

EU forum

The create project: EU support for the improvement of allergen standardization in Europe

Ronald van Ree and the CREATE partnership

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Since November 2001, the European Commission is funding a large project under the Fifth Framework Program in the field of allergen standardization. The project is best known under its acronym CREATE. The full title of the project is 'Development of Certified Reference Materials for Allergenic Products and Validation of Methods for their Quantification' (contract no.: G6RD-CT-2001-00582). The aim of this paper is to describe the background and experimental and organizational design of the project.

From overall potencies to exact quantification: state-of-the-art standardization

Current products for allergen-specific treatment of allergy are heterogeneous extracts of the allergen source. These extracts are biologicals of extremely complex composition with many different proteins, glycoproteins, and polysaccharides. Only a selection of the (glyco)proteins is allergenic. Because of their biologic origin, allergen extracts are not only complex but also variable in composition. The major allergens have been shown to be present in different amounts, depending on the origin of the source material used for extraction (1, 2). Intra-extract ratios of major allergens can differ up to a factor 1000 between different extracts. Present standardization protocols for allergen extracts have *in vivo* testing in allergic volunteers as starting point. In short, allergen extracts selected to serve as in-house references (IHR) are administered intradermally or by prick test to the skin of selected sensitized patients (3). The size of the resulting immunoglobulin (Ig)E-mediated skin reaction determines the allergenic potency of the IHR. Subsequently, production batches are compared with the IHR by *in vitro* IgE-based methods like radioallergosorbent test (RAST)-inhibition. For this purpose, pooled sera of allergic subjects are used. On average an allergen extract contains two to

three major allergens, being those proteins that are recognized by >50% of an allergic population. A good quality serum pool (>20 sera) will therefore always contain sufficient quantities of those IgE antibodies that are needed to detect the sum of all major allergens in an extract. In other words, by RAST-inhibition differences in overall major allergen potencies will be detected. Differences in individual major allergen quantities and major allergen ratios, however, remain hidden (1, 2). Both safety and efficacy are dependent on quantities of individual major allergens, and to lesser extent even minor allergens. High doses of individual allergens can cause serious side effects, including life-threatening reactions. Whether this occurs is at least partly dependent on a combination of individual IgE profiles and extract composition. In exceptional cases, even an extract with slightly higher content of a minor allergen can be dangerous for an individual patient. Of course the risk is highest when a major allergen is over-represented in an extract. At the same time, it is now generally accepted that effective treatment is only achieved when certain minimum quantities of major allergens are administered (5–20 µg) (4). Major allergen administration can of course only be well controlled if major allergen contents are known.

All companies on the allergen market use their own type of units based on their own methods for standardization and calculation. Only IHR are applied as standards. Consequently, products of different companies are not directly comparable. The present practice in standardization is not much more than a method to assess batch-to-batch consistency of overall potencies within a company. In this situation, switching between products of different companies is not easy for physician and patient. The need for a point of reference for comparison of different allergen extracts was recognized in the late 1970s. This resulted in the production of five certified WHO-reference materials for allergens (5–9). These references were essentially identical to the

products on the market: heterogeneous natural allergen extracts. These certified reference materials (CRM) never obtained a firm foothold among allergen producers, because the methods for standardization described above were also applied to these WHO references, and these methods are simply not suitable for achieving real comparability.

Over the past decade the major allergens of the most important allergen sources were identified, purified and characterized (10, 11). On the basis of this know-how, it is now possible to decide which allergens should be quantified for standardization of allergen extracts. In the same period mono-specific antibody reagents (monoclonal antibodies) were produced against most major allergens (1). These reagents are applied in assays for quantification of major allergens. Up to date, several hundred allergens have been cloned and expressed as recombinant proteins (for further information: <http://www.allergen.org> and <http://www.allergome.org>). Finally, application of various different expression systems has facilitated production of the recombinant proteins that are close to equivalent to their natural counterparts (12–15).

Purified natural and recombinant allergens in combination with accurate assays for their quantification form the key elements of state-of-the-art standardization of

allergen products (1, 2). This can end the present situation where active ingredients (major allergens) are not declared and products of different companies cannot directly be compared. Standardization on ground of mass-units of major allergen requires certified international references based on purified natural or recombinant allergens. In the future, they can replace the existing WHO-references based on crude allergen extracts. Another prerequisite for standardization based on mass units of major allergen is the availability of validated major allergen tests. It is these two tasks that CREATE has set out to achieve:

1. Development of candidate certified reference materials, consisting of purified natural or recombinant major allergens.
2. Validation of existing sandwich enzyme-linked immunosorbent assay (ELISA) for the measurement of major allergens.

