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by Sitti Wahyuni

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While interpreting our results, we acknowledge certain limitations. Although we used a cross-sectional study design, a longitudinal study following individual patients over time would be valuable in testing causality. Food-allergic patients had an increased prevalence of atopic dermatitis, allergic rhinitis, and asthma as would be expected, and differences in Treg cells may be important in the atopic march process and not solely food allergy. Therefore, some of our findings could have broader implications for the general predisposition of atopy.

In summary, we found that young, atopic food-allergic children have lower percentages of strictly defined Treg cells compared with healthy controls of similar age. Moreover, age-related increases in Treg-cell expression of CCR6 were observed in healthy controls but not food-allergic children, which may be important for Treg-cell migration to peripheral sites of inflammation in the maintenance of tolerance. Our study supports the concept that Treg-cell frequency is associated with the maintenance of tolerance in allergy.

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Molecular diagnostics and lack of clinical allergy in helminth-endemic areas in Indonesia

To the Editor:

Parasitic helminths and allergens are potent inducers of T_H2 responses that lead to high levels of IgE.[1] Despite the similar immunological profiles of helminth infections and allergic disorders, several cross-sectional and intervention studies have indicated a negative association between helminth infections and skin prick test (SPT) positivity to allergens.[2] In addition to the possible role of immune-regulatory networks induced by chronic helminth infections, cross-reactive IgE to helminths and environmental/food allergens with low biological activity may prevent mast cell degranulation.[3]

The allergen that results in the induction of the original allergic responses is the primary sensitizer, whereas others are considered cross-reactive allergens. IgE cross-reactivities can be due to homologous proteins, or to complex N-glycans on plant and invertebrate glycoproteins, known as cross-reactive carbohydrate determinants (CCDs).[4] The characterization of IgE in helminth-endemic areas has largely been based on allergen extracts. IgE against CCD has a very broad spectrum of cross-reactivity across the plant kingdom, invertebrates such as insects (venoms), and even parasites. Importantly, oral challenge with a CCD-bearing allergen in patients with IgE to CCD proved that CCD-specific IgE is of poor biological activity and has limited, if any, clinical relevance.[5] To fully understand the association between helminths and allergies, it is important to characterize IgE reactivity to allergen components at the molecular level.

The ImmunoCAP-ISAC microarray has recently been developed with individual purified natural and recombinant inhalant and food allergens.[6] This allows testing for individual molecular IgE recognition profiles, providing comprehensive information about likely sources of primary sensitization and cross-reactivity and helps elucidate the process of sensitizations and identify risk and protective factors for clinical phenotypes.

To elucidate the molecular basis of the extremely high prevalence of sensitization to common allergen sources (65% to 85%) among Indonesian school children, living in a helminth-endemic area, who have very low SPT reactivity and virtually absent clinical allergy, the IgE profiles to allergen components on ImmunoCAP-ISAC chips was studied. The study population selection from the ImmunoSPIN (www.immunospin.org) program,[7] and all methods are given in this article's Online Repository at www.jacionline.org. Of the study subjects, 93% were infected with soil-transmitted

