

Letter to the Editor

Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates

To the Editor:

Recent studies regarding the prevalence and type of respiratory and food allergies in Asian-Pacific populations have revealed not only parallels but also interesting differences concerning the occurrence of allergies in populations in these countries when compared with Westernized populations. Clinically relevant peanut and tree nut allergies occurred rarely in Asian-Pacific countries such as the Philippines, whereas fish allergy was very frequent.^{1,2} However, the “Allergies in Asia-Pacific Survey” indicated that allergic rhinitis, in particular seasonal allergic rhinitis, may be very common in the Asian-Pacific countries.³ Moreover, a high prevalence of IgE sensitizations to pollen extracts prepared from pollen of local tropical grasses was reported for a population from the Philippines.⁴

The International Study of Asthma and Allergies in Childhood Questionnaire on Allergic Screening (see the [Appendix](#) in this article’s Online Repository at www.jacionline.org) was used to recruit symptomatic subjects who reported symptoms indicative of allergy and asymptomatic subjects without such symptoms in the Philippines.⁴ An in-depth analysis of the molecular IgE reactivity profiles in 79 sera from symptomatic subjects and in 20 sera from asymptomatic subjects was performed by using an allergen microarray containing 103 allergen molecules (see [Table E1](#) and [Methods](#) in the Online Repository at www.jacionline.org). IgE antibodies were found to at least 1 of the 103 allergens in 48% symptomatic subjects and, interestingly, in 40% of the asymptomatic subjects. Unlike grass pollen-allergic patients from temperate climate areas who are sensitized to the major grass pollen allergens, Phl p 1, Phl p 2, Phl p 5, and Phl p 6,⁵ 25% of symptomatic patients showed an almost exclusive IgE reactivity to natural glycosylated grass pollen allergens nCyn d 1 or nPhl p 4 whereas nonglycosylated recombinant grass pollen allergens were almost not recognized ([Fig 1, A](#)). nCyn d 1 and nPhl p 4, and several other glycoproteins present on the microarray ([Fig 1, A](#), underlined) contain N-linked carbohydrates, which in most cases are $\beta(1,2)$ -xylose and $\alpha(1,3)$ -fucose motifs. These motifs occur as cross-reactive carbohydrate determinants (CCD) in various allergen sources (eg, plants, insects, and animals) and react with IgE antibodies without inducing relevant clinical symptoms.⁶ Only 2 sera reacted with nonglycosylated recombinant grass pollen allergens (ie, rPhl p 1, Phl p 7, and rPhl p 11) ([Fig 1, A](#)). Interestingly, asymptomatic subjects also showed IgE reactivity to nCyn d 1 and/or nPhl p 4 ([Fig 1, A](#)). Similar results were obtained for other pollen allergens where both symptomatic and asymptomatic subjects showed an almost exclusive IgE reactivity to natural glycosylated pollen allergens (eg, nCry j 1, nCup a 1, nOle e 1, and nPla a 2) but did not react with nonglycosylated pollen allergens ([Fig 1, A](#)). Rice is a frequently grown plant in the Philippines but only 1 of the sera containing IgE antibodies to nCyn d 1 reacted with the homologous recombinant major rice allergen Ory s 1 (data not shown).

Natural glycosylated major bee venom allergen nApi m 1 was also frequently recognized by 14% and 15% sera from

symptomatic and nonsymptomatic subjects, respectively ([Fig 1, A](#)). Interestingly, none of the subjects showed IgE reactivity to Bromelain ([Fig 1, B](#)), which because of its carbohydrate chain similarities with many N-linked glycoproteins has been suggested as a marker for carbohydrate sensitization. Weed pollen, animal, plant food, latex, parasite and mould allergens were either rarely or not recognized ([Fig 1, A](#) and [B](#); see [Table E1](#)). House-dust mites (HDM) represented the second major allergen source after grass pollen. Similar to data obtained for Middle Europe, twenty percent of the symptomatic patients recognized HDM allergens, in particular group 1 and 2 allergens, but none of the asymptomatic subjects showed IgE reactivity to HDM ([Fig 1, A](#) and [B](#)), which is similar to Middle Europe.⁷ Animal allergens were also recognized only by symptomatic subjects ([Fig 1, B](#)). Furthermore, tropomyosin from shrimp and mites was quite frequently recognized by symptomatic subjects ([Fig 1, A](#)). Thus, symptomatic individuals were discriminated from asymptomatic subjects through IgE reactivity to non-glycosylated allergens.

By using antibody probes specific for group 1, 2, 5, 6, 7, and 13 grass pollen allergens, we searched for related allergens in extracts from 5 tropical grasses prevalent in the Philippines (ie, *Cynodon dactylon*, *Saccharum spontaneum*, *Chloris barbata*, *Sporobolus indicus*, and *Imperata cylindrica*), rice (*Oryza sativa*), and maize (*Zea mays*) and used timothy grass (*Phleum pratense*) pollen extract for control purposes (see [Fig E1](#) in the Online Repository at www.jacionline.org; see [Methods](#)). Each of the 6 allergen groups could be detected in timothy grass, whereas only group 7 allergens, which are highly cross-reactive calcium-binding proteins, were found in the tropical grasses, rice, and maize. The major group 1 allergens were detected in rice, *C dactylon*, *C barbata*, *S indicus*, and maize, and group 13 allergens were found in *C dactylon*, *S spontaneum*, *C barbata*, *S indicus*, and maize. Group 2 and group 5 allergens could not be detected in any of the tropical grasses or rice. A weak reactivity of the anti-group 6 antibodies was observed in *C barbata*, with bands of approximately 15 and 23 kDa, which however did not match the molecular weight of group 6 allergens (ie, approximately 12 kDa). The band detected by group 2-specific antibodies at approximately 30 kDa in maize most likely results from cross-reactivity with group 1 allergens, which contain similar regions of sequence to group 1. Our results demonstrated that the allergen profiles of the tropical grasses resemble that of Bermuda grass in-as-much as they contained group 1, group 7, and group 13 allergens. Because the sequence and structural similarities of group 1 allergens among grasses are very high,⁸ the lack of sensitization to the protein backbone of group 1 allergens cannot be explained by lack of group 1-related allergens in grasses growing in the Philippines. We therefore must consider alternative explanations for the lack of clinically relevant sensitizations toward grass pollen. One possibility would be parasitic infestations inducing mainly IgE antibodies to carbohydrate epitopes as reported for other tropical populations,⁹ but according to the International Study of Asthma and Allergies in Childhood questionnaire used to select the individuals, worm infestations were excluded.

