

Analysis of the sensitization profile towards allergens in central Africa

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Summary

Background Almost no information is available regarding the prevalence of IgE-mediated allergies and the disease-eliciting allergens in tropical Africa.

Objective To study IgE-mediated allergies and the allergen profile in allergic patients from Zimbabwe.

Methods The frequency of sensitization to common environmental allergen sources was determined by skin prick testing in 650 allergic patients from Zimbabwe. Fifty representative sera were analysed for IgE reactivity to 20 respiratory and 20 food allergen extracts by multiallergen extract testing. The IgE reactivity profiles to recombinant pollen and mite allergens were compared between grass pollen- and mite-sensitized patients from Zimbabwe and central Europe. Sera from grass pollen-allergic patients were also analysed for IgE reactivity to nitrocellulose-blotted natural timothy grass and Bermuda grass pollen allergens.

Results IgE-mediated allergies were found to be common in Zimbabwe. Similar to the situation in central Europe, mites and grass pollens represented the most prevalent allergen sources. However, the IgE reactivity profiles determined with single recombinant pollen and mite allergens revealed interesting differences between the European and African patients, which most likely reflect the local allergen exposure.

Conclusions The striking differences regarding sensitization to grass pollen and mite allergens between African and European patients revealed by recombinant allergen-based testing emphasize the need for component-resolved allergy testing to optimize allergy prevention and therapy in different populations.

Keywords Africa, allergy, diagnosis, recombinant allergens

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Introduction

Due to a lack of immunological and allergological research facilities in central Africa, relatively few data are available regarding the prevalence of IgE-mediated allergies and the disease-eliciting allergens in this continent [1]. Current prevalence rates reported for type I allergy in industrialized countries vary from 15% to 44% [2–4] depending on environmental factors, such as local aeroallergens and degree of exposure, air pollution, temperature and humidity, as well as the age and the genetic disposition of the population. For westernized countries, epidemiological studies have demonstrated a progressive increase in the prevalence of IgE-mediated allergies [5–9]. A similar increase of allergic symptoms depending

on urbanization has also been reported for Africa recently [10, 11].

The spectrum of important allergen sources in developed countries is relatively well described. House dust mites and pollens represent the most important allergen sources, both because of their world-wide distribution and the heavy release of airborne allergenic materials [12–14]. It is well established that, depending on socioeconomic habits (e.g. housing, profession, eating habits) and regional differences regarding flora and fauna, individual allergen sources may become important. The application of recombinant allergen molecules for refined allergy diagnosis has recently demonstrated sophisticated differences in the sensitization profiles of allergic patients from different areas. For example, different rates of sensitization to recombinant birch pollen allergens rBet v 1 and rBet v 2 have been reported for birch pollen-allergic populations from northern and southern parts of Europe [15]. These differences are not detectable with allergen extract-based forms of diagnosis but may have strong implications for prevention and for selecting optimal forms of specific immunotherapy.

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Almost no data are available regarding disease-eliciting allergen sources and allergens in central Africa.

In this study we have analysed the allergen spectrum in a population of 650 allergic patients from central Africa (Zimbabwe). An in-depth serological analysis by testing multiple allergen extracts, recombinant timothy grass pollen and house dust mite allergens was carried out in 50 individuals. Results from our study demonstrate that, similar to the industrialized countries, grass pollen and mites represented the most prevalent allergen sources in Zimbabwe. However, the allergen profiles established with recombinant grass pollen and mite allergens differed substantially from those in central Europe and those reported for other parts of the world. The importance of the latter finding for prevention and therapy of allergy in Africa is discussed.

