dropped very significantly (results not presented). These results are probably linked to the fact that both types of antibodies recognize shared determinants of IgE.

Despite the slight differences in the standard curves presented in Fig. 5, the two assay systems provided similar results when applied to the determination of total IgE in serum. Fig. 6 presents the results of a correlation study for the two PRISTs with 52 randomly selected sera from normal and atopic patients. A good correlation between both measurements was obtained.

When monoclonal antibodies are used, the dilution curves for individual sera are all strictly parallel to the standard curve in order that identical results were obtained independently of the dilution used in the assay (Fig. 7). More interesting was the finding that the serum dilution curves for the measurement of spe-

cific or total IgE were parallel. This is presented in Fig. 8A in which PRIST and RAST dilution curves from the same serum were compared with a PRIST standard curve. However, with PRIST and RAST kits (Pharmacia) in the conditions prescribed by the manufacturer, parallelism between the RAST dilution curve and the PRIST standard curve was not observed (Fig. 8B).

The results obtained by the monoclonal system indicated that IgE was recognized by BS17 equally well, independently of how they were immobilized on the disc, thus providing the basis for a quantitative determination of specific IgE, with the following assumptions: (1) during the first step of both the PRIST and the RAST there is a *total* immobilization of IgE on the solid phase, (2) BS17 and Le27 monoclonal antibodies recognize a determinant that is present in all IgE molecules, and (3) all the IgE molecules, whether immobilized by IgE/anti-IgE or by allergen/antiallergen interactions, have the same capacity to bind iodinated BS17 antibody during the second

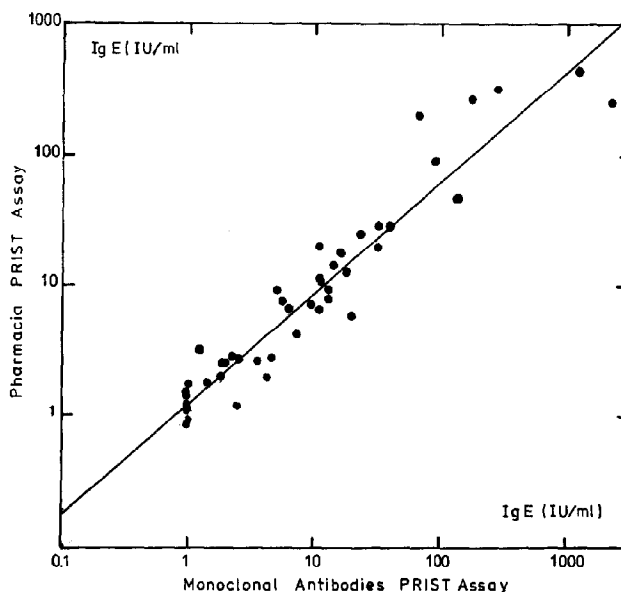


FIG. 6. Correlation between PRIST measurements made with polyclonal (Pharmacia) or monoclonal-tandem (BS17 plus Le27) assays. The total IgE content of 52 randomly selected sera from normal and atopic subjects were measured with either polyclonal or monoclonal tracers. Each type of assay was performed in the conditions described in Fig. 5 (5×10^4 cpm/disc). Linear regression analysis of the results elicited the following equation:

$$Y = 0.84 X + 0.282 \quad (r = 0.9)$$

where X and Y represent monoclonal and polyclonal measurements, respectively.

step of the assay. Under such conditions it would be possible to compare the amount of specific IgE contained in a given serum, and totally immobilized on the allergen disc, with the standard curve of the PRIST (assuming the second steps of both assays have been performed under identical conditions). Fulfillment of the first condition implies that Le27 and allergens are present in excess on the solid phase and that the first incubation is performed under conditions (temperature and duration) allowing a complete immobilization of total or specific IgE.

In order to test the completeness of the reaction between the solid phase and IgE, supernatants of a first incubation were reincubated for 24 hours with fresh discs. At the end of this second incubation period, the process was repeated once. The amount of IgE bound during each step was quantified with the use of iodinated BS17, as in a classic test. The results obtained with a pool of sera and discs coated with Le27 or *D. glomerata* are presented in Table I as a function of the duration of the first incubation. It is clear that immobilization of both total and specific

IgE is incomplete whatever the duration of the first incubation period. Even with a 24-hour incubation, not more than 90% (disc Le27) and 67% (*D. glomerata*) of the total radioactivity bound in three successive 24-hour incubations is bound during the first incubation period. Under the conditions conventionally used for the PRIST and the RAST (3-hour incubation), the corresponding values are 56% (disc Le27) and 33% (*D. glomerata*). As a consequence, a quantitative determination of specific IgE made under these conditions, and based on the use of the PRIST standard curve, would lead to an underestimation of the specific IgE content of the corresponding pool since Le27-coated discs appear more effective in immobilizing IgE than *Dactylis*-coated discs. When identical experiments were performed with discs coated with other allergens and with different pools of serum or individual sera, we observed significant variations in the percentage of radioactivity bound during the first 24-hour incubation period; the obtained values varied from 40% to 95%.

