The European Union CREATE Project: A model for international standardization of allergy diagnostics and vaccines

Martin D. Chapman, PhD,[a] Fatima Ferreira, PhD,[b] Mayte Villalba, PhD,[c] Oliver Cromwell, PhD,[d] Donna Bryan, PhD,[e] Wolf-Meinhard Becker, PhD,[f] Montserrat Fernández-Rivas, MD, PhD,[g] Stephen Durham, MD, PhD,[h] Stefan Vieths, PhD,[i] Ronald van Ree, PhD,[j] and the CREATE consortium *Charlottesville, Va, Salzburg, Austria, Madrid, Spain, Reinbeck, Borstel, and Langen, Germany, Potters Bar and London, United Kingdom, and Amsterdam, The Netherlands*

Allergen measurements are used extensively in the formulation of allergy diagnostics and vaccines, yet no purified international allergen standards are available for calibration purposes. The aims of the European Union CREATE project were to develop international standards with verifiable allergen content. Purified natural and recombinant allergens were analyzed by means of SDS-PAGE, mass spectrometry, circular dichroism spectra, and small-angle x-ray scattering. IgE reactivity was assessed by means of direct RAST, RAST inhibition, immunoblotting, and basophil histamine release with sera from 961 allergic patients. Three recombinant allergens, rBet v 1, rPhl p 5a, and rDer p 2, were structurally indistinguishable from their natural counterparts and showed excellent IgE reactivity suitable for use as certified reference materials. A second tier of allergens (rPhl p 5b, rOle e1, rDer p 1, rDer f 1, and rDer f 2) was identified that could provide suitable candidates for certified reference materials with minor improvements to the recombinant proteins. Only rPhl p 1 was considered unsuitable as a reference material. Quantitative ELISAs were identified that accurately measured each allergen, except for rPhl p 1. The CREATE project has provided a major step forward in allergen standardization and provides a model for the development of a comprehensive panel of international reference preparations that will harmonize allergen measurements worldwide.
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From [a]Indoor Biotechnologies, Inc, Charlottesville; [b]the Department of Molecular Biology, University of Salzburg; [c]the Department of Biochemistry and Molecular Biology, University of Complutense, Madrid; [d]Allergopharma Joachim Ganzer KG, Reinbek; [e]the National Institute for Biological Standards and Control, Potters Bar; [f]Forschungszentrum Borstel, Borstel; [g]Fundación Hospital Alcorcón, Madrid; [h]the National Heart & Lung Institute, Imperial College, London; [i]Paul-Ehrlich Institute, Langen; and [j]the Department of Experimental Immunology, Academic Medical Center, Amsterdam.

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Reprint requests: Martin D. Chapman, PhD, Indoor Biotechnologies, Inc, 1216 Harris St, Charlottesville, VA 22903. E-mail: mdc@inbio.com.

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The widespread use of allergen measurements in the fields of allergy, indoor air quality, and environmental exposure assessment has created an urgent need for internationally recognized purified allergen standards and for validated and certified immunoassays. Although allergenic products used for immunotherapy are licensed based on their total potency, allergists increasingly use specific allergen measurements for dosing of immunotherapy. Maintenance doses of 5 to 20 μg of major allergen are associated with clinical improvement after immunotherapy, and natural allergenic products are being formulated in part based on specific allergen content.[1-3] Allergens and IgE responses are the immunologic targets for allergen-specific immunotherapy. Allergen concentrations should be monitored to establish dose-response relationships between allergens and treatment efficacy, to compare allergenic products from different manufacturers, and to formulate recombinant allergen diagnostics and vaccines.[3-6]

Allergen measurements have been used most extensively to assess sensitization and exposure to environmental allergens (dust mite, animal allergens, cockroach, and molds). Epidemiologic studies, population surveys, and birth cohort studies have clearly defined levels of allergen exposure in Western populations and have found strong associations between exposure and the development of asthma.[7-14] Guidelines have been developed to indicate exposure levels that are risk factors for sensitization.[7] Allergen assays are used for testing the efficacy of allergen avoidance

Abbreviations used
CD: Circular dichroism
CRM: Candidate reference material
ESI-QTOF-MS: Electrospray-ionization quadrupole time-of-flight mass spectrometry
GMP: Good manufacturing practice
SAXS: Small-angle x-ray scattering
WHO: World Health Organization

procedures and devices and for monitoring clinical trials of avoidance and the efficacy of remediation.[11,15,16] They are used in the US indoor air quality industry for evaluation of allergen exposure in homes, the workplace, and public buildings.