Methods

Characterization of allergic patients

The study population comprised 650 allergic individuals (281 males, 369 females, age 1 to 63 years, mean age 24.7) from the urban areas of Zimbabwe. The diagnosis of allergy in the African population was based on case history and skin prick testing with nine allergen extracts: house dust mite (*Dermatophagoides pteronyssinus*), grass pollen mix (Kentucky bluegrass, Orchard, redbud, timothy grass, sweet vernal), tree pollen mix, *Cynodon dactylon*, maize pollen, cat dander, dog dander, feather mix, mould mix (ALPYRAL, Bayer, Pittsburgh, PA, USA). Sera from 50 randomly chosen allergic patients and 15 non-allergic persons were analysed for IgE antibodies specific for 20 airborne and 20 food allergen extracts using multiallergen tests (Hitachi Chemical Diagnostics, Mountain View, CA, USA) according to the manufacturer's instructions. Positive reactions are graded from CLA (chemiluminescence assay) classes 1 (low) to 4 (high) as defined by the manufacturer.

Determination of the IgE reactivity profiles using purified recombinant and natural allergens

Out of the 50 allergic patients from Zimbabwe, 25 grass pollen-allergic and 20 mite-allergic patients were subjected to in-depth analysis of their IgE reactivity profiles. The latter two groups and, for comparison, equally sized grass pollen-allergic and mite-allergic Austrian patient groups were tested for reactivity to dot-blotted recombinant allergens. Recombinant birch pollen allergens (rBet v 1, rBet v 2) and recombinant timothy grass pollen allergens (rPhl p 1, rPhl p 2, rPhl p 5) were obtained from BIOMAY (Vienna, Austria). Recombinant birch pollen allergen, rBet v 4, and purified timothy grass pollen allergens (rPhl p 6, rPhl p 7, rPhl p 12, nPhl p 4) were from Pharmacia (Pharmacia Diagnostics, Uppsala, Sweden). Recombinant mite allergens (rDer p 2, rDer p 5, rDer p 7, rDer p 10) were expressed in *E. coli* and purified as described [16–18]. IgE reactivity profiles of grass pollen-allergic and mite-allergic patients from Austria and Zimbabwe were analysed by dot blotting. Nitrocelluloses containing dot-blotted purified allergens and human serum albumin (negative control) (0.5 µg of each protein/dot) were blocked two times for 10 min and once for 30 min in buffer A (50 mmol/L sodium phosphate (pH 7.5), 0.5% v/v Tween-20, 0.5% w/v BSA, and 0.05% w/v NaN₃). Afterwards

membranes were incubated with sera diluted 1:10 in buffer A overnight at 4 °C, washed as described for blocking, and bound IgE antibodies were detected with ¹²⁵I-labelled anti-human IgE antibodies (RAST; Pharmacia) as described [19].

Natural grass pollen extracts IgE immunoblots

Pollens from *Phleum pratense* and *Cynodon dactylon* were purchased from Allergon (Vällinge, Sweden) and extracted as described [20]. Lyophilized extracts were analysed by sodium dodecylsulphate-polyacrylamide gel electrophoresis (SDS-PAGE) [21] and Coomassie Brilliant Blue (Bio-Rad, Richmond, CA, USA) [22] staining to estimate quality and quantity of proteins.

Approximately 200 µg/cm gel of *Phleum pratense* and *Cynodon dactylon* grass pollen extracts were separated by SDS-PAGE and blotted on nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) [23]. IgE immunoblot analyses were performed as described [19, 24].

Results

House dust mites and grass pollen represent the most frequently recognized allergen sources in Zimbabwe

Skin prick testing performed in 650 allergic individuals from the urban areas of Zimbabwe identified house dust mite and grass pollen as the major allergen sources (Table 1). Fifty-two per cent of the patients showed positive skin reactions to *Dermatophagoides pteronyssinus* extract and 38% reacted with *Cynodon dactylon* (Bermuda grass) pollen extract. The high prevalence of skin test reactivity to grass and corn pollen was also emphasized by the fact that 28% and 26% of the patients exhibited immediate skin reactions to maize and mixed grass pollen allergen extracts, respectively (Table 1). The other sensitization rates were as follows: tree pollen mix, 16.6%; cat dander, 18.4%; dog dander, 15.8%; feather mix, 15.8%; moulds, 15.6% (Table 1).