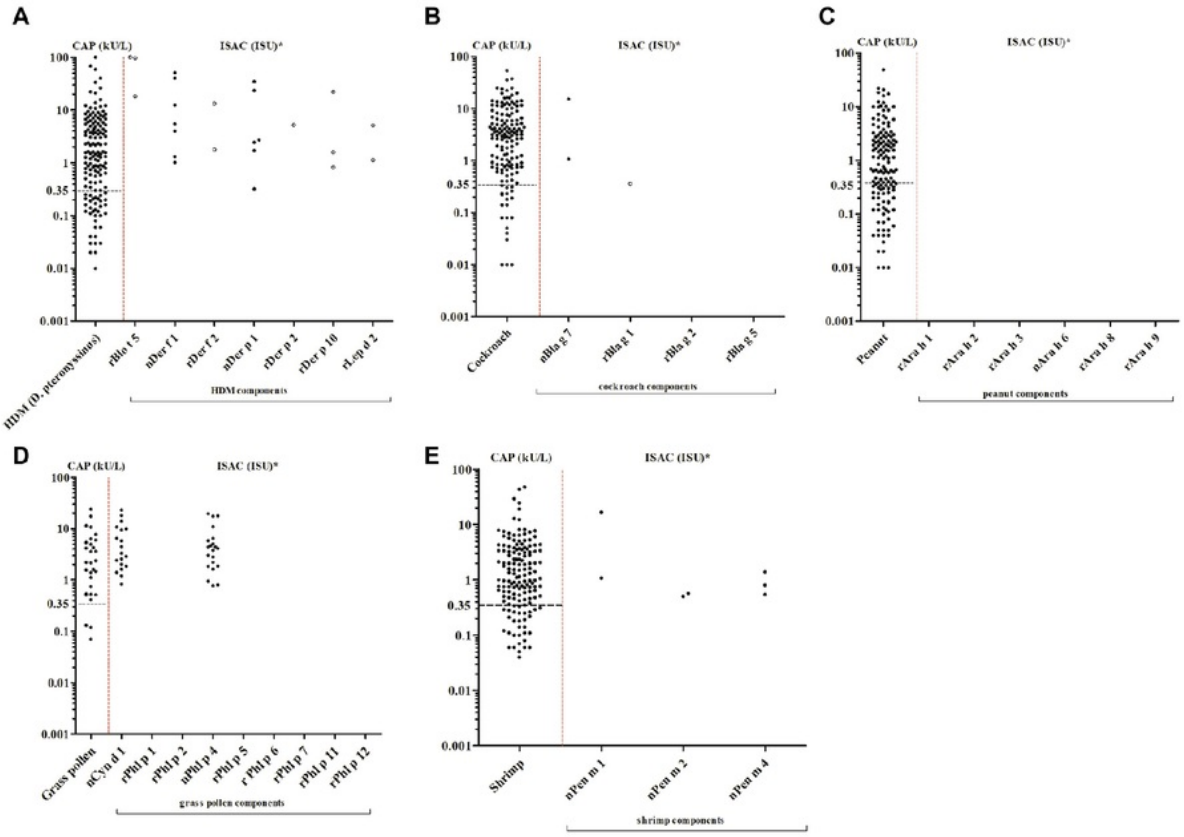

FIG 1. IgE measured by ImmunoCAP and ImmunoCAP-ISAC. IgE to HDM (*D pteronyssinus*) **(A)**, cockroach **(B)**, peanut **(C)**, grass pollen **(D)**, and shrimp **(E)** measured by ImmunoCAP and ImmunoCAP-ISAC. The single allergens depicted for ISAC are related to the whole allergen extracts tested in CAP. Solid circles represent natural (n) component allergens, and open circles represent recombinant (r) component allergens. *Horizontal dotted lines* represent the cutoff value for ImmunoCAP positivity. IgE levels are given as KU/L for ImmunoCAP and as ISAC standardized units (ISU)/L for ImmunoCAP-ISAC. *For ImmunoCAP-ISAC assay, only positive results are shown.

helminths (hookworm, *Ascaris lumbricoides* and *Trichuris trichiura*) with high total IgE (geometric mean, 2816 IU/mL). Sensitization to common inhalant and food allergen sources by ImmunoCAP assay was high: grass pollen, *Phleum pratense* (90%); cockroach, *Blattella germanica* (87%); shrimp, *Penaeus aztecus* (79%); house dust mite (HDM), *Dermatophagoides pteronyssinus (D pteronyssinus)* (74%); and peanut, *Arachis hypogaea* (65%) (see Table E1 in this article's Online Repository at www.jacionline.org; Fig 1). However, SPT reactivity was considerably lower than sensitization assessed by IgE: HDM (*D pteronyssinus* and/or *D farinae*) (27%), followed by cockroach (24%), peanut (6%), and shrimp (5%) (Table E1). Wheeze or eczema in the past 12 months was reported for only 5 children. None of the study subjects reported any symptoms of food allergy.