Another possibility would be that everyday contact of an individual with plant materials such as grass pollen grains may favor the development of anti-CCD antibodies.⁶ This could explain the detected predominance of anti-CCD IgEs in both

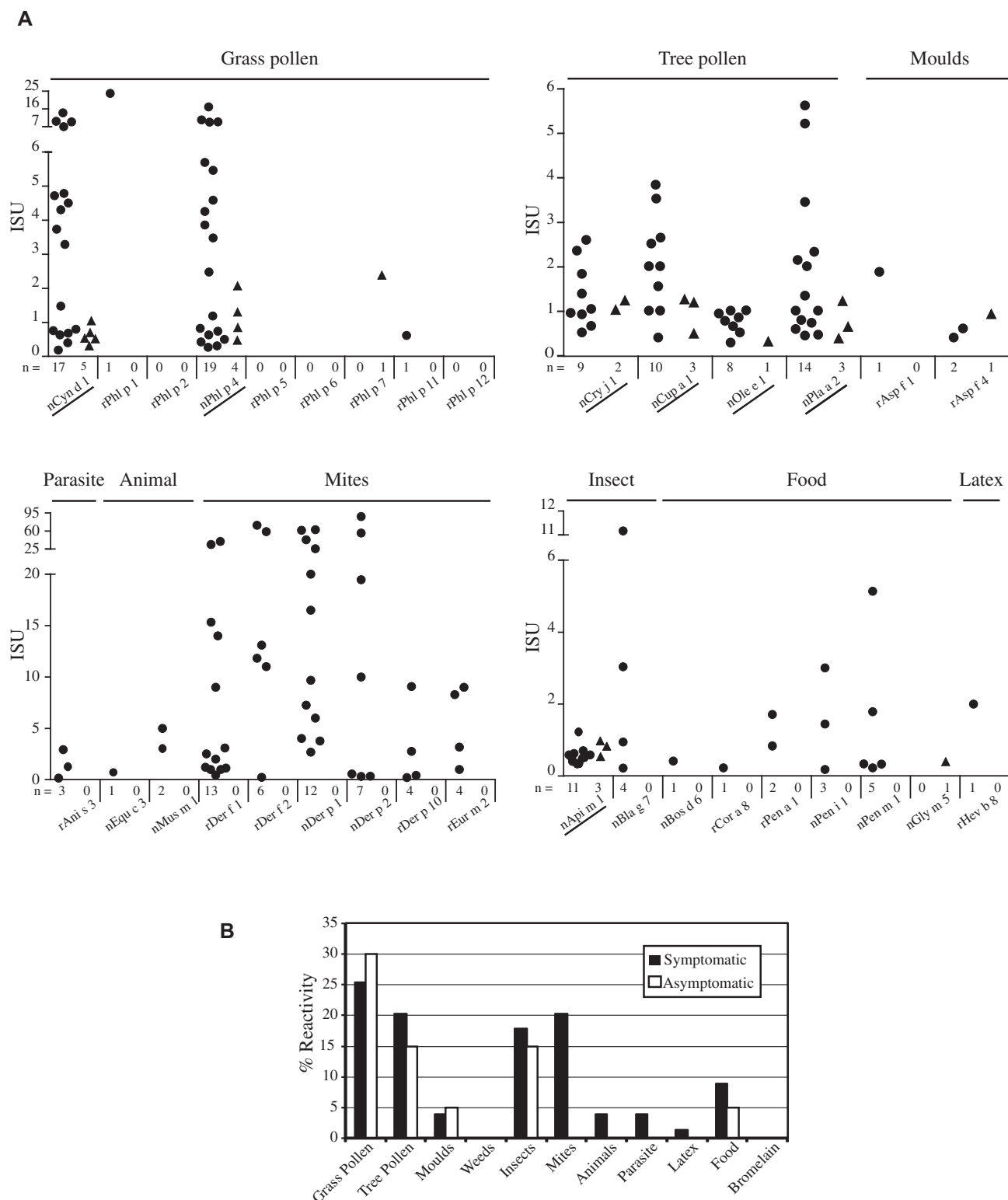


FIG 1. IgE reactivities of symptomatic and asymptomatic individuals from the Philippines to micro-arrayed allergens. **A**, IgE reactivities (y-axes: ISU) of symptomatic (dots) and asymptomatic individuals (triangles) to allergens on the chip (x-axes: allergens, allergen sources, number of positive sera, allergens with known glycosylation are underlined). **B**, Summary of the frequencies of IgE reactivities in symptomatic and asymptomatic individuals according to allergen sources.

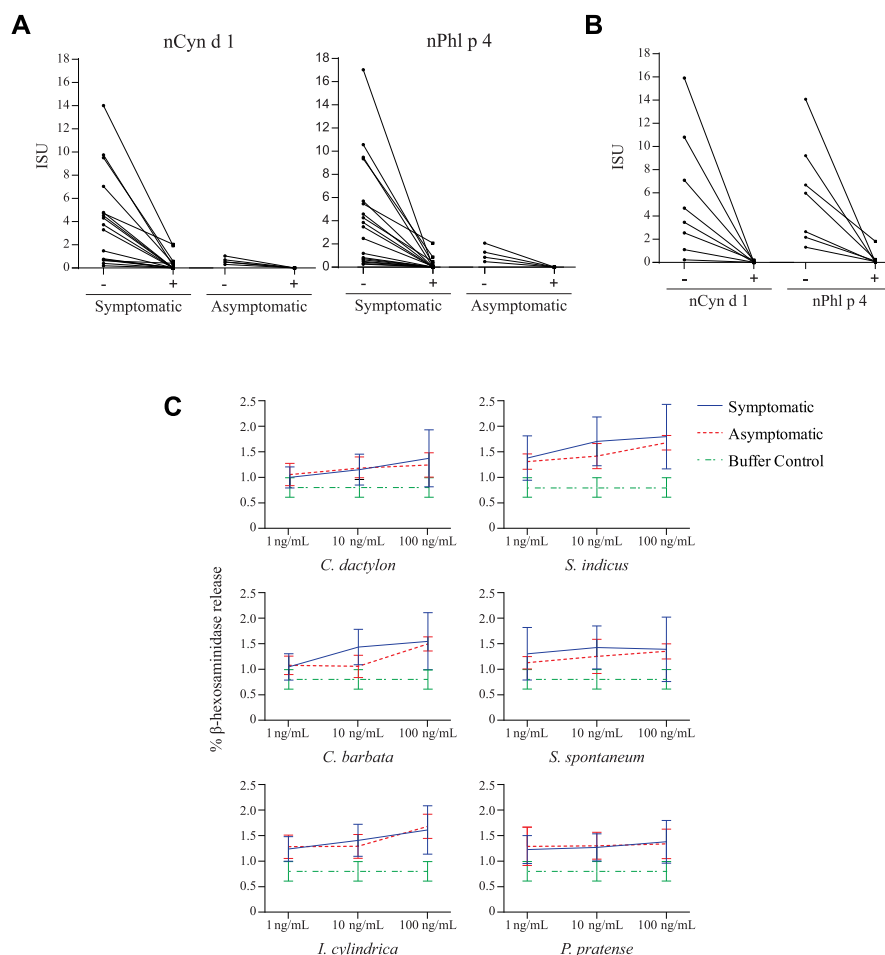


FIG 2. A, IgE reactivity (y-axis) of sera from subjects with grass pollen-specific IgE to nCyn d 1 (left panel) and nPhl p 4 (right panel) after preincubation with (+) or without (-) nPhl p 4. B, IgE reactivity (y-axis) of sera of untreated (-) and deglycosylated (+) nCyn d 1 and nPhl p 4. C, Mediator release induced by grass pollen allergens (x-axis) displayed as percentages of total β -hexosaminidase (y-axis) for symptomatic and asymptomatic subjects or buffer.