Although allergen measurements have become routine, few international standards are available for calibration purposes.[3] Recombinant allergens are being used to develop new diagnostics and vaccines, yet the structural and immunologic properties of the recombinant allergens had not been systematically compared with those of their natural counterparts in international collaborative studies. The World Health Organization (WHO)/International Union of Immunological Societies Allergen Standardization Sub-committee has been influential in coordinating international standardization. The committee established WHO-approved international standards for dust mite, dog hair, and birch, timothy, and short ragweed pollens and produced the WHO position paper that recommended the use of standardized allergen vaccines of defined allergen content for dosing in immunotherapy.[17,18] The approach was also endorsed by a position statement from the American Academy of Allergy, Asthma & Immunology.[19]

In 1999, the WHO/International Union of Immunological Societies Allergen Standardization Sub-committee initiated a program to develop highly purified allergens that could be used for standardization of *in vitro* assays. This provided the genesis for a European Union–funded study entitled "Development of certified reference materials for allergenic products and validation of methods for their quantification" (acronym: *CREATE*).[3,20] The aim of the European Union CREATE project was to produce international standards of purified allergens with verifiable allergen content. Such standards would enable allergen manufacturers, academic organizations, and government and regulatory agencies to use a common international standard for specific allergen measurements. A second aim was to compare the specificity, sensitivity, and reproducibility of ELISAs for allergen analysis.

Allergens were selected for the project based on the following criteria: (1) the allergen was a major allergen of well-documented clinical importance; (2) purified natural and recombinant forms of the allergen were available in greater than 20-mg amounts from academic or commercial laboratories; (3) there was strong evidence that the recombinant allergen had equivalent IgE binding to its natural counterpart and there was extensive structural data on the allergen; and (4) ELISA kits to measure the allergen were available from 1 or more laboratories.

Purified natural and recombinant allergens, as follows, were compared in the study: pollens, Bet v 1, Phl p 1, Phl p 5, and Ole e 1; mites, Der p 1, Der f 1, Der p 2, and Der f 2.

A detailed account of the aims, scope, and methods used in the CREATE project has been published elsewhere.[21] This rostrum highlights the clinical relevance of the CREATE project and discusses how the principles applied to allergen standardization in CREATE can become a model for harmonization of allergen measurements worldwide.

CREATE PROJECT OUTLINE

Participating organizations and study design

The CREATE consortium comprised 28 organizations: 9 research laboratories, 11 clinical research groups, 6 allergen manufacturers, and 2 biotech companies from 9 European countries (Austria, Denmark, France, Germany, Italy, The Netherlands, Spain, Sweden, and the United Kingdom; see Table E1 in this article's Online Repository at www.jacionline.org). The consortium included 3 laboratories from governmental institutions involved in regulatory affairs: the Paul-Ehrlich Institute (Germany), the Istituto Superiore di Sanita (Italy), and the National Institute of Biological Standards and Control (United Kingdom), a WHO-approved laboratory for international standards. The aims of the study were (1) evaluation of the suitability of purified recombinant allergens as candidate certified reference materials (CRMs) and (2) evaluation of ELISAs for measuring specific allergens using the CRM as the standard. To achieve these goals, 20 mg or more of each natural or recombinant pollen or mite allergen was purified, and the structural properties of the natural and recombinant forms were compared by using state-of-the-art proteomic analyses (Fig 1). The IgE reactivity of the purified allergens was compared by using *in vitro* assays and by means of histamine release. IgE antibodies were obtained from allergic patients by the clinical research groups, and a CREATE serum bank was established. Immunologic reactivity was further compared in several ELISAs for each allergen by using mAbs available from the CREATE partners.

Allergen purification and analysis

Natural allergens were purified from birch, timothy, or olive pollen or from *Dermatophagoides pteronyssinus* or *Dermatophagoides farinae* spent mite medium by using standard chromatographic techniques. The recombinant allergens were produced in *Escherichia coli* expression systems, with the exception of the group 1 mite allergens (rDer p 1 and rDer f 1) and rOle e 1, which were produced in *Pichia pastoris*. In all, 18 purified allergens in lots of 20 mg or more were produced (8 natural allergens and 10 recombinant allergens), and 2 allergens, rPhl p 5a and rPhl p 5b, were produced under conditions of good manufacturing practice (GMP).

Purity of the allergens was assessed by means of SDS-PAGE, and protein content was determined by using amino acid analysis, extinction coefficient at 280 nm, and colorimetric assays. Amino acid composition and partial amino acid sequencing of allergen peptides by means of tandem mass spectrometry were used to confirm the identity of the allergens.[22] The degree of homogeneity was assessed by means of analytic size-exclusion HPLC and small-angle x-ray scattering (SAXS), which determined the extent of aggregation of the proteins and whether they were monomers, dimers, or trimers.[23] Secondary structure was compared by using circular dichroism (CD) spectroscopy.[24] The isoform composition of purified natural allergens and the extent of posttranslational modifications to the proteins were evaluated by means of electrospray-ionization, quadrupole time-of-flight mass spectrometry (ESI-QTOF-MS).[22,23]

Real-time and accelerated degradation studies were performed with allergen formulated in normal saline solution containing