ImmunoCAP-ISAC microarray results indicated that IgE against commonly accepted major allergens of HDM (*D pteronyssinus*), cockroach, grass pollen, shrimp, and peanut was observed in only 0% to 5% of cases (Figs 1 and 2; see Table E2 in this article's Online Repository at www.jacionline.org). However, many individuals recognized several natural purified glycoprotein allergens from pollen (nCyn d 1, nPhl p 4, nCry j 1, nCup a 1, nOle e 1, nPla a 2) and from food (nJug r 2), all carrying typical CCD glycans (Fig 2), reflected in positivity of most plasma to MUXF3, representing a common CCD glycan. IgE responses to these natural glycoproteins and to MUXF3 were highly correlated ($0.60 < r < 0.77$), supporting CCD-based cross-reactivity (see Fig E1 in this article's Online Repository at www.jacionline.org). To further confirm CCD reactivity, a subgroup of 30 subjects, with sufficient plasma, recognizing natural glycoproteins, was tested for IgE against bromelain, which is a glycoprotein carrying the MUXF3 glycan structure. All were positive to bromelain and these responses correlated (see Fig E2 in this article's Online Repository at www.jacionline.org) with IgE to grass pollen and peanut ($r = 0.87$ and $r = 0.84$, respectively). Association with cockroach and shrimp was still significant ($r = 0.53$ and $r = 0.48$, respectively), which was not the case for HDM (*D pteronyssinus*), implying that typical CCD structures can most likely not (fully) explain reactivity to these allergen sources. CCD-specific IgE, titrated ImmunoCAP inhibition assays confirmed that CCD-based cross-reactivity was significant for peanut and grass pollen