symptomatic and asymptomatic subjects. Therefore, we performed IgE inhibition studies (see [Methods](#)) to investigate whether Phl p 4-associated carbohydrate epitopes represent major IgE-reactive structures. Preincubation of sera with nPhl p 4 caused a more than 90% inhibition of IgE binding to nCyn d 1 ([Fig 2](#), A, left panel). Moreover, nPhl p 4 strongly inhibited IgE binding to the other glycosylated pollen allergens (ie, nCry j 1: 78.6%; nCup a 1: 82.3%; nOle e 1: 100%; and nPla a 2: 99.1%) and to the venom allergen Api m 1 (100%) but had no relevant effect on IgE binding to the nonglycosylated allergens on the chip. Autoinhibition of a similar degree of IgE binding to nPhl p 4 was obtained by preincubation of the sera with nPhl p 4 ([Fig 2](#), A, right panel). Furthermore, the deglycosylation of the allergens on the chip by periodate treatment caused a more than 90% reduction in IgE binding to nCyn d 1 and nPhl p 4 ([Fig 2](#), B) but had no relevant effects on IgE binding to nonglycosylated allergens (see [Fig E2](#) in the Online Repository at www.jacionline.org).

Galactose alpha-1,3-galactose (α -Gal), an animal-derived glycan, has been described as a frequent cause of clinically relevant as well as asymptomatic sensitization in allergic patients.⁹ However, only 2 of the 11 tested sera from symptomatic

or asymptomatic patients showed weak IgE reactivity to α -Gal (ie, <0.48 kU_A/L) whereas sera from α -Gal-positive patients (positive control) displayed strong IgE reactivity (>20 kU_A/L) (see [Fig E3](#) in the Online Repository at www.jacionline.org).

We therefore assumed that the tropical flora, and in particular the tropical grasses, produce highly glycosylated allergens where protein epitopes are hidden by the carbohydrates and as a result primarily induce clinically irrelevant IgE sensitizations against carbohydrate epitopes. This assumption was supported by experiments studying the possible allergenic activity of carbohydrate IgE epitopes in tropical grasses. Rat basophil leukemia-2H3 cells expressing the human high-affinity receptor for IgE were loaded with serum IgE from subjects who had shown IgE reactivity to Phl p 4 and then exposed to natural pollen extracts from the tropical grasses ([Fig 2](#), C) and to timothy grass (*P. pratense*) extract. Although each of the tested sera had shown IgE reactivity to pollen extracts from the tropical grasses, no basophil degranulation was observed ([Fig 2](#), C). When serum from an Austrian timothy grass pollen allergic patient was used to load the rat basophil leukemia cells followed by incubation to timothy grass pollen extract, a dose-dependent degranulation was obtained (see [Fig E4](#) in the Online Repository at www.jacionline.org).

Our results thus reveal an astonishing high frequency of IgE sensitization to nonallergenic, grass pollen–derived carbohydrate epitopes and the absence of IgE reactivity to highly allergenic grass pollen protein allergens in the Philippines, indicating that the local flora consisting of tropical grasses does not induce clinically relevant sensitizations. We consider these data important because they reveal that a certain environment (ie, the presence of certain plant pollens and a certain climate) seems to protect against clinically relevant sensitization to plants.

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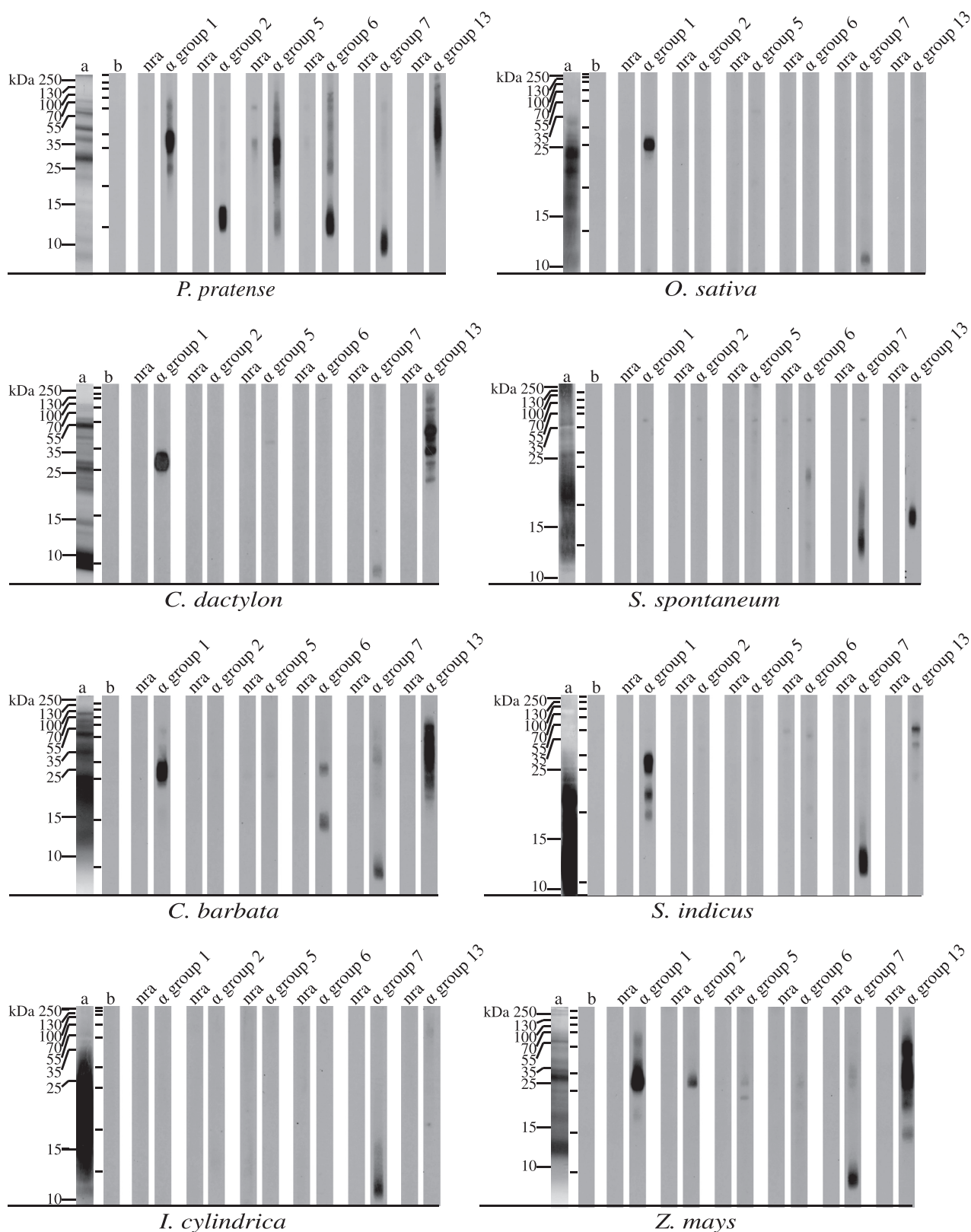


FIG E1. Presence of allergens in timothy grass and tropical grasses. Pollen extracts were subjected to SDS-PAGE and silver stained (lanes a), and after Western blotting, analyzed by using rabbit antisera specific for grass pollen allergen groups (lanes α group 1, 2, 5, 6, 7, and 13), normal rabbit antibodies (lanes nra), or buffer (lanes b). Molecular weights are indicated on the left margins.