Thus, it appears that it is not possible to obtain

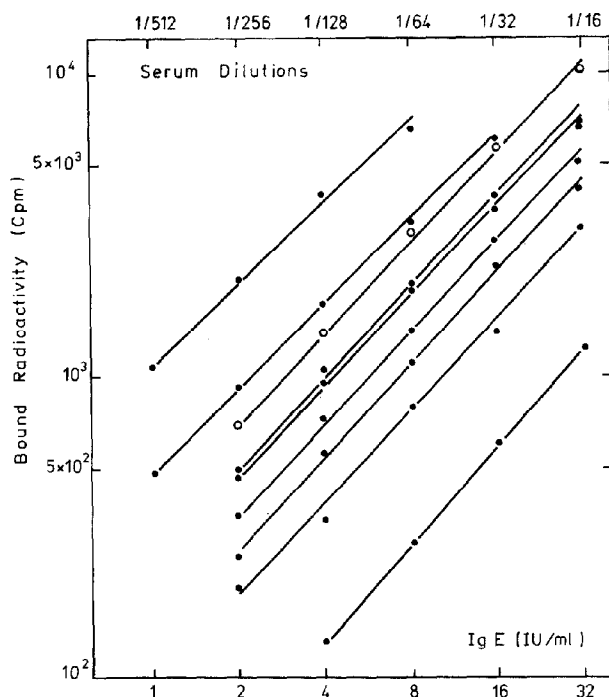


FIG. 7. Parallelism between PRIST standard curves and PRIST dilution curves with the monoclonal tandem assay. All PRIST measurements were made with the condition described in Fig. 5. ●—●, Individual sera; and ○—○, standard curve.

complete immobilization of specific IgE under the conditions we used. As this poor reactivity could be due to presence of insufficient amounts of allergens on the solid phase, we tried to improve it by use of solid phases of greater capacity. Two different sets of experiments were performed with either several discs or Sepharose-immobilized allergens (see Methods). The results obtained with each technique with pooled sera are presented in Tables II and III. It is clear that both approaches allow a significant increase in the efficiency of IgE immobilization, indicating that the amount of immobilized allergen was limiting during the first step of the RAST. This is clearly presented in Table II, since it appeared that the use of an increasing number of discs induces a progressive increase in the efficiency of IgE immobilization. It appeared, however, that a more effective immobilization was obtained with Sepharose-immobilized allergens, as presented in Table III for different pools of serum. In most cases the reaction between specific IgE and insolubilized allergen appeared quite complete during the first 24-hour incubation period (>85%). It should

be noted, however, that a lower efficiency may be observed with a few individual sera. In these cases, the RAST dilution curves are never parallel to the PRIST standard curve (data not presented). Differences between the results obtained with discs or the gel could be more pronounced when individual sera were tested. In a few cases the quantity of IgE bound to the discs during a 24-hour incubation period represented no >25% of the quantity bound to the corresponding gel.

DISCUSSION

In the present study we have presented evidence that total IgE may be easily determined with a tandem of monoclonal antibodies (namely, Le27 and BS17) in a conventional PRIST assay. In the conditions we used, <0.5 IU/ml could be quantified precisely. This sensitivity is sufficient to allow the measurement of total IgE in adult sera. When low IgE levels must be measured, such as in the serum of infants or in tissue-culture fluid, an assay of higher sensitivity would be desirable. According to our experimental findings, we