Next we analysed sera from 50 randomly chosen patients for the presence of IgE antibodies specific for 20 respiratory and 20 food allergen sources (Table 2). Results from the serological analysis were in good agreement with the skin test results. House dust mite (60%) and grass pollen allergen extracts (50%) represented the most frequently recognized allergen

Table 1. Frequency of sensitization in 650 allergic patients from Zimbabwe determined by skin prick testing. Patients were tested with nine allergen extracts. The percentages of patients reactive to each extract are displayed

Allergen extract	Skin reactivity (%) (n = 650)
<i>Dermatophagoides pteronyssinus</i>	52
<i>Cynodon dactylon</i> pollen	38
Maize pollen	28
Grass pollen mix	26
Cat dander	18.4
Tree pollen mix	16.6
Dog dander	15.8
Feather mix	15.8
Mould mix	15.6

Table 2. Frequency of IgE-reactivity to natural allergen extracts in 50 allergic patients from Zimbabwe. Mean IgE levels for each allergen source were calculated for the number of reactive sera and are displayed as mean CLA class

Allergen source	Frequency of reactivity (%)	Mean IgE level (CLA class)	Allergen source	Frequency of reactivity (%)	Mean IgE level (CLA class)
<i>D. pteronyssinus</i>	60	3.5	Egg white	8	1.3
<i>D. farinae</i>	60	3.5	Yolk	6	2.5
<i>Aspergillus</i> species	16	1.5	Milk	8	1.7
<i>Cladosporium</i>	4	1.5	Casein	8	1.2
<i>Alternaria</i>	8	1.2	Sesame	16	1.6
<i>Penicillium</i>	6	2	Wheat flour	16	1.2
Horse dander	2	1	Rye flour	16	1.3
Dog dander	8	1.7	Soy	22	1.6
Cat dander	8	2	Almond	14	1.1
Rabbit	4	1	Hazelnut	16	2.1
Guinea-pig	2	1	Peanut	20	2.1
Hamster	4	1	Walnut	12	1
Feather mix	2	1	Peach	18	2.1
Plantain pollen	20	1.8	Celery	12	1.6
Rye pollen	42	2.7	Tomato	24	1.6
Timothy grass pollen	50	2.8	Potato	14	1.4
Mugwort pollen	16	2.1	Apple	24	1.7
Birch pollen	14	1.7	Carrot	18	1
Elder pollen	16	1.5	Cod	10	2.4
Hazel pollen	20	1.5	Crab	22	1.9

sources. The prevalence of IgE reactivity to other pollens was as follows: plantain, 20%; mugwort, 16%; hazel, 20%; birch, 14%; elder, 16%.

The most frequently detected food allergen extracts were apple (24%), tomato (24%), soy (22%), crab (22%) and peanut (20%) (Table 2).

The highest levels of IgE antibodies were detected in sera from patients sensitized to house dust mite extracts (86.6% of positive sera class 3 or more; mean 3.5) and to timothy grass pollen extract (60% class 3 or more; mean 2.8) (Table 2).

Patients from Zimbabwe exhibit a different IgE reactivity profile to mite and grass pollen allergen molecules than patients from central Europe

The sensitization rates to mite and grass pollen extracts determined in the allergic patients from Zimbabwe were comparable with results obtained for populations in Central Europe (Austria, Switzerland) and the USA [12–14]. However, when we further analysed the IgE reactivity profiles of the sera with purified natural and recombinant timothy grass pollen allergens, interesting differences were found. While rPhl p 2, a member of the group 2 allergen family, was recognized by 68% of Austrian grass pollen-allergic patients, none of the 25 grass pollen-allergic patients from Zimbabwe contained rPhl p 2-specific IgE (Table 3). A similar discrepancy was noted for the major timothy grass pollen allergens, Phl p 5 and Phl p 6. Sensitization rates to rPhl p 5 and rPhl p 6 in Austrian patients were 100% and 52%, respectively. Only a few patients from Zimbabwe reacted with these allergens (rPhl p 5, 27%; rPhl p 6, 15%). Differences regarding the IgE recognition frequency of rPhl p 1 and nPhl p 4 were less pronounced between patients from Austria (rPhl p 1, 96%; nPhl p 4, 84%) and Zimbabwe (rPhl p 1, 80%; nPhl p 4, 92%) (Table 3). The component-resolved analysis of the IgE reactivity profile to purified grass