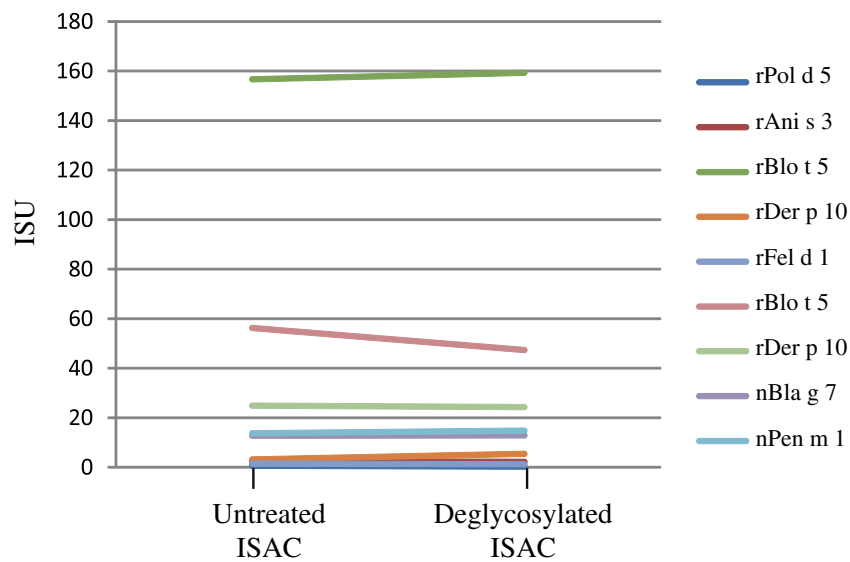


FIG E2. IgE reactivity of individual sera (*y-axis*: ISU) to allergens on ImmunoCAP ISAC after periodate treatment (deglycosylated ISAC) or after exposure to buffer alone (untreated ISAC).

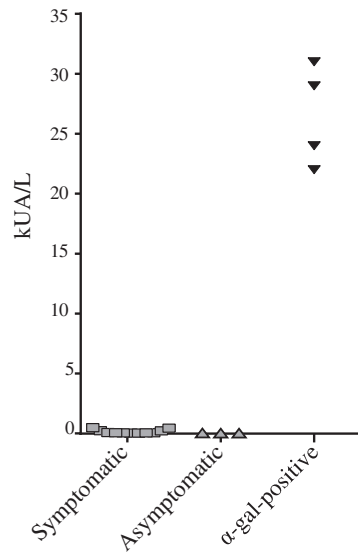


FIG E3. Quantitative IgE binding (*y-axis*: kUA/L) of 11 nPhl p 4-positive symptomatic, 3 asymptomatic subjects, and 4 α -Gal-positive sera (positive controls) (*x-axis*) to α -Gal.

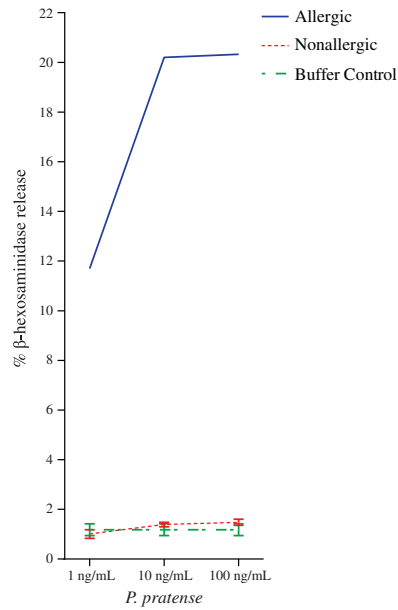


FIG E4. Induction of rat basophil leukemia cell degranulation (*y-axis*) with different concentrations of timothy grass pollen allergens (*x-axis*) when cells were loaded with serum IgE from a patient allergic to timothy grass pollen versus lack of degranulation when serum from a nonallergic individual or buffer was used.

TABLE E1. List of purified allergen molecules of the ImmunoCAP ISAC 103, grouped according to allergen sources

Allergen Source	Allergen
Grass pollen	nCyn d 1
	rPhl p 1
	rPhl p 2
	nPhl p 4
	rPhl p 5
	rPhl p 6
	rPhl p 7
	rPhl p 11
	rPhl p 12
	rBet v 1
	rBet v 2
Tree pollen	rBet v 4
	rAln g 1
	rCor a 1.0101
	nCry j 1
	nCup a 1
	nOle e 1
	nOle e 2
	rPla a 1
	nPla a 2
	nAmb a 1
	nArt v 1
Weed pollen	nArt v 3
	rPar j 2
	nSal k 1
	rMer a 1
	rDer f 1
Mites	rDer f 2
	nDer p 1
	nDer p 2
	rDer p 10
	rEur m 2
Animals	rCan f 1
	rCan f 2
	nCan f 3
	nEqu c 3
	rFel d 1
	nFel d 2
	rFel d 4
Insect	nMus m 1
	nApi m 1
	nApi m 4
	rBla g 1
	rBla g 2
	rBla g 4
	rBla g 5
Latex	nBla g 7
	rHev b 1
	rHev b 3
	rHev b 5
	rHev b 6
Parasite	rHev b 8
	rAni s 1
	rAni s 3
Molds	rAlt a 1
	rAlt a 6
	rAsp f 1
	rAsp f 2
	rAsp f 3
	rAsp f 4
	rAsp f 6
	rCla h 8

(Continued)

TABLE E1. (Continued)

Allergen Source	Allergen
Non-plant derived food	nBos d 4
	nBos d 5
	nBos d 6
	nBos d 8
	nBos d (Bovine lactoferrin)
	nGal d 1
	nGal d 2
	nGal d 3
	nGal d 5
	rCyp c 1
	rGad c 1
Plant food	rPen a 1
	nPen i 1
	nPen m
	nAct d 1
	nAct d 2
	nAct d 5
	nAct d 8
	rApi g 1
	rDau c 1
	rMal d 1
	rPru p 1
	nPru p 3
	rAna o 2
	nAra h 1
	nAra h 2
	nAra h 3
	rAra h 8
	rBer e 1
	rCor a 1.0401
	rCor a 8
	nCor a 9
	rGly m 4
	nGly m 5
	nGly m 6
	nSes i 1
	nTri a 18
	nTri a (Wheat Gliadin)
	rTri a 19.0101
	nTri a a (Wheat trypsin inhibitor)
Carbohydrate marker	nAna c 2 (Bromelain, N-linked glycan from Pineapple)

N, Natural; r, recombinant.