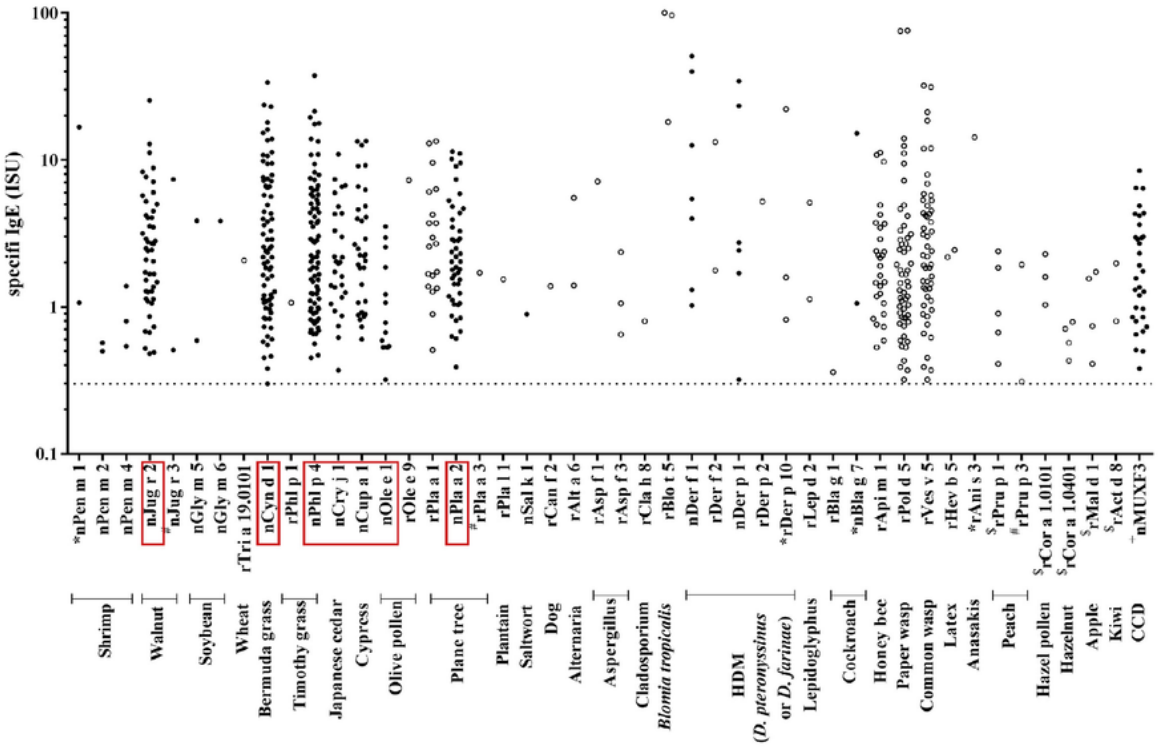

FIG 2. IgE to allergens. IgE to purified natural and recombinant allergens recognized on ImmunoCAP-ISAC (N = 150). *Solid circles* represent natural (n) component allergens, and *open circles* represent recombinant (r) component allergens. Cross-reacting allergen to *tropomyosin, #nonspecific lipid transfer protein (nsLTP), $the pathogenesis-related (PR)-10 protein, +CCDs. Each circle represents a single positive subject, and the dotted line indicates the cutoff value for the ISAC assay (0.3 ISU). *ISU,* ISAC standardized unit. The allergen abbreviations are given in Table E2. Indicated in *red squares* are allergens carrying CCD.

but was limited or absent for cockroach and HDM (*D pteronyssinus)*, respectively (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). A similar picture emerges on inhibition with soluble egg antigen of *Schistosoma haematobium* (Fig E3, *B*). However, *A lumbricoides* antigen was a very poor inhibitor of IgE binding to all 4 allergen sources (Fig E3, *C*). These data indicate that whereas schistosome eggs can be a source of cross-reactive glycans present on grass pollen (and less so on cockroach or HDM [*D pteronyssinus*]), this is not the case for *A lumbricoides* adult worms. In general, IgE reactivity to recombinant allergens not carrying CCD-type glycans was very rare (≤5 of 150 subjects per allergen). There were however 4 exceptions: rPla a 1, an invertase inhibitor from plane tree, recognized by 19 subjects (12.7%) and 3 insect venom allergens, that is, honey bee phospholipase A_2 (rApi g 1) recognized by 28 subjects (18.7%) and group 5 allergens from paper wasp (rPol d 5) and from common wasp (rVes v 5), recognized by 55 (36.7%) and 51 (34.0%) subjects, respectively. With the exception of a significant increase in odds of IgE reactivity to rVes v 5 in *A lumbricoides*–infected subjects (odds ratio, 3.42; 95% CI, 1.22-9.59; P = .019), no significant associations were found between helminth infections and IgE reactivity.

Taken together these data indicate that the observed IgE antibodies are not the result of primary sensitization to allergens tested, even though exposure can be great, for example, to peanut and shrimp, which are very common ingredients in the Indonesian diet. Using the allergen microarray, we found that the high degree of sensitization against common inhalant and food allergens is unique in that there is virtually no recognition of established major allergens. Instead there is evidence of CCD cross-reactivity, explaining most of the sensitization to pollen and peanut. For the high sensitization to HDM (*D pteronyssinus*) and cockroach, an explanation is still missing. It is however very likely that other cross-reactive protein or carbohydrate structures in helminths, other parasites, or insects are involved. These results are in line with observations from Ghana[8] and the Philippines[9] and can be used to design better microarrays for use in clinical diagnosis of allergic disorders not only in affluent but also in low- to middle-income countries.

We thank Anita Kober at ThermoFisher Scientific (Uppsala, Sweden) for the generous gift of the ImmunoCAP-ISAC microarrays that enabled this study and Paul van Rijn at HAL Allergy (Leiden, The Netherlands) for providing SPT reagents for the study. The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. This study would not been possible without enthusiastic cooperation of children, their parents, and teachers.

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Hematopoietic stem cell transplantation for RelB deficiency

To the Editor:

The nuclear factor kappa B (NF-κB) family of transcription factors regulates diverse biological processes, including innate and adaptive immunity, stress responses, apoptosis, and differentiation.[1,2] The NF-κB family includes the structurally homologous transcription factors NF-κB1 (p105/p50), NF-κB2 (p100/p52), RelA (p65), RelB, and c-Rel that interact to form homodimers and heterodimers with distinct gene regulatory functions.[3] NF-κB dimers are inactive in the cytosol, retained in a latent state through interaction with inhibitor of NF-κB proteins (IκB). The release of NF-κB dimers from IκB proteins is dependent on activation of the IκB kinase (IKK) complex.[3] Two NF-κB pathways are recognized, the classical, mediated by RelA (and c-Rel), and the alternative, mediated by RelB.[5] The canonical pathway responds to antigen receptors, cytokine receptors, and pattern recognition receptors,[4] and on receptor activation, the IKK complex phosphorylates IκBα, leading to its nuclear translocation of p50/RelA and p50/c-Rel dimers. Activation of the noncanonical NF-κB pathway is triggered by other receptors including BaffR and CD40, and leads to processing of the NF-κB2 precursor protein, p100, and subsequent activation of the p52/RelB dimer.[4]

Deleterious mutations in genes encoding various components of the NF-κB pathway, such as mutations in IKK-γ, IκBα, and inhibitor of NF-κB kinase subunit beta IKKβ (IKK2) and NF-κB1 or RelB, lead to a spectrum of combined immunodeficiencies with various severity.[3,5,6] Patients may also exhibit other features such as ectodermal dysplasia.[3]

The role of the noncanonical pathway in immunity was believed to be limited to transmitting signals downstream of only a few receptors, as compared with the classical pathway. It was therefore surprising that defects in this pathway resulted in equally significant immunodeficiencies. Heterozygous mutations in NF-κB2 were found to be associated with common variable immunodeficiency, and were indistinguishable from defects in NF-κB1. More recently, RelB deficiency was identified in patients with repeated infections, failure to thrive, and autoimmunity.[5] They were found to have a homozygous c.C1191A/p.Y397X mutation that creates a premature stop codon, which blocked translation of the last 3 exons.[6] Although the number of circulating lymphocytes was normal, the lack of T-cell responses to mitogens, low levels of T-cell receptor excision circles (TRECs), and the finding of a dysplastic thymus all supported the diagnosis of a profound T-cell immunodeficiency.[5]

Treatment options in these conditions usually involve immunoglobulin replacement and early empirical antibiotic treatment for antibody deficiency and infections. However, profound T-cell dysfunction observed in some patients has encouraged more radical solutions such as hematopoietic stem cell transplantation (HSCT), which has been tried infrequently with contrasting results.[2]

We report here, for the first time, the course and outcome of HSCT in 2 patients with RelB deficiency.

Patient 1 suffered repeated respiratory infections including multiple episodes of pneumonia, herpes simplex virus-1 skin infection, arthritis, ecthyma gangrenosum, and failure to thrive.[5] At the age of 1 year, he underwent HSCT from a 10/10 HLA-matched unrelated donor. He received myeloablative conditioning regimen consisting of busulfan (16 mg/kg) and cyclophosphamide (200 mg/kg). Graft versus host disease (GvHD) prophylaxis consisted of cyclosporine A (target levels, 175-200 mg/L) and methylprednisolone (2 mg/kg). The patient tolerated the conditioning with no complications. Neutrophil engraftment occurred +18 days post-HSCT. He developed grade I acute skin GvHD at +13 days post-HSCT, which resolved with a pulse of high dose of methylprednisolone (30 mg/kg) and suffered

METHODS

Study population

This study was part of a longitudinal study of the ImmunoSPIN programme (www.immunospin.org) investigating the effect of anthelminthic treatment on malarial parasitemia and allergy, described in detail elsewhere.[E1,E2] In short, skin prick testing was performed, blood was drawn for serological determinations, and a stool sample was collected for diagnosis of soil-transmitted helminth infections. In addition, parents/guardians were asked to fill out a questionnaire regarding allergy symptoms. Written informed consent was obtained from parent or guardian of each child. This study was approved by the Ethical Committee of the Medical Faculty, University of Indonesia, Jakarta, and filed by the ethics committee of the Leiden University Medical Center, the Netherlands. The study was registered as a clinical trial (ISRCTN83830814).

A total of 1674 schoolchildren aged 5 to 15 years were included in the study of allergic outcomes. Total and specific IgE levels have been measured in 883 subjects, of which 576 had sufficient plasma for ImmunoCAP-ISAC analysis. Because of budgetary constraints, only 150 of these plasma samples could be analyzed in the present study. To increase the chance of finding differences in IgE recognition profiles of SPT positive versus SPT negative, we overrepresented plasma samples from SPT positives, that is, 52 out of 150 (34% instead of 22% in original 576).