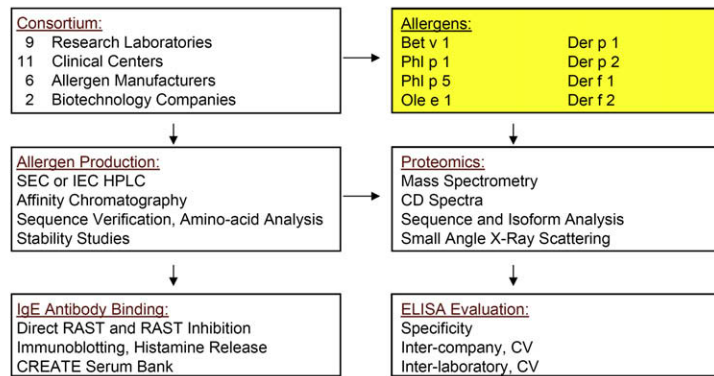

FIG 1. Outline of the CREATE consortium members, allergens, and study protocols. *SEC*, Size exclusion; *IEC*, ion exchange; *CV*, coefficient of variation.

0.1% trehalose dehydrate and 0.2% human serum albumin. Ampoules were filled with 5 μg of allergen product and lyophilized under nitrogen. Accelerated degradation studies were carried out with ampoules stored at temperatures ranging from −150°C to +56°C and tested for allergen by means of ELISA at 1, 3, 6, 9, 12, and 24 months after lyophilization.

CREATE serum bank

Allergic patients were recruited from 11 clinical centers in Europe (London, Manchester, Madrid, Jaén, Barcelona, Rome, Borstel, Utrecht, Gothenburg, Strasbourg, and Vienna) by the researchers listed in Table E1. Ethical approval was obtained from the institutional review boards of participating centers, and each patient provided written consent. Patients were enrolled at 16 to 50 years of age with a skin prick test wheal of 5 mm in diameter or larger with the standardized skin prick test solutions that were available in the respective clinical centers. Patients provided a clinical history of rhinoconjunctivitis, asthma, or both on exposure to allergen, when relevant, and had not received immunotherapy for 5 years. For most of Europe, tree and grass seasons are clearly defined. Mite allergy was defined by symptoms during usual daily exposures to mites, including bed making, dusting, and vacuum cleaning, as well as a positive skin prick test response to mite (in most of the clinical centers, mite was a dominant allergen in dust compared with cockroach and pet allergens). Serum (approximately 10 mL) was obtained from 961 sensitized patients as follows: birch, n = 186; timothy, n = 342; olive, n = 155; and mite, n = 278. Subsequently, 155 selected patients sensitized to birch (n = 34), grass pollen (n = 60), olive (n = 20), and mite (n = 41) donated approximately 40 mL of serum based on their IgE antibody levels by means of RAST. Recruitment of patients was coordinated by Drs Stephen Durham (London, United Kingdom) and Montserrat Fernández-Rivas (Madrid, Spain). The CREATE serum bank is maintained by Dr Ronald van Ree at the Academic Medical Center, Amsterdam, The Netherlands, and is available to researchers on agreement of the CREATE partners. The sera are stored at −40°C.

Comparison of IgE binding to natural and recombinant allergens

Sera from allergic patients were compared for binding to the respective natural or recombinant allergens by means of direct RASTs with allergens coupled to Sepharose and by means of immunoblotting.[25] The ability of recombinant allergens to inhibit the binding of natural allergens to IgE antibodies was tested by means of RAST inhibition with serial 2-fold dilutions of allergen at concentrations of 0.001 to 1 mg/mL. Biologic activity was compared by using the histamine release assay with the stripped basophil technique.[26] Basophils from a nonallergic donor were stripped of membrane-bound IgE by using lactic acid and resensitized with IgE from 25 sera from allergic donors. Dose-response curves for histamine release by natural or recombinant allergens were compared at concentrations from 0.001 to 100 ng/mL.

ELISA assessment

ELISA kits for measuring purified allergens were supplied by members of the CREATE partnership (see Table E2 in this article's Online Repository at www.jacionline.org). In most cases, the ELISA used a capture mAb together with a biotinylated or enzyme-labeled detector mAb, although in some assays polyclonal antibodies were used for detection. Dose-response curves were used to compare binding of natural and recombinant allergens and to determine assay sensitivity. The protein content of the allergen standards was based on amino acid analyses. The concentration of allergen in 6 commercial allergen extracts (from different European manufacturers) was compared by using the natural or recombinant allergen standards in 5 laboratories of the CREATE consortium. Each laboratory determined the intra-assay and interassay coefficient of variation, and the interlaboratory coefficient of variation was determined by the project leaders for a given allergen.