E2. ISAAC Questionnaire on Allergy Screening

I. Questions on asthma

1. Has your child ever had wheezing or whistling in the chest at any time in the last 12 months? IF NO, please proceed to Question 6. If YES, please continue.	☐ Yes ☐ No
2. In the last 12 months, has your child had a dry cough at night, apart from a cough associated with a cold or chest infection? IF NO, please proceed to Question 6. If YES, please continue.	☐ Yes ☐ No
3. How many attacks of wheezing has your child had in the last 12 months?	☐ None ☐ 1 to 3 ☐ 4 to 12 ☐ More than 12
4. In the last 12 months, how often, on average, has your child's sleep been disturbed due to wheezing?	☐ Never woken with wheezing ☐ Once a week ☐ More than once a week
5. In the last 12 months, has wheezing ever been severe enough to limit your child's speech to only one or two words at a time between breaths?	☐ Yes ☐ No
6. Has your child ever had asthma?	☐ Yes ☐ No
7. In the last 12 months, has your child's chest sounded wheezy during or after exercise?	☐ Yes ☐ No
8. In the last 12 months, has your child has a dry cough at night, apart from a cough associated with a cold or chest infection?	☐ Yes ☐ No

II. Questions on Rhinitis and Conjunctivitis

All questions are about problems which occur when your child DOES NOT have a cold or the 'flu'.

1. Has your child <u>ever</u> had any of the following symptoms for at least one hour on most days (or on most days during the season if your symptoms are seasonal)? • Watery runny nose • Sneezing, especially violent and bouts • Nasal obstruction • Nasal itching • Conjunctivitis (red, itchy eyes) • Postnatal drip 2. <u>In the past 12 months</u>, does your child have a problem with sneezing or a runny or blocked nose when he/she DID NOT have cold or the "flu"? IF NO, Proceed to Question 6. 3. <u>In the past 12 months</u>, has your child's nose problem been accompanied by itchy-watery eyes?	☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No ☐ Yes ☐ No												
4. In which of the <u>past 12 months</u> did this nose problem occur? (Please tick any which apply) <table border="0"><tr><td>☐ January</td><td>☐ April</td><td>☐ July</td><td>☐ October</td></tr><tr><td>☐ February</td><td>☐ May</td><td>☐ August</td><td>☐ November</td></tr><tr><td>☐ March</td><td>☐ June</td><td>☐ September</td><td>☐ December</td></tr></table>		☐ January	☐ April	☐ July	☐ October	☐ February	☐ May	☐ August	☐ November	☐ March	☐ June	☐ September	☐ December
☐ January	☐ April	☐ July	☐ October										
☐ February	☐ May	☐ August	☐ November										
☐ March	☐ June	☐ September	☐ December										
5. In the past 12 months, how much did this nose problem interfere with your child's daily activity? 6. Has your child ever had hay fever?	☐ Not at all ☐ A little ☐ A moderate amount ☐ A lot ☐ Yes ☐ No												

III. Questions on Dermatitis

1. Has your child <u>ever</u> had an itchy rash that was coming and going for at least six months? If NO, proceed to Question 7. If YES, please continue.	☐ Yes ☐ No
2. Has your child had itchy rash at any time <u>in the last 12 months</u>? If NO, proceed to Question 7. If YES, please continue.	☐ Yes ☐ No
3. Has this itchy rash at any time affected any of the following places: the fold of the elbows, behind the knees, in front of the ankles, under the buttocks, or around the neck, ears, or eyes?	☐ Yes ☐ No
4. At what age did this itchy rash first occur?	☐ Under 2 years ☐ Age 2-4 years ☐ Age 5 or more
5. Has this rash cleared completely at any time <u>during the last 12 months</u>?	☐ Yes ☐ No
6. <u>In the last 12 months</u>, how often, on average, had you child been kept awake at night by this itchy rash? ☐ Never in the last 12 months ☐ Less than 1 night per week ☐ 1 or more nights per week	
7. Has your child <u>ever</u> had eczema?	☐ Yes ☐ No

E1. METHODS

Subjects

Using the local dialect-translated and modified questionnaire from The International Study of Asthma and Allergies in Childhood (ISAAC) Questionnaire on Allergic Screening (Supplemental document E1),^{E1} subjects who reported symptoms indicative of allergy (e.g., asthma, rhinitis, conjunctivitis and/or dermatitis) were identified. Information regarding subjects' demographic characteristics and allergic symptoms were included in the questionnaire. Symptoms for allergic asthma were identified as positive response to 3 or more of the questions related to asthma. Evidence for allergic rhinitis and/or conjunctivitis was provided by the presence of watery runny nose and/or presence of redness or itchiness in the conjunctiva and behind the eyelids. The presence of itchy, relapsing skin rash that had affected the skin flexural parts was considered as dermatitis. Each of the subjects was personally interviewed. For subjects below 18 years, parents were the main respondents to obtain the children's information.^{E2}

Using the questionnaire 141 subjects with allergy-like symptoms (i.e., "symptomatic") were recruited in the Philippines. A second age and gender-matched group of 141 subjects without symptoms (i.e., "asymptomatic") according to the ISAAC questionnaire was recruited for control purposes. The age range in each group was 3 to 79 years with a mean age of 26.4 years (SD $\pm$ 20.9) and a gender distribution of 1:2 (male:female). All subjects stated not to have been infected by parasites.

Purified allergens and grass pollen allergen-specific antibodies

Natural Phl p 4 was purified as described^{E3} and recombinant major rice allergen, rOry s 1 as described.^{E4}

Galactose alpha-1,3-galactose (α -Gal)-specific IgE reactivity was analysed by ImmunoCAP system (Phadia-Thermo Fisher Scientific, Uppsala, Sweden). For control purposes, sera from patients described to have α -Gal-reactivity were used.^{E5}

Rabbit antibodies specific for the protein backbone of group 1, 2, 5, 6, 7 and 13 allergens were produced by immunization of rabbits with purified recombinant timothy grass allergens (i.e., rPhl p 1, 2, 5, 6, 7 and 13).^{E6}

Determination of IgE reactivity to micro-arrayed and deglycosylated allergens and IgE inhibition studies using ImmunoCAP Immuno Solid-phase Allergen Chip (ISAC)

Seventy-nine randomly picked sera from symptomatic subjects and from 20 randomly picked sera from asymptomatic subjects were subjected to allergen microarray analysis containing 103 different allergens (see Table E1). Sera were tested on the ImmunoCAP ISAC (Phadia-Thermofisher, Uppsala, Sweden) according to the manufacturer's instruction.

In IgE inhibition assays, sera from symptomatic (n=18) and asymptomatic (n=6) were pre-incubated either with phosphate buffered saline (PBS, negative control) or with 25 μ g/mL of nPhl p 4. Displayed are the levels of allergen-specific IgE in ISAC Standard Units (ISU). The percentage inhibition of IgE binding was calculated as follows: Percent inhibition = $100 - (\text{ISU}^a \times 100 / \text{ISU}^b)$. ISU^a and ISU^b represent the extinctions after pre-incubation with allergen and without allergen (i.e., buffer alone), respectively.

For deglycosylation, allergen chips were treated with 10mM Sodium (meta) Periodate (Sigma-Aldrich, Munich, Germany) for 1 hour at RT and incubated in the dark. This was followed by 30min incubation in 50mM Sodium borohydride (BDH, Poole, England) (in PBS) at 23°C for 30min prior to incubation with available sera from subjects with IgE to nCyn d 1 and to nPhl p 4.^{E3} Untreated allergen chips were subjected to buffer alone (negative control) before sera were added.