pollen allergens suggests that patients from Zimbabwe are sensitized preferentially against grasses (e.g. Bermuda grass) lacking group 2, 5 or 6 allergens [24]. The surprising finding that 14% of the serologically tested patients from Zimbabwe reacted with birch pollen extract could be explained by sensitization to a highly cross-reactive allergen, profilin. All three birch pollen-reactive sera from Zimbabwe, that have been tested with single recombinant birch pollen allergens, contained IgE against birch profilin, Bet v 2, whereas more than 95% of birch pollen-allergic patients from Austria react with the major birch pollen allergen Bet v 1 and fewer than 10% contain Bet v 2-specific antibodies (Table 3).

Comparing the IgE reactivity profile to recombinant mite allergens revealed also striking differences between patients from Austria ($n = 20$) and Zimbabwe ($n = 20$). The IgE recognition frequency of patients from Zimbabwe to rDer p 5 (45%), rDer p 7 (35%) and rDer p 10 (55%) was much higher than that of Austrian patients (rDer p 5, 15%; rDer p 7, 0%; rDer p 10, 10%). The percentage of rDer p 2-reactive sera was comparable in patients from Austria (80%) and from Zimbabwe (70%) (Table 3).

The IgE reactivity profile of African grass pollen-allergic patients to nitrocellulose-blotted timothy grass and Bermuda grass pollen allergens suggests a primary sensitization to Chloridoideae

The low rate of reactivity to group 5 and group 6 grass pollen allergens as well as the lack of IgE antibodies to rPhl p 2 in the grass pollen-allergic patients from Zimbabwe suggest that they were sensitized preferentially to grasses lacking group 2, 5 and 6 allergens. The local grass flora in Central Africa is in fact dominated by grasses belonging to the Chloridoideae (e.g. Bermuda grass), that do not contain allergens cross-reactive with group 2, 5 and 6 allergens from timothy grass pollen [24]. We

Table 3. Origin, characteristics and IgE recognition frequency of purified allergens from timothy grass, birch pollen and house dust mites. The table displays allergen sources, allergen names (n, natural; r, recombinant), molecular weights (MW) and biological functions of the allergens. The IgE recognition frequency determined in central Europe (Austria) was compared with that found for the patients from Zimbabwe

Allergen source	Allergen	MW (kDa)	Biological function	Frequency of IgE recognition Austria (%)	Frequency of IgE recognition Zimbabwe (%)
<i>Phleum pratense</i> (timothy grass)	rPhl p 1	26.1	Similar to expansins	96	80
	rPhl p 2	10.7	Unknown	68	0
	nPhl p 4	55	Unknown	84	92
	rPhl p 5a	31	Ribonuclease (putative)	100	27
	rPhl p 6	11.8	P-particle associated	52	15
	rPhl p 7	8.6	Calcium-binding	8	0
	rPhl p 12	14	Actin-binding (profilin)	24	7
<i>Betula verrucosa</i> (birch)	rBet v 1	17.4	IPR-protein	95	0
	rBet v 2	14	Actin-binding (profilin)	10	100
<i>Dermatophagoides pteronyssinus</i> (house dust mite)	rDer p 2	14.1	Unknown	80	70
	rDer p 5	14	Unknown	15	45
	rDer p 7	23.7	Unknown	0	35
	rDer p 10	36	Tropomyosin	10	55