RESULTS

Structural comparisons of purified natural and recombinant allergens

All allergens showed greater than 95% purity when analyzed by means of SDS-PAGE (Fig 2). The molecular weights of allergens produced in *E coli* showed good agreement with their natural counterparts. Mite allergens produced in *P pastoris* (rDer p 1 and rDer f 1) had significantly higher molecular weights because they were glycosylated (by using carbohydrates incorporated by *P pastoris*). Partial amino acid sequencing by means of ESI-QTOF-MS confirmed the identity of the allergens and accounted for between 30% and 95% of the full sequence. For some allergens, rBet v 1,

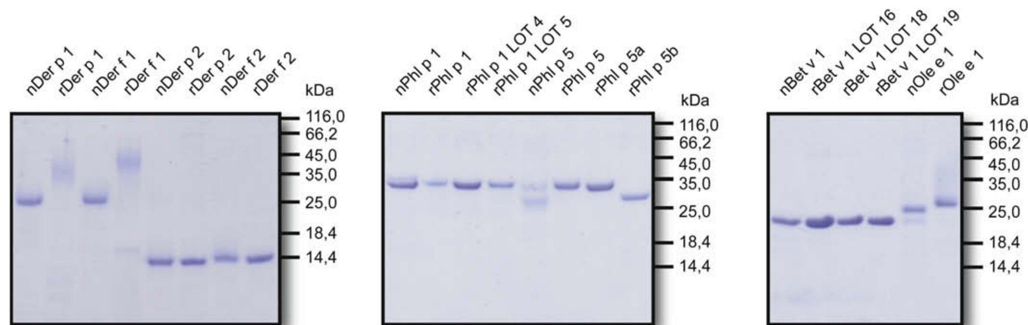

FIG 2. SDS-PAGE analysis of purified natural and recombinant allergens used in CREATE (Coomassie Blue staining).

rPhl p 5a, rPhl p 5b, and rDer f 2, greater than 95% of the sequence was confirmed. Identity was also confirmed based on the tight correlations between the observed and theoretic amino acid compositions, with one exception. Natural Phl p 1 had an unexpectedly high alanine content, which was attributed to contamination with trace amounts of Phl p 5 (an alanine-rich protein).

Analyses by means of ESI-QTOF-MS and size-exclusion HPLC confirmed the high degree of purity of rBet v 1, rPhl p 5a, rPhl p 5b, and rDer f 2 and suggested that these allergens were monomers. Other allergens, including nPhl p 1, rPhl p 1, nOle 1, and rOle 1, showed multiple peaks, indicating microheterogeneity. Similar heterogeneity was seen with natural and recombinant mite group 1 allergens. Natural and recombinant forms of Bet v 1 provided CD spectra in close agreement with each other and with the 3-dimensional structure of Bet v 1. Similar CD data were obtained for Phl p 5 and Der f 1. Ole e 1, Der p 1, and the group 2 mite allergens showed the expected CD spectra based on 3-dimensional structures. On heating to 95°C, the CD spectra of all allergens, with the exception of Phl p 1, changed to random coil, as expected for thermally unfolded polypeptides. The melting temperature was similar for natural and recombinant forms of the same allergen. On cooling to 25°C, Bet v 1, Phl p 5, and rDer f 1 showed CD spectra similar in shape and peak amplitudes to those recorded before heating, suggesting that these proteins were able to correctly refold after thermal denaturation. Both natural and recombinant Ole e 1 were partially refolded after thermal denaturation. Except for rDer f 1, natural and recombinant mite group 1 and 2 allergens were irreversibly denatured by means of heat treatment.

Five allergens (nBet v 1, nPhl p 1, nDer p 1, nDer f 1, and nDer p 2) could be assigned as monomeric or dimeric molecules based on SAXS analyses. Six allergens (rBetr v 1a, rPhl p 5a, rPhl p 5b, nOle e 1, rDer p 2, and nDer f 2) displayed some tendency to form oligomers at the rather high concentrations (3.3 mg/mL) used in the SAXS measurements. A nonaggregated state (ie, not multimerized) could be assigned to 11 of the 17 samples tested. Three preparations (rDer p 1, rDer f 1, and rDer f 2) showed moderate aggregation, and rOle e 1 showed extensive aggregation.

Stability of lyophilized allergens

Most allergens (15/17) remained stable at −20°C for 2 years, with an annual potency loss of less than 1%. Similar levels of stability were seen for allergens stored at +4°C. For natural and recombinant Der p 1, potency loss at 1 year was 1.6% and 4.2%, respectively, and 6.7% and 9.7%, respectively, at 2 years. In summary, allergens showed good stability when kept at −20°C and in most cases could be expected to be stable for 2 years.

IgE antibody binding to natural and recombinant allergens

Spearman rank analyses showed highly significant correlations ($P < .001$) between IgE antibody binding to natural and recombinant allergens by means of direct RAST (Bet v 1, n = 187 and r = 0.986; Phl p 1, n = 345 and r = 0.982; Phl p 5, n = 345 and r = 0.971; Ole e 1, n = 166 and r = 0.904; mite allergens (n = 280): Der p 1, r = .920; Der f 1, r = 0.903; Der p 2, r = 0.958; and Der f 2, r = 0.968). The strength of these correlations was analyzed by comparing the mean ratio of IgE antibody bound to recombinant and natural allergen. For Bet v 1, the correlation was 1.0, indicating that overall IgE binding to natural and recombinant allergen was indistinguishable. Ratios of greater than 0.7 were obtained for Phl p 5, Der p 2, and Der f 2, and ratios of 0.5 to 0.6 were obtained for all other allergens (indicating that for these allergens the IgE bound by the recombinant allergen was 50% to 60% of that bound to the natural allergen). Differences in IgE antibody binding were most pronounced at low IgE levels (<2 IU/mL). The results of IgE dot blots confirmed the RAST results, but overall dot blots were less sensitive than RASTs and resulted in semiquantitative data.