Questionnaire

Symptoms of asthma and atopic dermatitis in the past 12 months were recorded using a modified version of the International Study of Asthma and Allergy in Childhood questionnaire as reported before.[E2]

Skin prick test

HDM (*D pteronyssinus* and/or *D farinae*), peanut, shrimp (kindly provided by Paul van Rijn, HAL Allergy, Leiden, The Netherlands), and cockroach (*Blattella germanica*; Lofarma, Milan, Italy) were used for SPT. An SPT result was considered positive when the wheal size (mean of longest diameter and diameter perpendicular to that) was 3 mm or more as described previously.[E2]

IgE antibody measurement

ImmunoCAP (ThermoFisher Scientific, Uppsala, Sweden) was used to measure IgE against HDM (*D pteronyssinus*), cockroach, grass pollen, bromelain (as a marker for CCD), peanut, and shrimp, according to the manufacturer's instructions. Levels of 0.35 kU/L or more were considered positive.

The ImmunoCAP-ISAC 112 with 112 allergen molecules (kindly provided by Anita Kober, ThermoFisher Scientific) was used following manufacturer's instructions. Briefly, arrays were incubated with 30 μL of plasma for 2 hours. After washing and drying, arrays were incubated for 30 minutes with fluorescently labeled antihuman IgE. After further washing, arrays were read with a laser scanner. Results were expressed as ISAC standardized units (ISU)/L. Levels of 0.3 ISU/L or more were considered as positive.

Total IgE was measured by ELISA as described previously.[E1,E2] The results were expressed in International Units (IU/mL).

IgE inhibition assay

Four separate pools of plasma were prepared, an HDM (*D pteronyssinus*)-, a cockroach-, a grass pollen-, and a peanut-positive pool, which each contained equal volumes (n = 16, n = 11, n = 3, and n = 18, respectively) of plasma samples that had 5 kU/L or more of specific IgE for the respective allergen sources. Inhibitors used were bromelain (marker for CCD), soluble egg antigen of *Schistosoma haematobium* (a source of helminth CCD),[E3] and *Ascaris lumbricoides* antigen (locally endemic parasite). Each 75 μL of pooled plasma was mixed with equal volume of inhibitor at different dilutions (titrated inhibition) and preincubated at room temperature for 1 hour. Subsequently, samples were analyzed for HDM-, cockroach-, grass pollen-, and peanut-specific IgE, following the normal ImmunoCAP protocol. Results were expressed as percentages of an uninhibited control (PBS).[E3]

Parasitological examinations

Of the 150 studied subjects, 74 had provided stool samples for parasitological testing. Characteristics of subjects providing stool samples and those who did not showed no differences in atopic sensitization (SPT and IgE), age, or sex. *Trichuris trichiura* was detected by microscopy and a multiplex real-time PCR was used for detection of hookworms (*Ancylostoma duodenale*, *Necator americanus*), *A lumbricoides*, and *Strongyloides stercoralis* DNA as detailed previously.[E1,E2]

Statistical analysis

Statistical analyses were performed using SPSS (IBM Corp., Armonk, NY) version 20. Data were expressed as means ± SDs, frequency (percentage of measured data), and geometric means (95% CI). The correlations were examined using Spearman rank correlation coefficients. Logistic regression was used to examine the association between helminth infection and IgE reactivity to allergen component measured on ImmunoCAP-ISAC as well as the association between helminth infection and IgE reactivity to whole allergen extract tested on ImmunoCAP with adjustment for age, sex, and area. A P value of less than .05 was considered statistically significant.