IgE reactivity was determined according to the manufacturer's instruction. Final data processing was performed using the Phadia Microarray Image Analysis Software.

Determination of IgE reactivity to recombinant Ory s 1 and to α -Gal

Allergen-specific IgE levels to Ory s 1 were measure by ELISA by coating 5 μ g/mL of the recombinant allergen with PBS in ELISA plates (Nunc Maxisorb, Roskilde, Denmark). Plates were incubated overnight (ON) at 4°C. After blocking with 1% (w/v) bovine serum albumin (BSA) in PBST (PBS + 0.05% Tween 20), plates were incubated overnight with human sera (1:5 in PBST, 0.5% [w/v] BSA). Bound human IgE antibodies were detected with 1:2,000 diluted purified goat anti-human IgE, horseradish peroxidase–labelled (KPL, Gaithersburg, MD, USA) and the optical densities (OD at 405 nm, reference OD at 490 nm) corresponding to bound IgE was recorded in an ELISA reader (Molecular Devices, Sunnyvale, CA, USA). The cut-off value was based on the mean plus 2 SD (standard deviations) of the OD value measured from sera of 5 non-allergic individuals. IgE antibody levels to α -Gal were measured using the ImmunoCAP system.^{E7}

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68 Preparation and characterization of natural grass pollen extracts

69 Pollens from grass species were collected from mature anthers of *C. dactylon* (Bermuda
70 grass), *C. barbata* (feather finger grass), *I. cylindrica* (cogon), *S. indicus* (smut grass), *S.*
71 *spontaneum* (wild sugar cane), *O. sativa* (rice), and *Z. mays* as described.^{E8} The purity of the
72 pollen was checked through microscopy (Olympus BX50, Olympus Corp, Japan). *P. pratense*
73 (timothy grass) which was used as a positive control was purchased from Allergon (Vaelinge,
74 Sweden). The grains were defatted with diethyl-ether (1/10 w/v) and mechanically digested
75 by freezing with liquid nitrogen and crushed by mortar and pestle. PBS (1/10 w/v) was added
76 and constantly stirred ON at 4°C. After centrifugation at 14,000 rpm for 20 min, supernatants
77 were passed through a 0.45 µm syringe filter and stored at -20 °C until further use. The
78 protein contents of the extracts were quantified by Bradford assay (BioRad, Hercules, CA,
79 USA)^{E9} and subjected to reducing sodium dodecylsulfate-polyacrylamide gel electrophoresis
80 (SDS-PAGE)^{E10} followed by silver staining or immunoblotting.

81 For immunoblotting, 60 µg/cm total protein of the natural pollen extract were subjected to a
82 10cm preparative 20% SDS-PAGE and blotted onto nitrocellulose membrane.^{E11}
83 Nitrocellulose-blotted extracts were exposed to different rabbit anti-sera specific for
84 individual grass pollen allergens or for control purposes, with serum from a non-immunized
85 rabbit (dilution: 1:10,000). Bound specific antibodies were detected with ¹²⁵I-labeled donkey
86 anti-rabbit antiserum (Amersham, Buckinghamshire, U.K.) and visualized by
87 autoradiography.^{E12}

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91 **Determination of the allergenic activity of natural grass pollen extracts by RBL assay**

92 RBL-2H3 cells expressing the human FcεRI were passively sensitized with sera (1:30
 93 diluted) from symptomatic or from asymptomatic subjects. Cells were then exposed to
 94 increasing concentrations (1ng/mL, 10ng/mL and 100ng/mL) of allergen extracts from *P.*
 95 *pratense*, *C. dactylon*, *I. cylindrica*, *C. barbata*, *S. indicus*, and *S. spontaneum* or buffer alone
 96 as described.^{E13} For control purposes, cells were incubated with serum from a *P. pratense*
 97 pollen allergic patient (positive control), sera from healthy individuals (n=2) (negative
 98 control) or with buffer alone.

99

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Table E1.

List of purified allergen molecules of the ImmunoCAP ISAC 103<comma>
grouped according to allergen sources

Allergen Source,Allergen

Grass pollen,nCyn d 1

,rPhl p 1

,rPhl p 2

,nPhl p 4

,rPhl p 5

,rPhl p 6

,rPhl p 7

,rPhl p 11

,rPhl p 12

Tree pollen,rBet v 1

,rBet v 2

,rBet v 4

,rAln g 1

,rCor a 1.0101

,nCry j 1

,nCup a 1

,nOle e 1

,nOle e 2

,rPla a 1

,nPla a 2

Weed pollen,nAmb a 1

,nArt v 1

,nArt v 3

,rPar j 2

,nSal k 1

,rMer a 1

Mites,rDer f 1

,rDer f 2

,nDer p 1

,nDer p 2

,rDer p 10

,rEur m 2

Animals,rCan f 1

,rCan f 2

,nCan f 3

,nEqu c 3

,rFel d 1

,nFel d 2

,rFel d 4

,nMus m 1

Insect,nApi m 1

,nApi m 4

,rBla g 1

,rBla g 2

,rBla g 4

,rBla g 5

,nBla g 7

Latex,rHev b 1

,rHev b 3

,rHev b 5

,rHev b 6

,rHev b 8

Parasite,rAni s 1

,rAni s 3

Molds,rAlt a 1

,rAlt a 6

,rAsp f 1

,rAsp f 2

,rAsp f 3

,rAsp f 4

,rAsp f 6

,rCla h 8

Non-plant derived food,nBos d 4

,nBos d 5

table.csv.txt

,nBos d 6
,nBos d 8
,nBos d (Bovine lactoferrin)
,nGal d 1
,nGal d 2
,nGal d 3
,nGal d 5
,rCyp c 1
,rGad c 1
,rPen a 1
,nPen i 1
,nPen m
Plant food,nAct d 1
,nAct d 2
,nAct d 5
,nAct d 8
,rApi g 1
,rDau c 1
,rMal d 1
,rPru p 1
,nPru p 3
,rAna o 2
,nAra h 1
,nAra h 2
,nAra h 3
,rAra h 8
,rBer e 1
,rCor a 1.0401
,rCor a 8
,nCor a 9
,rGly m 4
,nGly m 5
,nGly m 6
,nSes i 1
,nTri a 18
,nTri a (wheat Gliadin)
,rTri a 19.0101
,nTri a a (wheat trypsin inhibitor)
Carbohydrate marker,nAna c 2 (Bromelain<comma> N-linked glycan from Pineapple)

