Production of a mouse/human chimeric IgE monoclonal antibody to the house dust mite allergen Der p 2 and its use for the absolute quantification of allergen-specific IgE

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A chimeric human IgE monoclonal antibody was developed against the house dust mite allergen Der p 2. This chimeric antibody (hIgE-Dp2A) was composed of the heavy-chain variable domains and light chains of the original murine monoclonal antibody retaining its binding characteristics, whereas the heavy-chain constant domains were exchanged with the human IgE heavy chain. The chimeric IgE expression level was IgE 600 IU/ml (1 IU = 2.4 ng/ml). The binding of the chimeric hIgE-Dp2A to mite extract was indistinguishable from that of the original mouse monoclonal antibody. Parallel dose-response curves were found when the binding of hIgE-Dp2A to mite extract and anti-IgE coupled to sepharose were compared. Binding levels were not identical; however, hIgE-Dp2A bound significantly better to the mite-extract sepharose. This result indicates that the commonly used anti-IgE on solid phase calibration systems may lead to an overestimation of the amount of allergen-specific IgE present in the serum sample. The less efficient binding of the detector anti-IgE in case of the anti-IgE sepharose is likely to be because of the occupation of epitopes of the IgE by the sepharose-bound anti-IgE. Dose-response curves of serial dilutions of patient samples were parallel with the hIgE-Dp2A dose-response curve, which indicates that hIgE-Dp2A behaves like natural IgE antibodies in binding to allergen coupled to solid phase. This antibody is well suited for use as a reference reagent in the RAST and enables the expression of the amount of allergen-specific IgE present in a patient sample in absolute amounts. (J Allergy Clin Immunol 1997; 99:545-50.)

Key words: *IgE, chimeric antibody, Der p 2, RAST, house dust mite, allergen-specific IgE*

Abbreviations used
DNA: Deoxyribonucleic acid
V_H: Heavy-chain variable

Quantitation of allergen-specific IgE in bodily fluids by RAST[1] or related procedures is dependent on the availability of both an efficient allergosorbent to extract specific IgE antibodies from the sample and a dependable reagent to calibrate the amount of bound IgE to the solid phase. In RAST several solutions have been proposed to calibrate the bound IgE amount such as the use of an anti-IgE adsorbent that binds all IgE in a sample. Because in this latter procedure calibration of allergen-specific IgE is done with non-allergen-specific IgE, any sample with a known amount of total IgE can be used as calibrator of the assay. However, the mode of binding IgE to the solid phase is different from that in the RAST and this difference is likely to affect calibration. The use of allergen-specific IgE as calibrator is preferable, but for this procedure to be practical, two problems have to be solved: how to establish the amount of allergen-specific IgE in the calibration and how to obtain a sufficient supply of the reagent for interlaboratory comparison. Hybridoma technology offers the availability of unlimited supplies of specific monoclonal antibodies.

We report on the construction of a mouse/human chimeric monoclonal IgE antibody by recombinant deoxyribonucleic acid (DNA) techniques from a mouse monoclonal antibody directed to Der p 2, a 14 kD monomeric major mite allergen.[2] This chimeric IgE antibody (hIgE-Dp2A) has the binding characteristics of the original mouse monoclonal antibody 2B12,[3] whereas the Fc domain is derived from human IgE.[4,5] More precisely, chimeric IgE consists of the heavy-chain variable (V_H) domains and the complete light chains both cloned from the mouse hybridoma, whereas the constant domains of the heavy chain are of human origin. Because all IgE in this preparation is allergen specific, it is useful as a reference reagent and enables the calculation of the amount of allergen-specific IgE in absolute IgE mass units.