therefore compared the IgE reactivity profile of sera from Zimbabwe to nitrocellulose-blotted Bermuda grass and timothy grass pollen extracts. All sera exhibited strong IgE reactivity to Bermuda grass pollen allergens ranging, from 10 to 70 kDa, whereas two sera failed to react and four showed only weak reactivity with timothy grass pollen allergens (Fig. 1a, b). The profile of allergens detected by the African sera in timothy grass pollen extract was different from that recognized by sera from central Europe (Fig. 1b). No serum showed reactivity to group 2 or group 6 allergens migrating at approximately 10 kDa. The only serum (#11) exhibiting IgE reactivity to a low molecular weight component in timothy grass contained IgE against profilin. All sera containing timothy grass pollen allergen-specific IgE reacted with high molecular weight components (30 to 70 kDa) in timothy grass pollen, containing group 1 and group 4 allergens (Fig. 1b).

The pattern of IgE-reactive components in Bermuda grass pollen extract was different from that in timothy grass pollen extract (Fig. 1a). All sera reacted with a 30 to 35 kDa component, presumably representing the major Bermuda grass pollen allergen, Cyn d 1. Nine sera exhibited IgE reactivity to low molecular weight (approximately 10 kDa) components in Bermuda grass pollen extract.

Discussion

In this study we have analysed the allergen spectrum defined by allergic patients from Zimbabwe, a country located in central Africa. Screening of 650 allergic patients by skin prick testing with nine different allergen extracts revealed the highest sensitization rates to house dust mite and grass pollen extracts. An in-depth *in vitro* serological analysis performed with 20 respiratory and 20 food allergen extracts also identified mites and grass pollen as the most important allergen sources and thus confirmed the results obtained by skin prick testing. The results obtained with allergen extracts in the African population are similar to those obtained for industrialized countries where

mites and grass pollen represent the most important allergen sources [12–14]. However, when the allergen profile was analysed in more detail with a panel of recombinant timothy grass pollen and house dust mite allergens, striking differences were noted between grass pollen-allergic and mite-allergic patients from Africa (Zimbabwe) and from central Europe (Austria).

While the recognition frequency of the individual recombinant grass pollen allergens in the Austrian population was comparable with that reported for other European countries [26], grass pollen-allergic patients from Africa showed little (rPhl p 5, rPhl p 6) or no (rPhl p 2) IgE reactivity to certain recombinant timothy grass pollen allergens, which are frequently detected in Europe [26]. On the other hand we noted a much higher prevalence of IgE recognition of Phl p 4 in the African population than in the Austrian patients. The unusual sensitization profile of the African patients is most likely due to sensitization to grasses belonging to the Chloridoideae (e.g. Bermuda grass: *Cynodon dactylon*), which may express species-specific allergens that do not cross-react with the established panel of recombinant timothy grass pollen allergens (i.e. rPhl p 2, rPhl p 5, rPhl p 6). The latter assumption was supported by two other findings: (i) the prevalence of skin reactivity in the African patients to *Cynodon dactylon* pollen extract (38%) was higher than that to a grass pollen mix (26%); (ii) all grass pollen-sensitive patients from Africa reacted with nitrocellulose-blotted Bermuda grass pollen extracts, whereas several sera failed to react with timothy grass pollen extract.

The testing with recombinant timothy grass pollen allergens therefore indicates that grass pollen-allergic patients from central Africa are sensitized preferentially against species-specific epitopes from Bermuda grass pollen. Diagnosis of grass pollen allergy in central Africa may hence be improved by the use of recombinant Bermuda grass pollen allergens.