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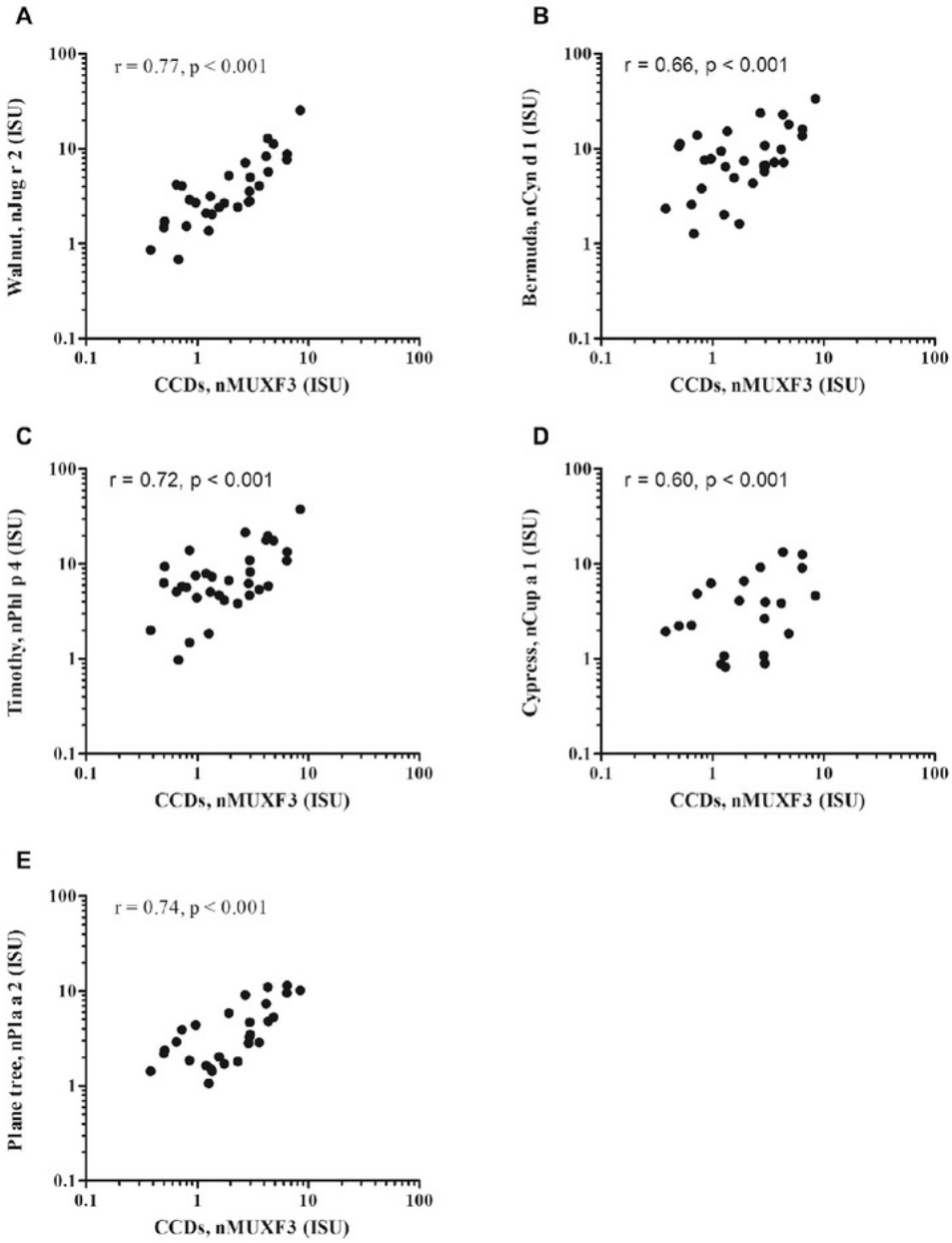

FIG E1. Correlation between IgE to CCD and natural allergens on ImmunoCAP-ISAC: walnut **(A)**, bermuda **(B)**, timothy **(C)**, cypress **(D)**, and plane tree **(E)**. nMUXF3 is a marker glycoprotein for CCD reactivity on ISAC assay. *ISU*, ISAC standardized unit; *r*, correlation coefficient.