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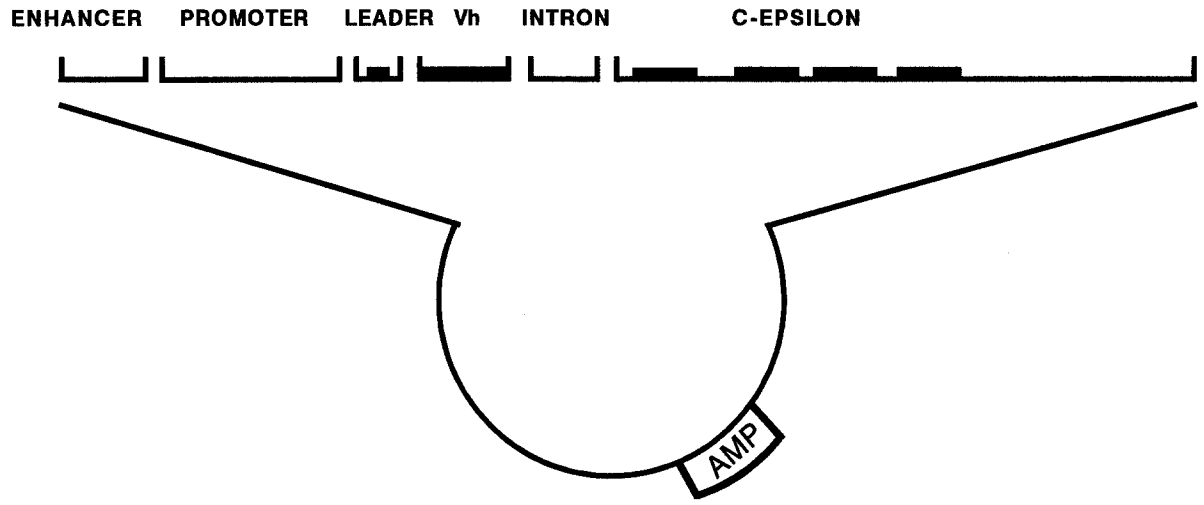

FIG. 1. Heavy-chain expression plasmid was prepared in vector pSV-SPORT and contained mouse IgH enhancer, IgH promoter, IgH leader sequence, V_H 2B12 domain, intron fragment, and human genomic C_ϵ gene.[4, 5, 11] *AMP,* Ampicillin resistance gene.

METHODS

Hybridoma cell line

Monoclonal antibody against Der p 2, 2B12, subclone B3 (mouse IgG2b with kappa light chains), has been described previously.[3]

Culture conditions

The 2B12 hybridoma cells, the heavy-chain loss variants, and the transfectomas were cultured in IMDM (GIBCO/BRL, Paisley, Scotland) containing 5% heat-inactivated fetal calf serum (Bodinco, Alkmaar, The Netherlands), 2-mercaptoethanol (50 μmol/L), penicillin (100 IU/ml), streptomycin (100 μg/ml), and rhIL6 (500 U/ml; see reference 6).

Selective medium was supplemented with Geneticin base, 500 μg/ml (Sigma Chemical Co., St. Louis, Mo.). Transfectomas were grown in 1 L roller bottles (Falcon, Becton Dickinson Labware) in culture medium without G418. The culture supernatants obtained were concentrated with use of a hollow fiber dialyzer (Hemoflow F5, Fresenius AG, Bad Homburg, Germany).

Preparation of a mouse/human chimeric IgE monoclonal antibody (hIgE-Dp2A) from monoclonal antibody 2B12

The chimeric hIgE-Dp2A antibody was prepared by combining methods described previously.[7, 8] A brief synopsis of the method follows.

First, a heavy-chain expression plasmid was prepared as follows. Total ribonucleic acid was isolated from 10^7 2B12 hybridoma cells, and cDNA was prepared with use of an RT-H kit (GIBCO/BRL) in combination with an oligo-dT primer (Boehringer, Mannheim, Germany).

The 2B12 V_H domain was then amplified in a polymerase chain reaction with the use of V_H domain–specific primers, V_H1FOR (5′TAGGAAGCTT CCTGAGGAGACGGTGACCGTGGTCCCTTGGCC) and V_H1BACK (5′TSMARCTGCAGSAGTCWGG, in which S = C or G, M = A or C, R = A or G, and W = A or T, according to Orlandi et al.[9] with minor modifications), which contained restriction enzyme recognition sequences used in cloning as described later. Restriction sites for *Hin*dIII, *Bsu*36I, and *Pst*I are underlined in the primer sequence. Restriction endonucleases were obtained from GIBCO/BRL except for *Bsu*36I, which was obtained from New England Biolabs (Beverly, Mass.). Plasmid isolations, restriction enzyme digestions, and agarose gel electrophoresis were done as described by Sambrook et al.[10] DNA sequence analysis of the V_H domain was done with dideoxysequencing by using Sequenase according to the manufacturer's suggested conditions (USB, Cleveland, Ohio).

The heavy-chain construct was prepared in the vector pSV-SPORT (GIBCO/BRL) and contained the IgH intron enhancer, IgH promoter, IgH leader sequence, V_H 2B12 domain, and intron fragment.[8, 11] A *Bam*HI fragment containing the human genomic C_ϵ gene[4, 5] was introduced in the unique *Hin*dIII site with use of *Hin*dIII-*Bam*HI adapters (New England Biolabs).