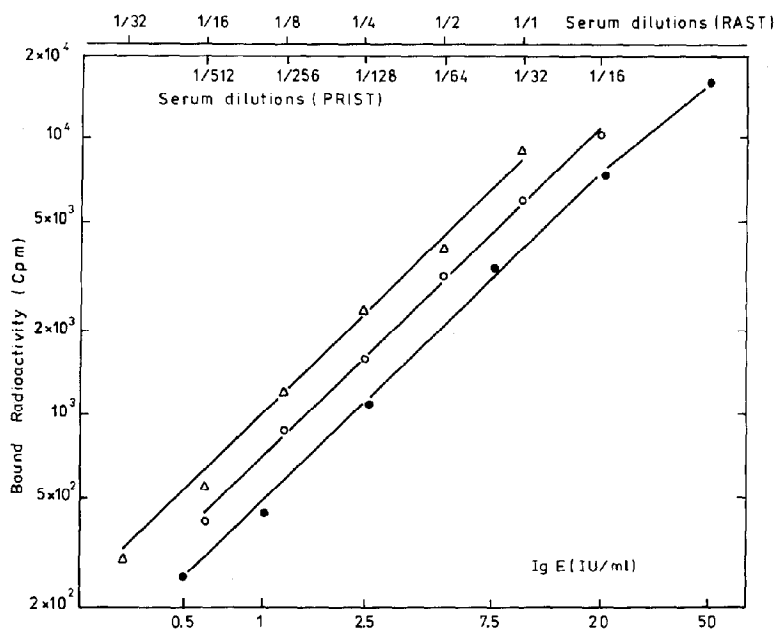


FIG. 8A. Parallelism between the PRIST standard curve, the PRIST dilution curve, and the RAST dilution curve with the monoclonal assay. PRIST measurements were made in the conditions described for Fig. 5. RAST measurements (*D. glomerata*) were performed in the conditions described in Fig. 3 with BS17 (12 Ci/gm, 5.10^4 cpm/disc) as tracer. Δ — Δ , Serum dilutions (specific IgE); $\circ$ — $\circ$, serum dilutions (total IgE); and $\bullet$ — $\bullet$, standard curve.

can propose several ways of enhancing the sensitivity of the IgE assay. A first possibility would be to use a tracer with a greater specific radioactivity (>15 Ci/gm) in order to increase the intensity of the signal to be measured. This approach, however, did not appear very attractive: first, because, as presented in Fig. 1, improvement of the assay was only obtained when large quantities of radioactivity ($>10^5$ cpm) were used, and second, because these corresponding tracers are rather unstable. In a second approach we can consider increasing the quantity of labeled BS17 introduced in the assay since, as presented in Fig. 5 and as already mentioned by several authors,^{7, 15, 16} this procedure induced an increase in tracer binding. This behavior, observed with both monoclonal and polyclonal antibodies, indicated that the corresponding assays did not work as ideal "excess reagents methods."^{38, 39} In contrast to Gleich et al.,^{15, 16} we think that this phenomenon might not only be related to the presence of excess IgE on the solid phase but also might reflect a limited affinity of anti-IgE antibodies. This opinion is supported by the fact that partial binding of labeled anti-IgE antibodies is observed even with substoichiometric concentrations of the tracer. This is presented, for instance, in curves

of Fig. 1, where maximum binding to the solid phase ($\approx 6 \times 10^4$ cpm) was not obtained unless a tenfold excess of tracer was used. Since we have demonstrated that this limited reactivity is not related to heterogeneity of the tracer (Fig. 2), it appeared very likely that the incomplete reaction was due to the limited affinity of the anti-IgE antibodies. As a consequence, the selection of monoclonal antibodies of greater affinity would represent an interesting alternative for increasing the sensitivity of the IgE assay. This approach would allow the quantity of radioactivity used in the assay to be limited. Another possibility would be to use enzyme-labeled antibodies, which can be used in great excess without posing the problems encountered when large quantities of radioactivity are handled. A highly sensitive assay of this type, with affinity-purified anti-IgE Fab' conjugated with β -galactosidase, has been developed by Imagawa et al.¹¹

The sensitivity of the assay performed with monoclonal antibodies was very similar to that obtained with commercially available polyclonal antibodies (Pharmacia) (Fig. 5). Both types of assays provided similar results when these were applied to the determination of total IgE in serum (Fig. 6). Standard curves obtained with the monoclonal system, how-