RAST inhibition dose-response curves (using 0.001-1 mg/mL allergen) showed comparable inhibition curves for Bet v 1 and Der f 2 when recombinant allergens were compared with their natural homologues. For other allergens, IgE antibody inhibition by recombinant allergens required more than 10-fold higher concentrations than for the natural allergens. These results appeared to be inconsistent with those obtained in direct RAST assays. Basophil histamine release was used to further compare IgE antibody binding. Dose-response curves were performed at allergen concentrations of 0.001 to 100 ng/mL by using IgE antibodies in sera from 25 allergic patients in the stripped basophil technique. Parallel dose-response curves were obtained for all allergens, and near-identical histamine release was obtained for natural and recombinant Bet v 1, Der p 2, and Der f 2. Surprisingly, several recombinant allergens were 5- to 10-fold more potent in histamine release assays than their natural counterparts (rPhl p5a, rPhl p 5b, and rOle e 1). The recombinant group 1 mite allergens showed 5-fold lower histamine release, and rPhl p 1 release was 100-fold lower. With the exception of rPhl p 1, all recombinant allergens produced significant histamine release (>10%) at concentrations of less than 1 ng/mL.

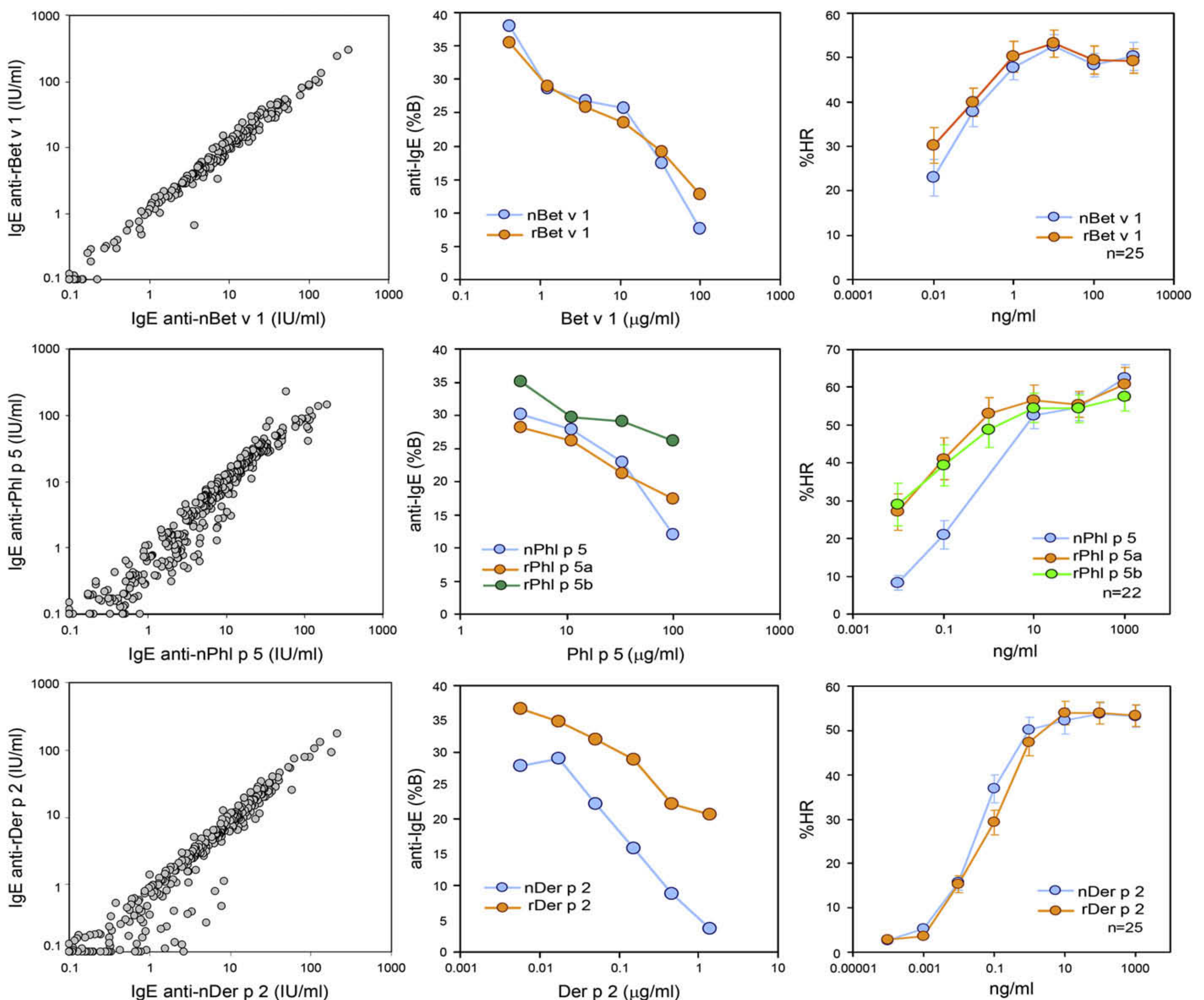

FIG 3. IgE antibody binding to natural and recombinant allergens. The allergens Bet v 1 *(top)*, Phl p 5 *(middle)*, and Der p 2 *(bottom)* were compared by means of direct RAST *(left panel)*, RAST inhibition *(center panel)*, and basophil histamine release *(right panel)*. Histamine release data are the means ± SDs of results of sera from 25 allergic patients.

A comparison of the direct RAST data, RAST inhibition, and histamine release for Bet v 1, Phl p 5a, and Der p 2 is shown in Fig 3.

Comparisons of ELISAs for allergen measurement

The outcomes of the ELISA evaluation were subject to a number of different variables that could affect the results, including the extent of structural homology between the natural and recombinant allergens to be used as CRMs, whether the mAbs used in ELISAs were raised against natural or recombinant allergens, and the isoform specificity