The network of CREATE

CREATE brings together a highly multidisciplinary consortium: allergen manufacturers ($n = 6$), biotech companies ($n = 2$), regulatory bodies ($n = 3$), clinicians

Table 1. Partnership of CREATE

Partner	Contact	Country
Sanquin Research (co-ordinator)	R. van Ree	NL
Universität Salzburg	F. Ferreira	AT
Biomay	A. Neubauer	AT
Allergopharma Joachim Ganzer	O. Cromwell/H. Fiebig	DE
Universidad Complutense Madrid	M. Villalba/R. Rodriguez	ES
ASAC Pharmaceutical Int.	F.M. Marco	ES
CBF Leti	E. Fernandez Caldas	ES
Indoor Biotechnologies	M. Chapman	UK
HAL Allergy	H. van Schijndel	NL
Imperial College (London)	S. Durham	UK
National Institute for Biological Standards and Control	D. Bryan/C. Dolman	UK
ALK-Abelló	R. Monsalve/D. Barber	DK
Forschungs Zentrum Borstel	WM Becker	DE
Berufsgenossenschaftliches Forschungsinstitut für Arbeitsmedizin	U. Lepp/F. Eberhardt	
Stallergènes	M. Raulf-Heimsoth	DE
Universität Wien	A. Didierlaurent	FR
Paul Ehrlich Institut	R. Valenta/S. Vrtala/M. Focke	AT
Istituto Superiore di Sanità	S. Vieths/K. Fötisch	DE
North West Lung Centre (Manchester)	G. di Felice/C. Pini	IT
University Medical Centre Utrecht	A. Custovic/B. Simpson	UK
Fundación Hospital Alcorcón	A. Knulst	NL
Institut Universitari Dexeus	M. Fernandez Rivas	ES
Adriano Mari (as private clinician/Rome)	A. Cistero/M. San Miguel	ES
University Medical School of Vienna	A. Mari	IT
Hospital Ciudad de Jaén	T. Kinaciyan	AT
Hôpitaux Universitaires de Strasbourg	J. Quiralte	ES
Sahlgrenska University Hospital (Göteborg)	G. Pauli/F. de Blay/A. Purohit	FR
European Allergen Manufacturers Group	S. Rak	SW
	A. Kroon	EU

NL, The Netherlands; AT, Austria; DE, Germany; ES, Spain; UK, The United Kingdom; DK, Denmark; FR, France; IT, Italy; SW, Sweden; EU, Europe.

($n = 11$) and research laboratories ($n = 6$). The full partnership is listed in Table 1. The broad set-up of the partnership was chosen to increase the chance of successful future implementation of allergen standardization based on mass units.

The allergens of CREATE

For CREATE a panel of major allergens originating from four of the most important inhalant allergen sources was selected: Bet v 1 from birch pollen, Phl p 1 and Phl p 5 from grass pollen, Ole e 1 from olive pollen and Der p 1 and 2 and Der f 1 and Der f 2 from house dust mite. Of all these allergens both purified natural and recombinant versions were included. For Phl p 5 two isoforms were selected i.e. Phl p 5a and Phl p 5b.

Overall work program structure

The work program (Fig. 1) spans 3 years, from March 2002 until February 2005. Essentially the CREATE program can be divided in two phases:

(A) Production and collection of materials and reagents, i.e. purified natural and recombinant allergens and serum samples of well documented allergic patients.

(B) Detailed comparison of natural and recombinant allergens with respect to physico-chemical characteristics and immune-reactivity and validation and comparison of ELISA for the measurement of major allergens.

The work program was divided into seven workpackages (WP). Workpackages 1–4 are dedicated to the four allergen sources in the project: birch pollen, grass pollen, olive pollen and house dust mite. Within these WPs, activities from phase A and B are carried out for each of the selected major allergens. Workpackages 1–4 will result in the selection of a candidate-CRM (either recombinant or natural) and validation reports of the different ELISA. The WP 5 (Clinical Evaluation) falls under phase B and is dedicated to *in vivo* evaluation by skin prick test of candidate CRMs. Workpackage 6 falls under phase B. Natural and recombinant allergens will be compared with respect to their lyophilization characteristics. Furthermore, the stability and homogeneity profile of the selected candidate CRMs will be investigated. The outcome of WPs 1–6 will be a set of candidate-CRMs with well-defined physico-chemical and immunological characteristics that form the basis for the specifications for the final CRM. In addition, WPs 1–4 and 6 will provide detailed validation reports on different major allergen ELISA. On the basis of these reports, ELISA can be designated as suitable for the