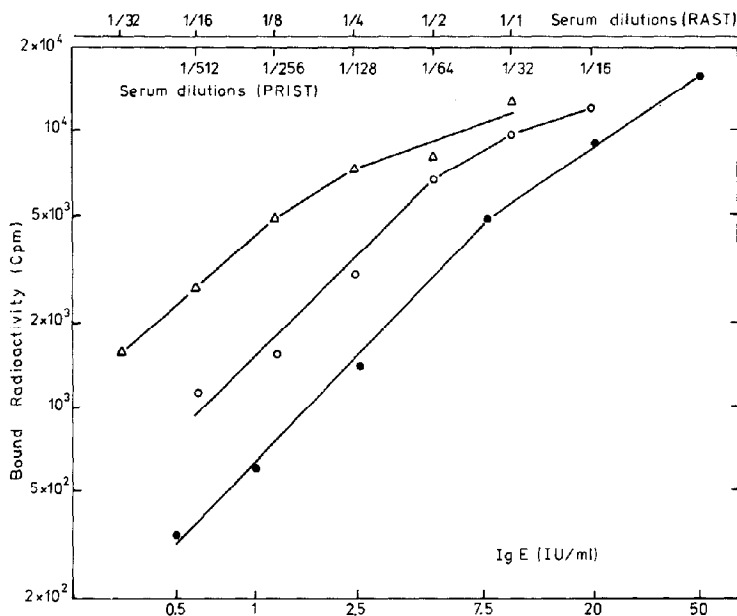


FIG. 8B. Lack of parallelism between the PRIST standard curve, the PRIST dilution curve, and the RAST dilution curve with the polyclonal assay (Pharmacia). PRIST and RAST measurements were performed in the conditions prescribed by the manufacturer. Δ — Δ , Serum dilutions (specific IgE); $\circ$ — $\circ$, serum dilutions (total IgE); and $\bullet$ — $\bullet$, standard curve.

ever, were linear over a wider range of IgE concentrations. In addition, the PRIST dilution curves for individual sera were always strictly parallel to the PRIST standard curve (Fig. 7). Such behavior was not always observed in the polyclonal system (Fig. 8B). We think that this divergence is linked to differences in the homogeneity of both reagents. Monoclonal BS17 is, indeed, a homogeneous reagent that recognizes, with a constant affinity, a unique determinant present on all IgE molecules. As a consequence, standard myeloma IgE and IgE contained in serum will be recognized identically. Polyclonal antibodies, on the other hand, may contain subpopulations of antibodies directed against epitopes that are not present in all serum IgE, thus explaining the lack of parallelism observed with any individual sera. Furthermore, the possibility that the commercial anti-IgE antisera cross-react to a slight degree with other immunoglobulin classes cannot be totally excluded, as presented by Spiegelberg et al.⁴⁰ for other polyclonal antibodies. This fact could also result in some non-linearity in the PRIST and RAST assays.

Monoclonal BS17 and Le27 may also be used to measure specific IgE immobilized with a conventional RAST procedure. The RAST dilution curves obtained with monoclonal antibodies were very different from

TABLE III. Comparative efficiency of Sepharose-immobilized allergens and allergen-coated discs in binding specific IgE

Allergen	Bound IgE (%) ^a	
	Sepharose-immobilized allergen	Allergen-coated discs
House dust	94	42
<i>Dermatophagoides pteronyssinus</i>	88	86
<i>D. glomerata</i>	99	79
Dog dander	99	38
Five grasses mixture	73	86

Different pools of serum from atopic patients were incubated (24 hours) with either Sepharose-immobilized allergens (see Methods) or with allergen-coated discs. Bound IgE was detected as described in Table I.

^aResults are expressed in percentages, taking the total amount of radioactivity bound during two successive incubation periods of 24 hours with Sepharose-immobilized allergens as reference.

those obtained with polyclonal tracer from Pharmacia. Polyclonal anti-IgE provided a larger signal for low concentrations of specific IgE but produced nonlinear dilution curves. Although monoclonal antibodies ap-

TABLE IV. Quantitative determination of total and specific IgE for an individual serum with Sepharose-immobilized Le27 or allergen

Dilution	Total IgE	House dust	DPT	DF	<i>D. glomerata</i>	
1/5	—	220	—	—	—	
1/10	—	190	98	150	220	
1/20	—	220	118	180	240	
1/40	1200	194	112	180	256	
1/80	1120	—	112	152	—	
1/160	1600	—	—	—	—	
1/320	1664	—	—	—	—	
1/640	1220	—	—	—	—	
	1360	206	112.5	165.5	239	Mean
	19	8	11	10	8	CV (%)
	100	15.2	8.3	12.2	17.6	Balance sheet (%)

DPT = *Dermatophagoides pteronyssinus*; DF = *Dermatophagoides farinae*; CV = coefficient of variation.

Serial dilutions of an individual serum were reacted (24 hours) with either Le27 or allergens immobilized on Sepharose. Total or specific bound IgE were quantified with iodinated BS17 (12 Ci/gm, 5×10^4 cpm/test, 18 hours), taking the PRIST standard curve as reference. Results are corrected for the dilution factor and are expressed in international units.