and affinity of the mAb. The study had too few laboratories (5 in all) to be considered a true ring trial, and the ELISA kits were not uniform. Some manufacturers provided matched antibody pairs, others provided complete immunoassay kits, and differences were reported in the types of microtiter plates, enzyme conjugates, and substrates used. Nonetheless, despite these shortcomings in study design, the evaluation was successful and allowed 1 or more ELISA methods to be validated for most allergens. For Bet v 1, 2 ELISA methods provided similar quantitative results for measuring Bet v 1 in allergen extracts, irrespective of whether natural or recombinant allergen was used as the standard (Table E2). ELISA results for Phl p 1 were disappointing, and none of the 3 ELISAs tested was considered suitable for assay purposes. rPhl p 1 did not fulfill the requirements of a candidate CRM. Four Phl p 5 ELISAs were evaluated, and 1 of these showed comparable measurements of nPhl p 5 and rPhl p 5. Another Phl p 5 ELISA performed well but showed 4.5 times greater reactivity with rPhl p 5, most likely because the mAbs were raised against the recombinant allergen. One ELISA for Ole e 1 showed equivalent binding for the natural and recombinant allergen. Another Ole e 1 ELISA showed reproducible binding for nOle e 1 but did not react strongly with rOle e 1.

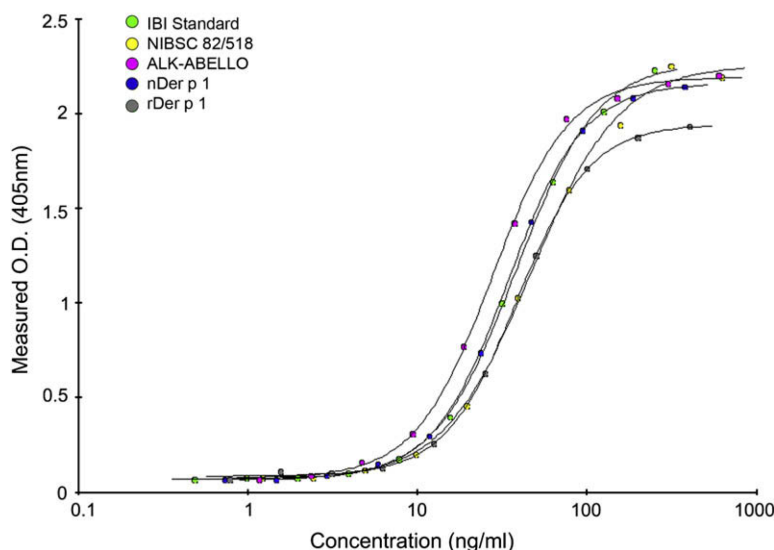

FIG 4. Dose-response curves for natural and recombinant allergens by means of ELISA for Der p 1. Curves for nDer p 1 and rDer p 1 produced for the CREATE project were compared with an International Reference Preparation (National Institute for Biological Standards and Control 82/518) and 2 company standards (IBL and ALK).

Parallel dose-response curves for Der p 1 (natural or recombinant) were obtained by using ELISAs from 2 different manufacturers (Fig 4 and Table E2). With improvement of the recombinant allergens, both sets of ELISAs will be suitable for allergen measurements. Differences in isoform specificity were observed in 2 ELISAs tested for Der p 2, but one of these assays was suitable for measuring total Der p 2. Two ELISAs were also identified as being suitable for measurement of Der f 2 (Table E2).