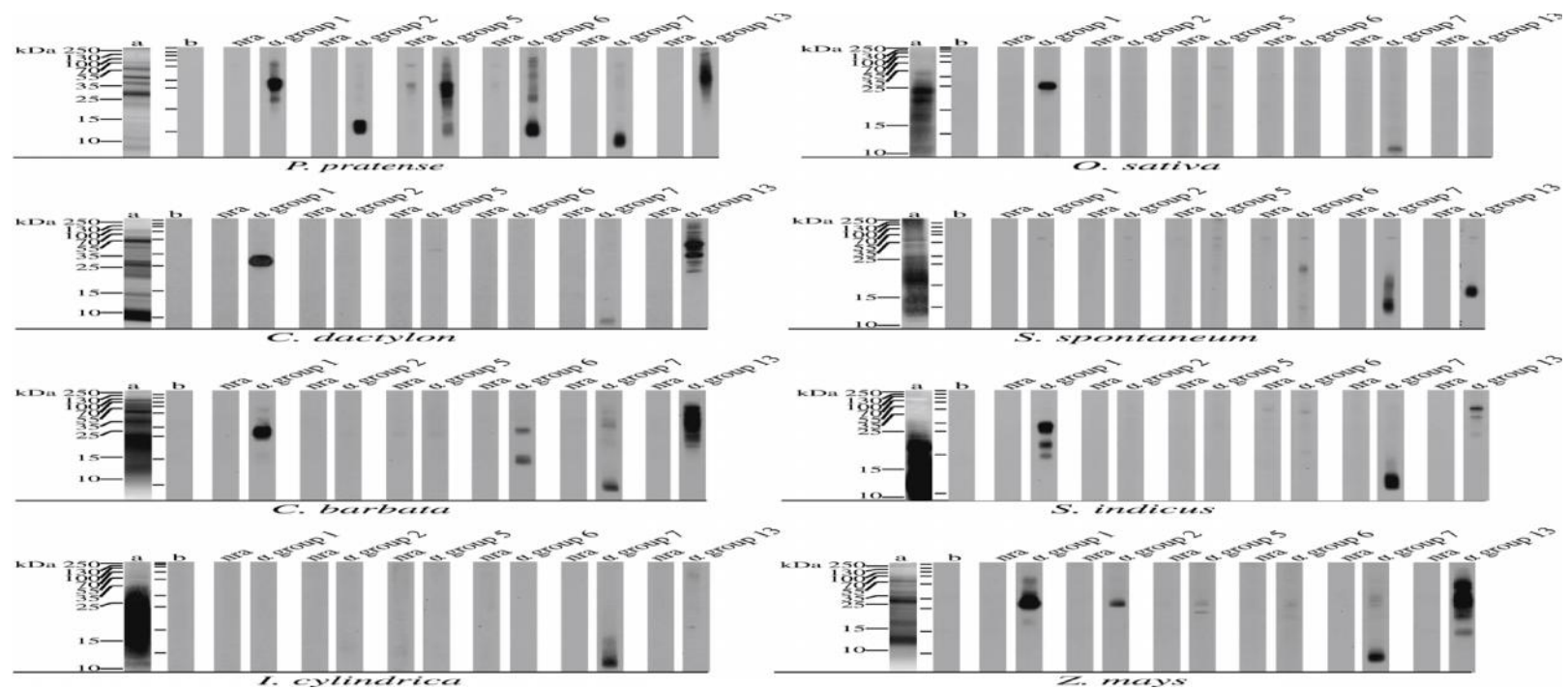


Fig E1 Presence of allergens in timothy grass and tropical grasses. Pollen extracts were subjected to SDS-PAGE and silver stained (lanes a), and after Western blotting, analyzed by using rabbit antisera specific for grass pollen allergen groups (lanes α gr...

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Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates

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<http://dx.doi.org/10.1016/j.jaci.2013.10.004>

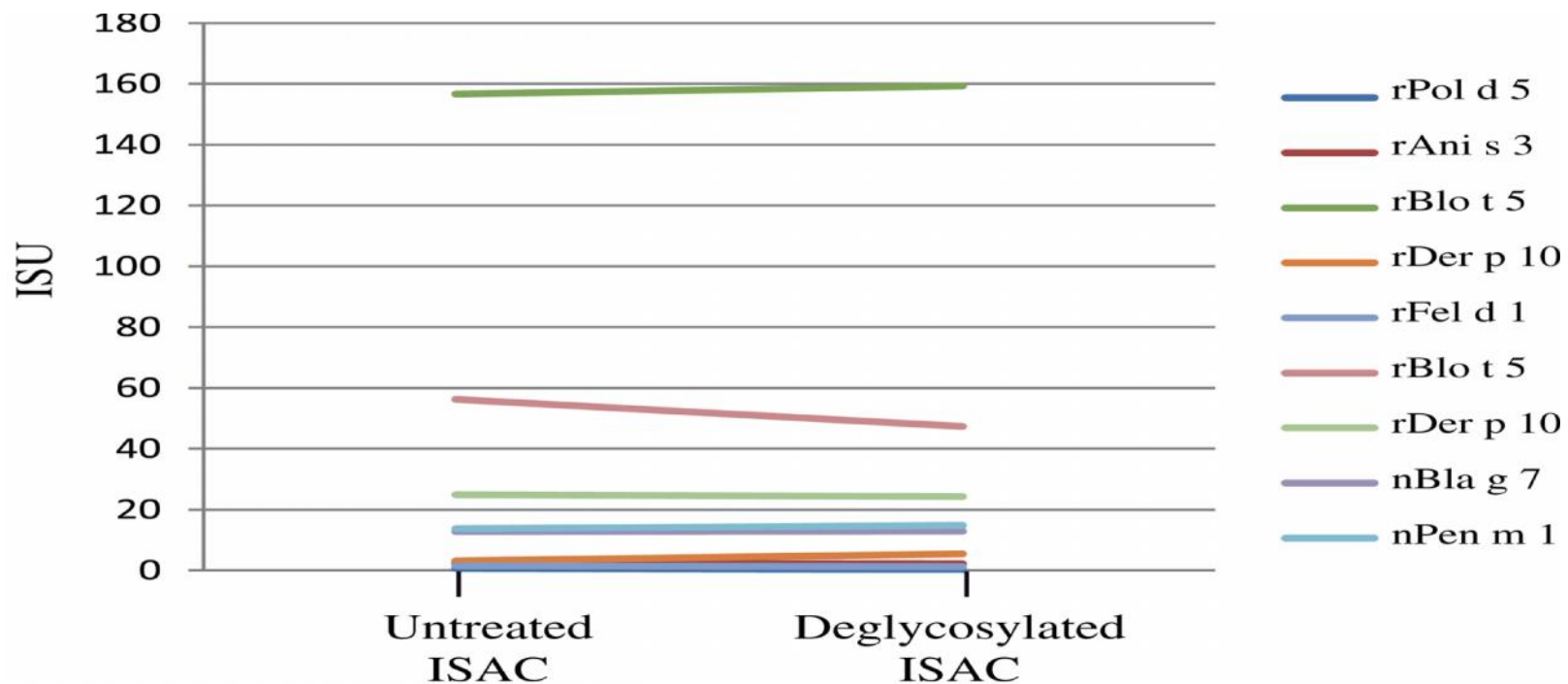


Fig E2 IgE reactivity of individual sera (y-axis : ISU) to allergens on ImmunoCAP ISAC after periodate treatment (deglycosylated ISAC) or after exposure to buffer alone (untreated ISAC).