Second, several heavy-chain loss variants (secreting light chains only) were isolated from a population of 2B12 hybridoma cells that was cultured for 6 months during which period the cells were transferred two to three times per week.[8, 12] This population of cells was further enriched for clones producing light chains only by sorting for heavy-chain negative clones on a fluorescence-activated cell sorter. Hybridoma cells producing light chains but not heavy chains were then indentified by ELISA.[13] Production of light chains only was confirmed by biosynthetic labeling with ^{35}S-methionine and analysis by sodium dodecylsulfate-polyacrylamide gel electrophoresis.

Third, heavy-chain plasmid was introduced in a heavy-chain loss variant by cotransfection with pSV2-neo.[14] Heavy-chain (75 μg) and pSV2-neo (7.5 μg) plasmid DNA were mixed with 10^7 heavy-chain loss variant cells in 800 μl ice-cold phosphate-buffered saline solution. The suspension was electroporated in a 0.4 cm cuvette with use of a Biorad Gene Pulser instrument with capacity extender (Biorad Laboratories, Richmond, Calif.) at 960 μF and 250 V. The cells were plated in two 48-well culture plates (Costar, Cambridge, Mass.) in culture medium. Selective medium containing Geneticin base, 500 μg/ml, was added after 2 days. Clones were screened for hIgE production and for binding to mite extract coupled to sepharose by RAST. Mite-positive clones (transfectomas) were subcloned by limiting dilution.

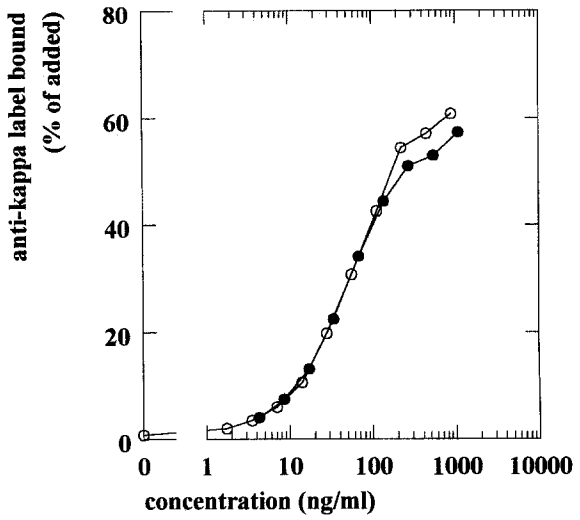

FIG. 2. Comparison of binding of hIgE-Dp2A *(open circles)* and original mouse monoclonal antibody 2B12, mIgG2b-Dp2A *(solid circles)* to mite extract coupled to sepharose. Serial dilutions of antibodies were incubated with mite-sepharose. Detection of antibodies bound was done with ^{125}I-labeled anti–light chain antibodies. Light chain of both antibodies is identical.

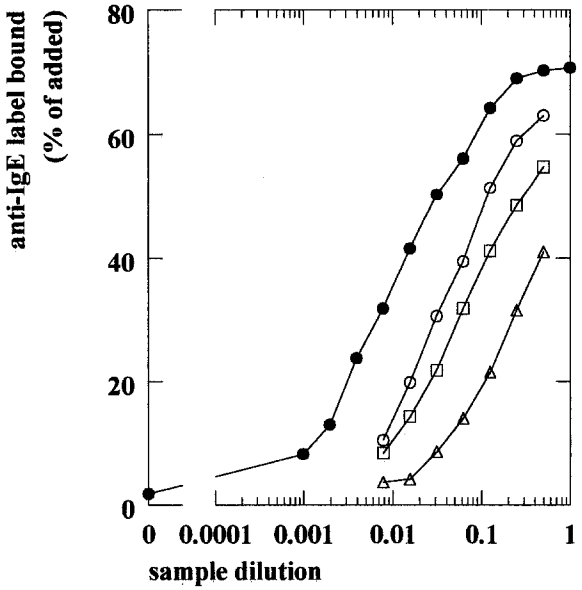

FIG. 3. Binding of hIgE-Dp2A *(solid circles)* and three patient samples *(open circles, squares, triangles)* to mite extract coupled to CNBr-activated sepharose. Sample dilutions (1 = undiluted) were incubated with sepharose and binding was detected with ^{125}I–anti-IgE antibodies. IgE concentration in culture supernatant was 600 IU/ml (1 IU = 2.4 ng/ml).