Interesting differences were also noted regarding the recognition frequency of recombinant mite allergens in the African and Austrian population. Again the IgE reactivity profile of recombinant mite allergens in the Austrian sera reflected closely that reported for other countries [27], whereas the African sera

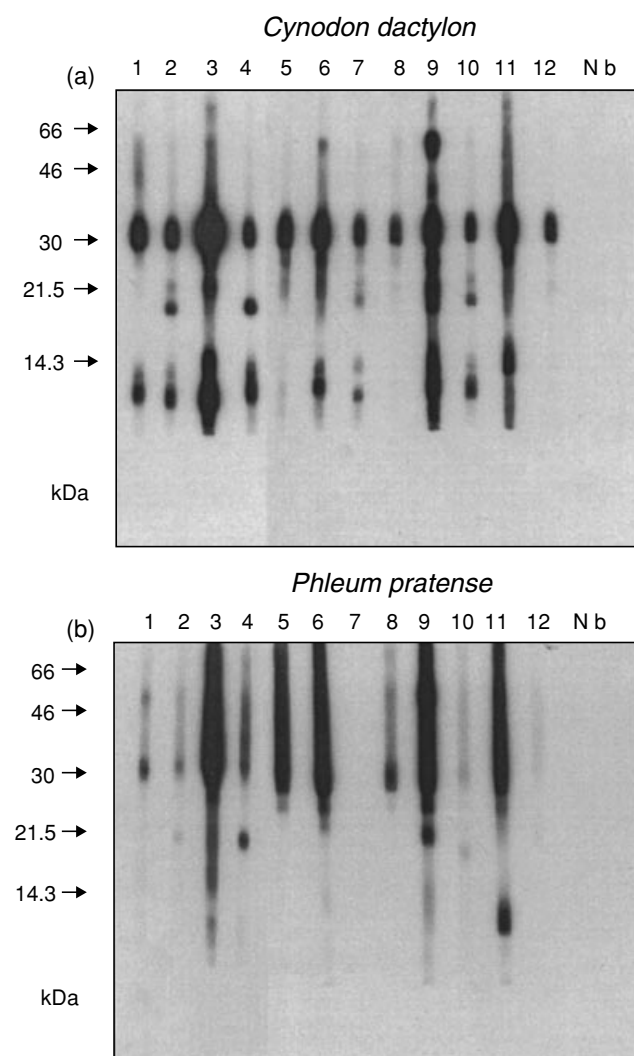


Fig. 1. IgE reactivity of grass pollen-allergic patients from Zimbabwe to nitrocellulose-blotted grass pollen allergens. Sera from 12 grass pollen-allergic patients (lanes 1–12), a non-atopic individual (lane N) and buffer alone (lane b) were exposed to Bermuda grass (a, *Cynodon dactylon*) and timothy grass (b, *Phleum pratense*) pollen extract. Molecular weights (kDa) are shown on the left margin.

reacted frequently with mite allergens that represent rather rare allergens (e.g. Der p 10) [27, 28].

The unusual high rate of IgE positivity to Der p 10 (55%) may reflect sensitization by other invertebrates containing tropomyosin as cross-reactive allergen, for example: insects (cockroaches); crustaceans (shrimp, lobster, crab, crawfish); and molluscs, squid [29, 30]. This hypothesis would be in agreement with the rather high frequency of IgE reactivity (22%) to crab detected with the multiallergen test in the African population.

The imprecision of extract-based diagnosis also became evident when the IgE reactivity profiles of the patients containing IgE antibodies to birch pollen extract were analysed with recombinant allergen molecules. Although birch is very uncommon, perhaps even non-existing in Zimbabwe, we found that 14% of the allergic patients contained IgE antibodies against birch pollen extract. These sera did not contain IgE against the major birch pollen allergen, Bet v 1, but reacted with the highly

cross-reactive birch profilin, Bet v 2 [25]. The latter finding indicates that these patients were originally sensitized against profilins from plant species that are more common in Africa and cross-reacted with birch profilin [19].

Our study thus demonstrates that IgE-mediated allergies are common in central Africa and strongly reflect the local exposure to prevalent allergen sources (grass pollen, mites). However, the detailed analysis of the IgE reactivity profiles performed with recombinant allergens demonstrates interesting differences regarding the sensitization to allergen molecules in Africa vs. Europe. The latter finding exemplifies the importance of using defined allergen molecules (i.e. recombinant allergens) from the sensitizing environment for refined diagnosis and its possible impact on allergy prevention and specific immunotherapy.

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