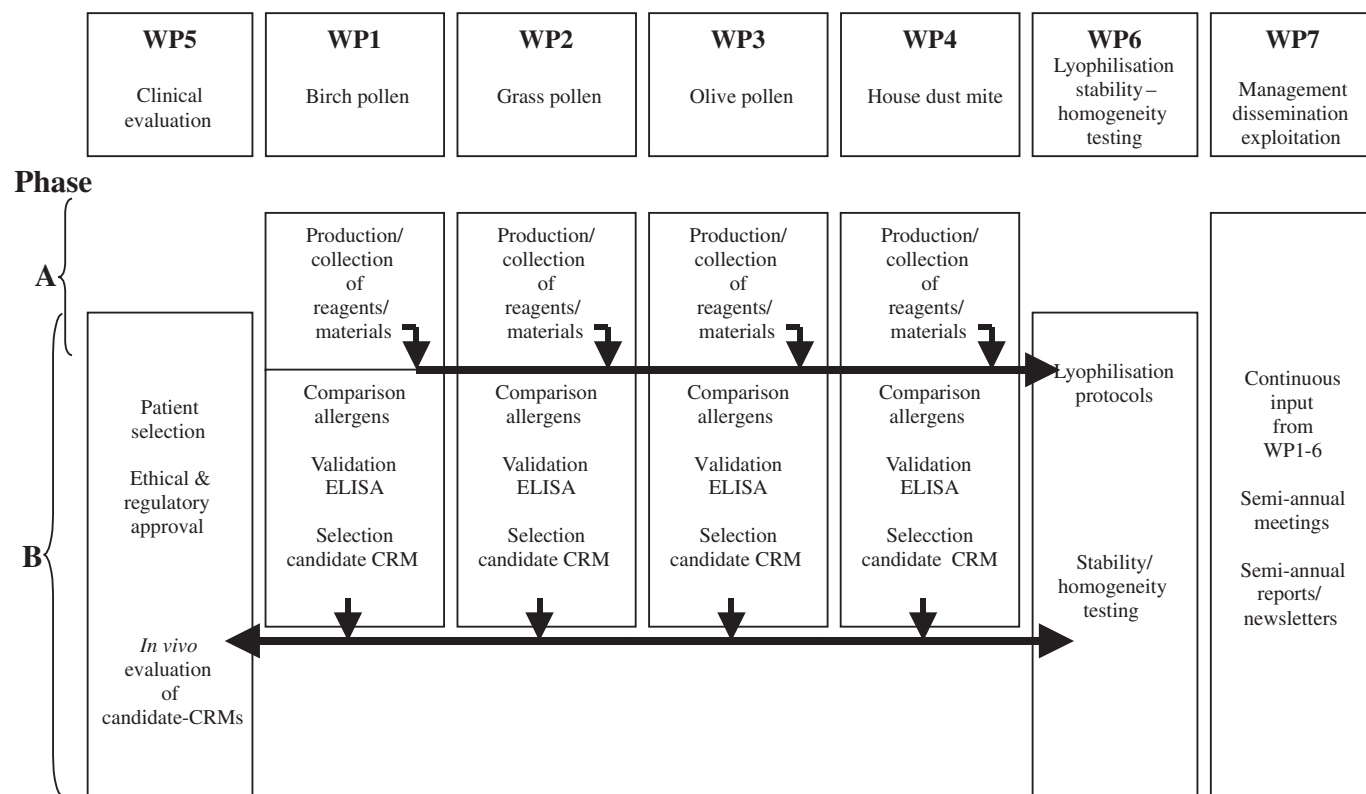


Figure 1. Overall work program structure.

intended use or not suitable. In the latter case, recommendations for improvements will be formulated. Finally, there is a WP 7 that is active throughout the entire project and is dedicated to management, dissemination and exploitation.

Status of activities

Phase A of the CREATE project has been successfully completed. All natural and recombinant allergens were produced in sufficient quantities to perform all tasks in phase B. Eleven clinicians in eight EU countries included a total of 971 patients. A detailed standardized questionnaire was used to document clinical histories. All patients were tested with a standard panel of inhalant allergens and serum samples were collected. After completion of phase A, serum samples and allergens were distributed over the various partners responsible for the characterization of the allergens in

phase B. At present data from phase B are being collected. The project is on target.

Concluding remarks

The CREATE project has successfully produced allergen reagents that can in the future serve as reference materials for standardization of allergen extracts in mass units. In addition, these reagents will serve the same reference purpose for the expected future generation of products based on recombinant allergens. At the same time, CREATE will increase the knowledge on the performance of available ELISA tests for the measurement of major allergens. This will allow selection of assays with the best performance. By funding this project the European Commission has given great support to the improvement of the quality of allergen products in Europe and to the competitiveness of European industry in the world.

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