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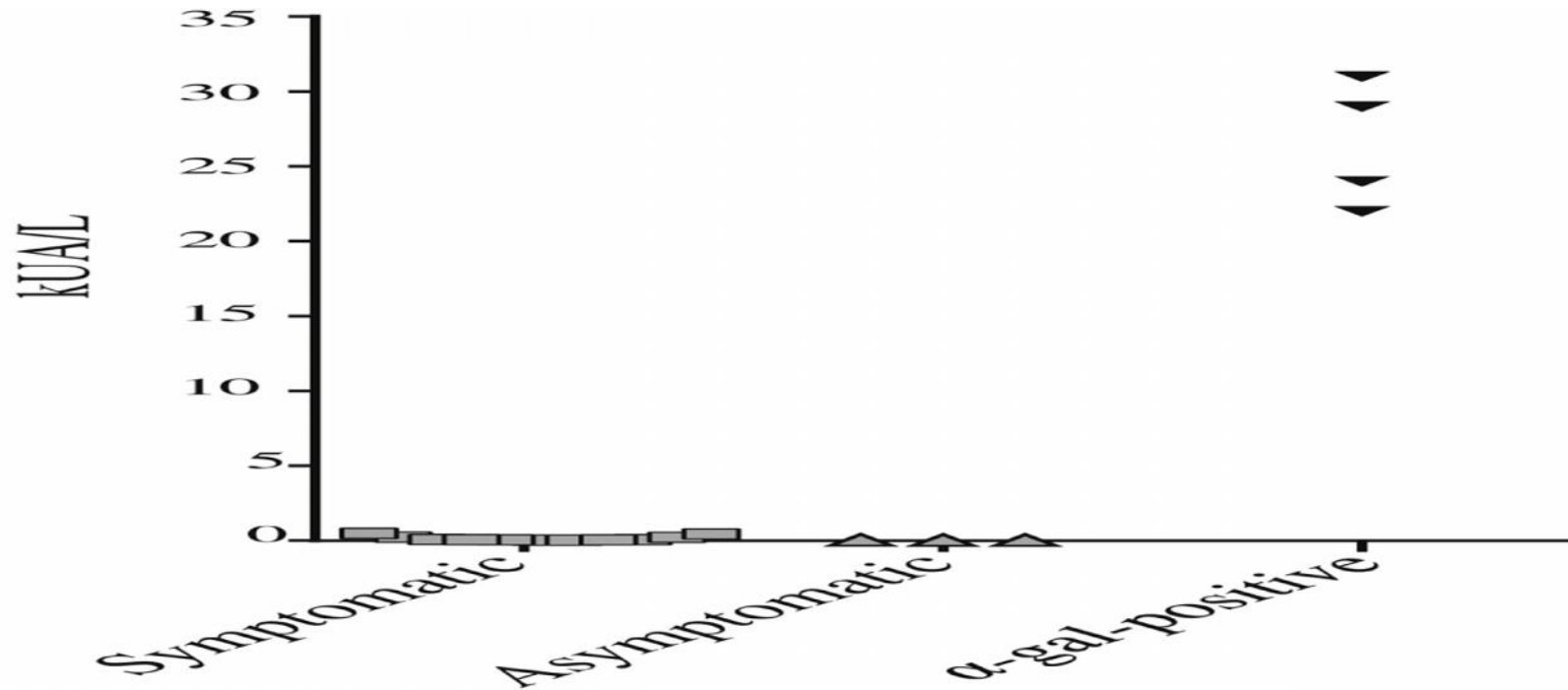


Fig E3 Quantitative IgE binding (y-axis : kU A /L) of 11 nPhl p 4-positive symptomatic, 3 asymptomatic subjects, and 4 α -Gal-positive sera (positive controls) (x-axis) to α -Gal.

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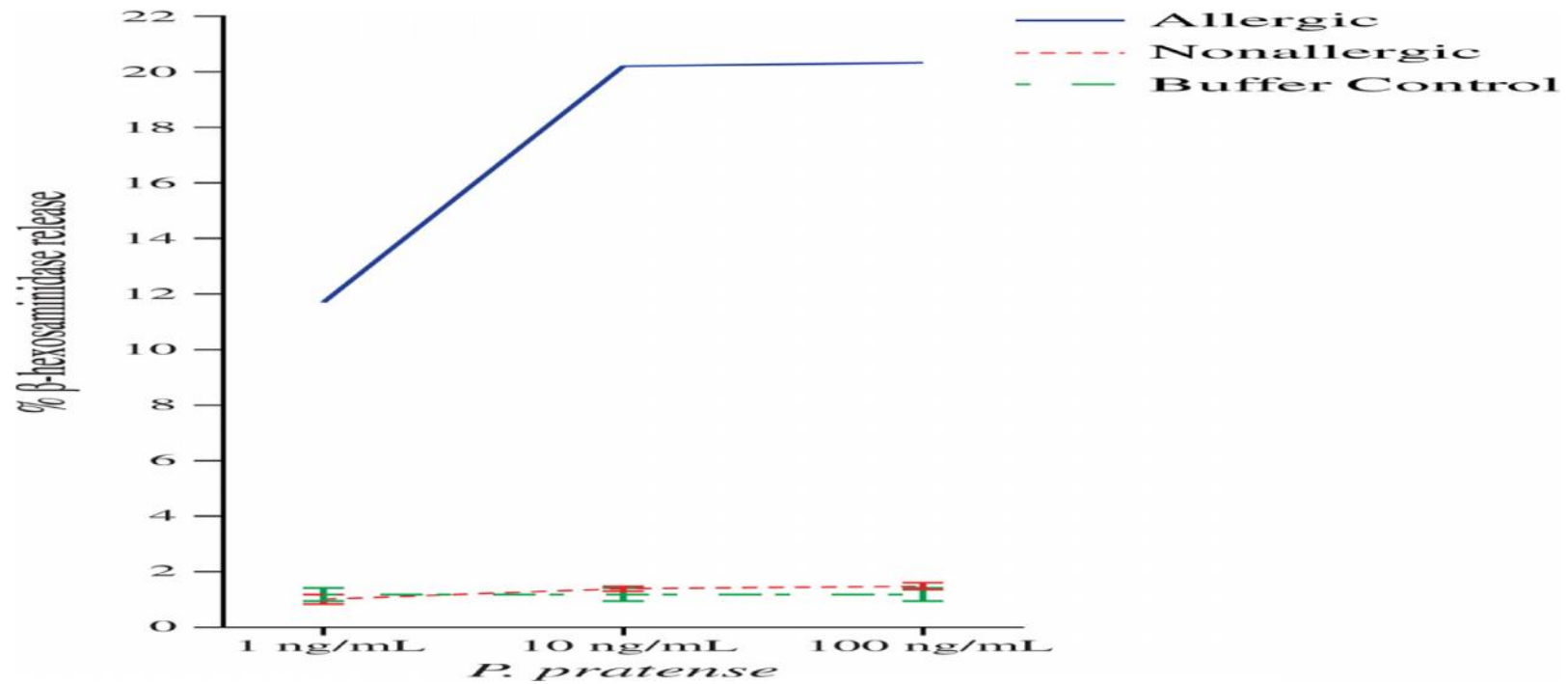


Fig E4 Induction of rat basophil leukemia cell degranulation (y-axis) with different concentrations of timothy grass pollen allergens (x-axis) when cells were loaded with serum IgE from a patient allergic to timothy grass pollen versus lack of degranul...

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