TABLE V. Quantitative determination of total and specific IgE for an individual serum with Le27- or allergen-coated discs

Dilution	Total IgE	House dust	DPT	DF	<i>D. glomerata</i>	
1/5	—	75	—	135	11.6	
1/10	—	54	110	140	12.8	
1/20	1120	65	124	166	8.4	
1/40	1360	60	144	184	11.7	
1/80	1280	—	120	192	—	
1/160	1328	—	128	—	—	
1/320	1536	—	—	—	—	
	1325	63.5	125	163.5	11	Mean
	11	14	10	16	17	CV (%)
	100	4.8	9.5	12	0.8	Balance sheet (%)

DPT = *Dermatophagoides pteronyssinus*; DF = *Dermatophagoides farinae*; CV = coefficient of variation.

Experiments were performed as described in Table IV with the same individual serum. The duration of the first incubation period for both PRIST and RAST measurements was 3 hours. Results are expressed as in Table IV.

peared less effective in "detecting" very low concentrations, they allow a sensitive determination of specific IgE, as demonstrated by their performances in the PRIST. The linear response curve they provided appears advantageous in many respects. For instance, it allows discrimination between high IgE levels that would all be categorized as class IV by the Pharmacia assay. From this point of view, the monoclonal assay provides more information.

To increase the sensitivity of the monoclonal assay, we may consider raising the concentration of tracer, as already suggested for the total IgE assay. In addition, we may use a 1/1 mixture of the two monoclonal antibodies (Fig. 4).

A more interesting characteristic of the dilution curves obtained with monoclonal BS17 is the fact that they were usually parallel to the PRIST standard curve (Fig. 8A). We think that this behavior also reflected the homogeneity of the reagent, which recognized solid phase-bound IgE whether IgE was linked to the solid phase by its variable region or was held by a second monoclonal antibody. This observation might provide the basis for a quantitative determination of specific IgE with a PRIST standard curve as reference. Such a procedure would be very attractive, since in theory all types of specific IgE could be quantified with a unique standard curve.

Such an approach implies that the second steps of

the PRIST and the RAST are actually performed under identical conditions, thus excluding the use of a mixture of Le27 and BS17 as tracer in the RAST. In addition, the principle of this quantitative assay for specific IgE implies that immobilization of IgE during the first steps of the PRIST and the RAST is total. The reincubation experiments we have performed demonstrate that these conditions were never fulfilled in the RAST when paper discs were used, regardless of the incubation time. This limited reaction of specific IgE may be due to the absence of sufficient amounts of active allergen on the solid phase. In addition, low affinity antiallergen IgE may be partially bound even in the presence of excess allergen. In both cases, a more efficient immobilization would be expected with greater quantities of allergen during the first step of the RAST. This is actually obtained when Sepharose-immobilized allergen of greater binding capacity is used. With this solid phase, with a 24-hour incubation, immobilization of specific IgE appears quite complete for most of the allergens and sera we have tested. When this condition is fulfilled, all the RAST dilution curves are strictly parallel to the PRIST standard curves. In any case, the lack of parallelism observed with paper discs has been avoided with the use of Sepharose. In these conditions we think that an absolute determination of immobilized specific IgE with the PRIST standard curve as reference may reasonably be made. Such an example is presented in Table IV where weights of total IgE, as well as of specific IgE for one serum and of different allergens, have been determined with the PRIST standard curve as reference. The good correspondence observed between the different values obtained with various serum dilutions demonstrated that a close parallelism was observed for all curves. These results allow a real balance sheet to be drawn up for specific IgE. The results obtained with the same serum with paper discs and a 3-hour incubation are presented in Table V as a comparison. It is worth noting that the combined use of paper discs and a short incubation period induced a highly significant underestimation of specific IgE for two allergens, whereas very good correspondence existed for total IgE and two other allergens.

We are aware of the fact that the procedure we propose cannot be generalized, since total immobilization of specific IgE during the first step of the RAST, as well as parallelism between the RAST dilution curves and the PRIST standard curve, are not always obtained, even when gel is used. As a consequence, further studies involving the analysis of a greater number of samples and allergens (including reincubation experiments) are still necessary to establish the validity of the method. If it proves to be applicable in most

cases, we believe it would provide an interesting tool for casting light on the role played by IgE in allergic diseases.

In conclusion, we think that the determination of absolute amounts of specific IgE may actually be obtained if an adequate anti-IgE tracer and a high-capacity solid phase are used. The first requirement will be more easily (but not exclusively) fulfilled with the use of a high-affinity monoclonal antibody. Better characterization and better purification of allergens, the use of a high-capacity solid matrix, and long incubation times are the prices to be paid in order to fulfill the second requirement.

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