DISCUSSION

The CREATE project had several successful outcomes. The project conducted a thorough structural comparison of purified natural and recombinant allergens and identified several recombinant allergens that are suitable as biologic reference preparations. CREATE provided a strong body of data on the IgE-binding activity of natural and recombinant allergens and has identified ELISA reagents that are suitable for allergen measurement. Three recombinant allergens were considered to be suitable as candidate CRMs: rBet v 1, rPhl p 5a, and rDer p 2. These allergens had almost identical purity, protein folding, aggregation states, solubility, and stability as their natural counterparts (structural equivalence) and showed comparable binding in IgE antibody assays and biologic potency. Some reduced uptake of IgE was seen for rDer p 2 in direct RASTs in approximately 5% of the 280 sera tested. A second tier of allergens was identified that could become reference preparations if improvements were made to the recombinant proteins: rPhl p 5b, rOle e1, rDer p I, rDer f 1, and rDer f 2. Such improvements include engineering to reduce glycosylation in *P pastoris*–expressed allergens. For example, the recombinant mite group 1 allergens used in CREATE were glycosylated and contained partial proregion sequences, which are now known to interfere with IgE antibody binding.[27] Mature deglycosylated forms of the group 1 allergens lacking the proregion sequences have since been produced, with comparable IgE reactivity to natural allergens. The only recombinant allergen with poor structural and IgE-binding characteristics was rPhl p 1. However, since the initiation of CREATE, correctly folded rPhl p 1 has been produced by a European allergen manufacturer under GMP conditions and has been successfully used in clinical trials of immunotherapy.[28,29]

Excluding rPhl p 1, all of the recombinant allergens showed strong reactivity in IgE-binding assays. The extent to which IgE binding of the recombinant allergens matched that of the natural allergens was correlated with the degree of structural identity. In most cases greater than 90% of patients with IgE to natural allergen had IgE to the recombinant allergen by means of direct RAST, confirming the approach of using recombinant allergens for diagnostic purposes. For the second-tier recombinant allergens, the mean level of IgE antibody binding was approximately 2-fold lower than that to natural allergens. Minor modifications of expression systems to produce better folded, more homogeneous molecules with less aggregation will improve IgE binding of these allergens to the level of the natural proteins. Differences in the methods used for IgE evaluations were observed in CREATE. Specifically, the apparent dichotomy between strong IgE binding of recombinant allergens in direct RASTs and basophil histamine release assays compared with the generally weak activity of the recombinant allergens in RAST inhibition. On the one hand, the recombinant allergens produced positive results in histamine release assays at concentrations of 1 ng/mL, yet in many cases the dose-response curves in RAST inhibition were several orders of magnitude higher. This could reflect the fact that the RAST inhibition system used was relatively insensitive (natural allergens needed to be used at 1 mg/mL to achieve 90% inhibition). Alternatively, it could be argued that oligomerization and a degree of aggregation seen in some allergens (natural and recombinant) enhanced their ability to cross-link in histamine release assays. The reproducibility and consistency of the histamine release data were striking given the number of allergic sera ($n = 25$) used in the evaluation. A detailed analysis of the CREATE IgE-binding studies will be published elsewhere.

A MODEL FOR INTERNATIONAL ALLERGEN STANDARDIZATION

The CREATE project has provided a major step forward in allergen standardization and provides a coherent strategy for the future development of a comprehensive panel of international allergen standards. The criteria of allergen purity and IgE binding used in CREATE can readily be applied to other allergens. Improvements that have been made in the production of recombinant allergens since the CREATE project was initiated will make it easier to produce high-quality allergens with optimal IgE antibody binding and biologic potency. Over the past 5 years, the crystal structure of several important allergens has been determined (Fel d 1, Der p 1, and Bla g 2), which required allergens of high purity.[30] Increasingly, allergens are being produced under GMP conditions that facilitate production of gram quantities of reproducible materials for standardization purposes. These developments provide a pipeline through which the second-tier allergens identified in CREATE will become reference preparations and that can be used for important allergens that were not originally included in CREATE (eg, Fel d 1). Recombinant allergens need to be used together with validated ELISAs that measure natural and recombinant allergens with similar performance characteristics. This allows the same designation of potencies in mass units of specific allergen. The CREATE project identified suitable ELISAs for measurement of all allergens, except Phl p 1.

Two recombinant allergens tested in CREATE, rPhl p 5 and rBet v 1, have recently been used in clinical trials of allergen immunotherapy.[28,31] These allergens have been produced under GMP conditions and are now being developed into biologic reference preparations by the Biological Standardization Programme of the European Directorate for the Quality of Medicines. Under this program, 200 mg of each allergen will be formulated, analyzed according to CREATE protocols, and lyophilized in 10,000 vials. These vials will be assayed in a formal ring trial by using 2 ELISAs for each allergen from CREATE, which will include 8 Official Medicines Control Laboratories from Europe. The objective of the European Directorate for the Quality of Medicines project is to establish both of the recombinant allergens as biologic reference preparations in conjunction with certified ELISAs for allergen measurement. Once the recombinant allergen reference preparation and ELISAs are established, they will become part of the European Pharmacopoeia and will serve as international standards for licensing of allergen products. This will mean that all European measurements of Bet v 1 and Phl p 5 will be made relative to these standards by using the certified ELISAs. Consequently, for the first time, it will be possible to directly compare levels of these allergens in products produced by manufacturers from Europe and from other countries. Application of the CREATE principles to other allergens will provide a road map toward the production of a comprehensive panel of purified recombinant allergen reference preparations and certified ELISAs that will result in verifiable allergen measurements.