Radioimmunoassays

RAST was done as described previously.[15] *Dermatophagoides pteronyssinus* mites (1.5 gm, Commonwealth Serum Laboratories, Melbourne, Australia) were extracted in phosphate-buffered saline solution (3% wt/vol) supplemented with Tween 20 polysorbate (0.1% vol/vol) and sodium azide (0.1% vol/vol) for 4 hours. After filtration (black ribbon filter, Schleicher and Schüll, Dassel, Germany) the extract was coupled to 10 gm CNBr-activated sepharose 4B (Pharmacia, Uppsala, Sweden). Per test 1.5 mg sepharose was used (about 170 ng = 170 KU Der p 2/test). Per test 50 μl hIgE-2B12 dilution or human serum was added to sepharose-coupled allergen. Detection was done with use of ^{125}I-labeled sheep antibodies to human IgE. To compare the binding of the chimeric hIgE-Dp2A and the original mouse monoclonal antibody 2B12 (mIgG2b-Dp2A) to mite extract, detection of the amount of antibody bound was done with rat anti-(mouse) kappa light-chain antibodies (RM19, CLB, Amsterdam) making use of the identical mouse light chain of both antibodies. Polyclonal sheep anti-human IgE serum (10 ml) coupled to 20 gm CNBr-activated sepharose 4B was used in comparing the binding of total IgE to mite-specific IgE antibodies. In this assay 1 mg sepharose per test was used.

Results were expressed as the amount of radioactive label bound relative to the total amounts added.

RESULTS

Expression of mouse/human chimeric IgE monoclonal antibody (hIgE-Dp2A) directed to Der p 2

Mouse/human chimeric IgE-Dp2A antibodies were obtained by combining methods described previously.[7-9] The V_H domain was cloned from the hybridoma cell line 2B12[3] and inserted into a mammalian expression vector with a human epsilon heavy-chain constant region.[4, 5] Fig. 1 shows the construction of the heavy-chain plasmid. From a long-term culture of 2B12 cells we isolated several spontaneously arising "heavy-chain loss variants" (producing light chains only). The heavy-chain construct was transfected into the heavy chain loss variant. After 2 weeks of tissue culture, Geneticin base–resistant clones were examined for expression of IgE anti–Der p 2 antibodies. IgE-producing clones with high reactivity to mite sepharose were subcloned and expanded in roller bottles. The culture supernatant of this IgE-producing clone contained IgE, 600 IU/ml, which corresponds to IgE, 1.4 μg/ml, assuming 1 IU IgE = 2.4 ng/ml.[16]

Comparison of the binding to mite extract of the original mouse monoclonal antibody mIgG2b-Dp2A and the chimeric IgE monoclonal antibody hIgE-Dp2A

The binding of the hIgE-Dp2A to mite extract coupled to sepharose was compared with the binding of the original mouse monoclonal antibody 2B12,[3] mIgG2b-Dp2A. Fig. 2 shows dose-response curves of both antibodies incubated with mite extract coupled to sepharose. Detection was done with ^{125}I-labeled anti–light chain antibodies. The light chains of the chimeric hIgE-Dp2A and the mouse mIgG2b-Dp2A antibodies were identical. In the steepest part of the dose-response curve the binding of the two antibodies to mite extract was indistinguishable.