In summary, the European Union CREATE project has successfully established 2 recombinant allergen reference materials that are being evaluated as biologic reference preparations and that should ultimately be included in the European Pharmacopoeia as international standards. CREATE provides a model that can be followed for other purified allergens, and it is anticipated that this approach will be widely adopted in Europe and in other countries. Harmonization of allergen measurements will have important benefits for allergists and will improve the accuracy and precision of dosing for immunotherapy, which, ultimately, will improve the management of allergic diseases.

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TABLE E1. Members of the CREATE partnership

Research laboratories		
Sanquin Research (coordinator)	R. van Ree, M. Aalbers, S. Notten, and P. Ooievaar-de Heer	The Netherlands
University of Salzburg, Salzburg	F. Ferreira, G. Gademaier, and M. Wallner	Austria
Universidad Complutense, Madrid	M. Villalba and R. Rodriguez	Spain
Research Centre Borstel, Borstel	W-M. Becker, F. Eberhardt, and U. Lepp	Germany
BGFA Ruhr University Bochum, Bochum	M. Raulf-Heimsoth	Germany
University of Vienna, Vienna	R. Valenta and M. Focke	Austria
Research laboratories with regulatory role		
National Institute for Biological Standards and Control, Potters Bar	D. Bryan, C. Dolman, and R. G. Das	United Kingdom
Paul-Ehrlich-Institut, Langen	S. Vieths and K. Fötisch	Germany
Istituto Superiore di Sanità, Rome	G. di Felice and C. Pini	Italy
Allergen manufacturers		
Allergopharma Joachim Ganzer KG, Reinbek	O. Cromwell, H. Fiebig, and B. Weber	Germany
HAL Allergy BV, Haarlem	H. van Schijndel and J. W. Dorpema	The Netherlands
ASAC Pharmaceutical International SA, Alicante	F. M. Marco	Spain
ALK-Abelló, Madrid	R. Monsalve and D. Barber	Spain
Laboratorios Leti, Madrid	E. Fernandez Caldas	Spain
Stallergènes SA, Anthony	P. Moingeon, A. Didierlaurent, and C. André	France
European Allergen Manufacturers Group, Haarlem	A. Kroon	The Netherlands
Biotech companies		
Biomay AG, Vienna	A. Neubauer	Austria
Indoor Biotechnologies Ltd, Warminster	M. Chapman, L. Vailes, and A. Tsay	United Kingdom
Clinical researchers		
Imperial College (London)	S. Durham	United Kingdom
North West Lung Centre (Manchester)	A. Custovic and B. Simpson	United Kingdom
University Medical Centre (Utrecht)	A. Knulst	The Netherlands
Fundación Hospital Alcorcón (Madrid)	M. Fernández Rivas	Spain
Ramon y Cajal Hospital (Madrid)	E. Gonzalez Mancebo	Spain
Forschungszentrum Borstel	Forschungszentrum Borstel	Germany
Institut Universitari Dexeus (Barcelona)	A. Cistero Bahima and M. M. San Miguel Moncin	Spain
Adriano Mari (as private clinician/Rome)	A. Mari	Italy
Medical University of Vienna	T. Kinaciyan and M. Focke	Austria
Hospital Ciudad de Jaén	J. Quiralte	Spain
Hôpiteaux Universitaires de Strasbourg	G. Pauli, F. de Blay, and A. Purohit	France
Sahlgrenska University Hospital (Göteborg)	S. Rak	Sweden

TABLE E2. ELISA kits for allergens evaluated in CREATE

Allergen	Supplier
Bet v 1	**ALK-Abelló**, **Stallergènes**, Sanquin*
Phl p 1	Allergopharma, Sanquin
Phl p 5	**ALK-Abelló**, Allergopharma,* Sanquin
Ole e 1	**ALK-Abelló**,* Stallergènes, Sanquin
Der p 1	**Indoor Biotechnologies**,* **ALK-Abelló**†
Der f 1	**Indoor Biotechnologies**,* **ALK-Abelló**†
Der p 2	**Indoor Biotechnologies**,* Sanquin
Der f 2	**Indoor Biotechnologies**,* **Sanquin**

Although ELISAs from some other suppliers were included in CREATE, they are not included in this table because their performance in preliminary evaluations was deemed unsuitable for the study. ELISAs that were considered suitable for allergen standardization are highlighted in boldface.
*ELISA used in stability studies.
†Assay with polyclonal rabbit antibodies for detection.