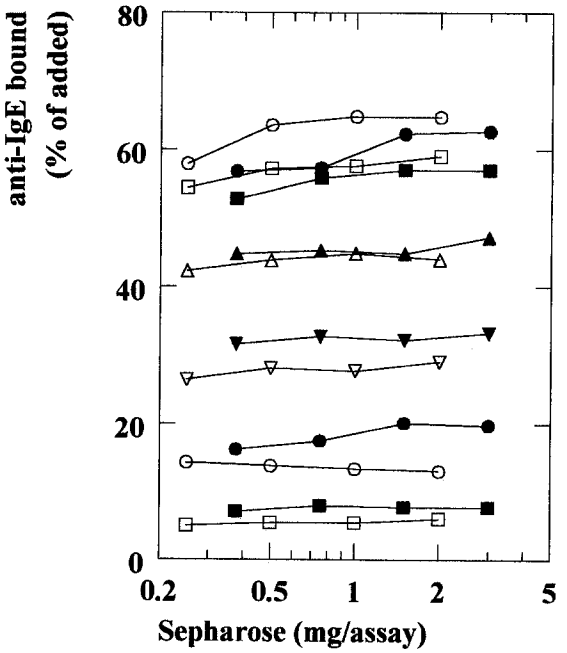

FIG. 4. Adsorbent in excess conditions. Several hIgE-Dp2A concentrations (*squares* 1.2 IU/ml, *circles* 3.7 IU/ml, *inverted triangles* 11 IU/ml, *triangles* 33 IU/ml, *squares* 100 IU/ml, *circles* 300 IU/ml) were incubated with anti-IgE *(open symbols)* or mite extract *(solid symbols)* coupled to sepharose. Binding was detected with use of ^{125}I-labeled anti-IgE antibodies.

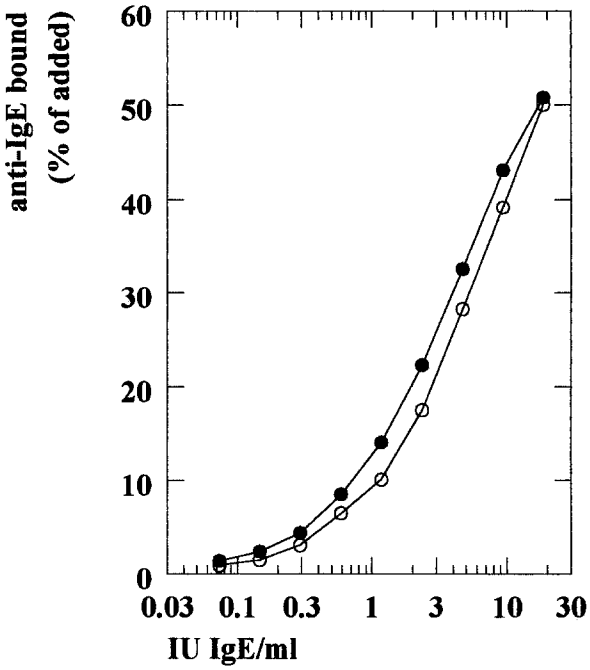

FIG. 5. Binding of chimeric hIgE-Dp2A to mite and anti-IgE coupled to sepharose. Serial diluted hIgE-Dp2A supernatant was incubated with mite extract *(solid circles)* or anti-IgE *(open circles)* coupled to sepharose. Bound IgE was detected with ^{125}I-labeled sheep–anti-IgE antibodies. Results shown are representative for seven independent experiments.

Comparison of the dose-response curves of hIgE-Dp2A and mite-positive sera

Fig. 3 shows the binding of the supernatant of hIgE-Dp2A to mite extract coupled to CNBr-activated sepharose in combination to the binding of three mite-positive sera. Dose-response curves were assessed for 11 mite-positive patient sera and compared with hIgE-Dp2A (not shown). In the steepest part of the dose-response curve of hIgE-Dp2A doubling the antibody concentration led to an increase of 8.9% in binding of the anti-IgE label (expressed as percentage binding of the total amount of anti-IgE label added). All the 11 patient sera tested showed similar increases of 9.4% ± 0.85% (standard deviation) binding. All patient samples were within a range of 2 standard deviations of the mean.

Amount of adsorbent added in excess

The amount of adsorbent required for maximal binding of hIgE-Dp2A was tested. This is important because under nonsaturating adsorbent conditions the binding of hIgE-Dp2A to mite extract and anti-IgE coupled to sepharose cannot meaningfully be compared. Sepharose-coupled mite and sepharose-coupled anti-IgE were both titrated and incubated with serial dilutions of hIgE-Dp2A antibody. As shown in Fig. 4, maximal binding of radiolabeled anti-IgE was found at 1.5 mg mite sepharose per test or 1 mg anti-IgE sepharose per test.

Comparison of the binding of chimeric hIgE-Dp2A to anti-IgE sepharose and mite sepharose

The binding of chimeric hIgE-Dp2A to sepharose-bound allergen extract was compared with the binding to sepharose-bound anti-IgE (Fig. 5). If all IgE is indeed allergen specific, and the adsorbents are added in excess, one would expect similar binding curves. Although the dose-response curves of hIgE-Dp2A to mite and anti-IgE coupled to sepharose are parallel, the comparison of the binding of radiolabeled anti-IgE to the two adsorbents showed a small but significant difference in the amount of tracer binding: mite-sepharose gave a higher response than anti-IgE–sepharose at the same IgE dose (ratio [counts bound to anti-IgE–sepharose]/[counts bound to mite-sepharose] 0.76 ± 0.06 [standard error of the mean]; n = 7). The less efficient binding of the detector anti-IgE in case of the anti-IgE–sepharose indicated the occupation of epitopes of the IgE by sepharose-bound anti-IgE.

DISCUSSION

The absolute quantitation of IgE antibodies to allergens is even more complicated than the quantitation of antibodies in general because of the heterogeneity of the allergens and the presence of potentially interfering antibodies of other isotypes directed to this allergen. This paper focuses on the quantitation of the amount of IgE bound to the solid phase. For the calculation of

absolute amounts of IgE bound to the solid phase an allergosorbent with a known amount of IgE attached is required. IgE attachment can be achieved in various ways: covalent coupling, coupling via anti-IgE antibodies, or coupling via allergen. The mode of attachment may change the reactivity of the IgE toward the detecting antibody, as we found for anti-IgE capture compared with allergen capture (Fig. 5). Comparing the IgE binding to mite extract (allergen) and anti-IgE coupled to sepharose, hIgE-Dp2A showed parallel dose-response curves but a less efficient binding of the detector anti-IgE in the case of anti-IgE sepharose (ratio [counts bound to anti-IgE–sepharose]/[counts bound to mite-sepharose] 0.76 ± 0.06 [standard error of the mean]; n = 7). This indicates the engagement of epitopes of the IgE by sepharose-bound anti-IgE probably resulting in the binding of about three instead of four tracer anti-IgE antibodies. This less efficient binding would lead to an overestimation of the amount of allergen-specific IgE in patient sera in case of solid phase anti-IgE. Covalent attachment (unpublished observations) may also result in a change of reactivity toward the detecting antibody, making this procedure less suitable for calibration. Antibody capture can presumably be made more efficient by careful selection of the capture antibody, for example, by using anti–light chain antibodies in combination with IgE myeloma protein as calibrator or by using a non-competing (monoclonal) anti-IgE antibody.

The first step of the RAST, involving the efficiency of the allergosorbent to extract specific IgE antibodies from the sample, is influenced by the avidity of the antibody-allergen interaction and by allergen-specific non-IgE antibodies present in the patient sample. These complications can be circumvented by performance of the test in a sufficiently large excess of antigen, but generally this is not a realistic option. It is therefore important to control the capture by retesting the serum after the first incubation or by testing the serum with variable amounts of allergosorbent.

In the latter case, if the same test result is found by a twofold variation in the allergosorbent dose, it is safe to assume that the allergen is not limiting. This condition was obtainable with the chimeric antibody (Fig. 4), but not for all of the human sera tested (data not shown). On the basis of the results of RAST inhibition experiments using mite extract it is unlikely that the natural IgE antibodies have a lower avidity than the chimeric antibody (Kd = 0.9 nmole/L). The more likely explanation is that minor allergens are underrepresented on the allergosorbent and contribute significantly only at the higher allergosorbent doses. At high allergosorbent doses, the dose-response curves of serum samples and the chimeric calibrator were parallel (Fig. 3). If a serum shows a strong dependency on the allergosorbent dose, quantitation of the IgE antibody level is not feasible by any calibration procedure (but a lower limit estimate can obviously be made).

Controlling the antibody capture by retesting hIgE-Dp2A after incubation with mite-sepharose showed that when up to 94 IU/ml antibody was added >95% of the IgE was bound to the sepharose. The part of the curve used for calibration is normally below 100 IU/ml.

The use of chimeric antibodies as reference material has several advantages compared with the use of serum antibodies. The production by a hybridoma-like cell line provides unlimited access to well-defined antibodies. The only immunoglobulin present in the culture supernatant is allergen-specific IgE, although some overexpression of light chains was observed. However, light chains did not bind to the adsorbent (data not shown). This hIgE-Dp2A is stable under various conditions of storage (data not shown).

Another interesting application of these chimeric antibodies is the possible comparison of IgE and IgG antibodies. Isotypic variants of this anti–Der p 2 antibody (e.g., IgG1 and IgG4 antibodies) have been produced. With the use of anti–light chain antibodies for detection it is possible to compare directly the dose-response curves of IgE, IgG1, and IgG4 antibodies. In this way the conversion factors for the antibody units can be determined and the quantities of isotype-specific anti-allergen antibodies can be measured.

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