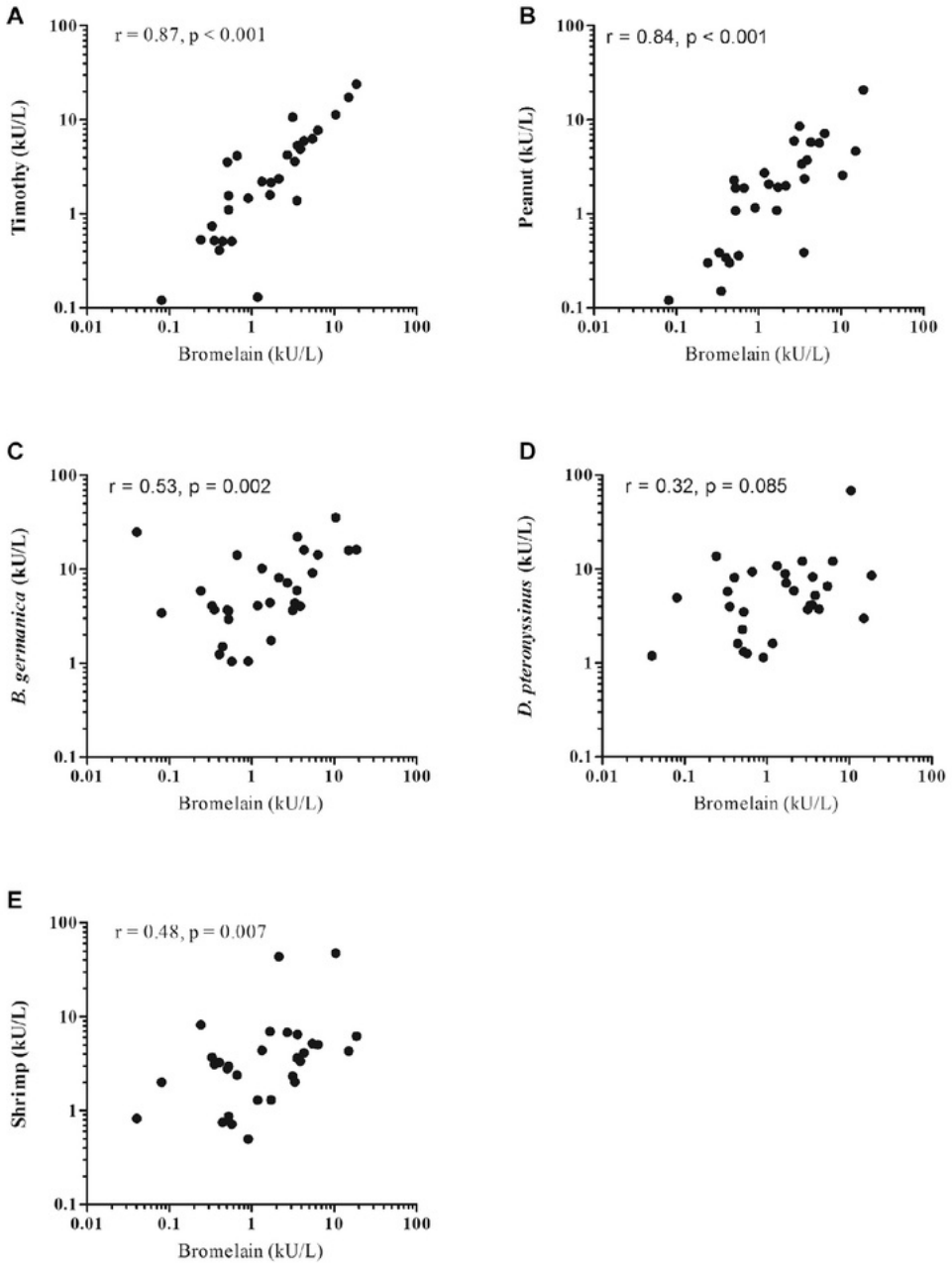

FIG E2. Correlation between IgE levels to bromelain and allergens on ImmunoCAP: timothy **(A)**, peanut **(B)**, cockroach **(C)**, HDM (*D pteronyssionus*) **(D)**, and shrimp **(E)**. Bromelain is a marker glycoprotein for CCD reactivity. *P* values and *r* (correlation coefficient) are given.

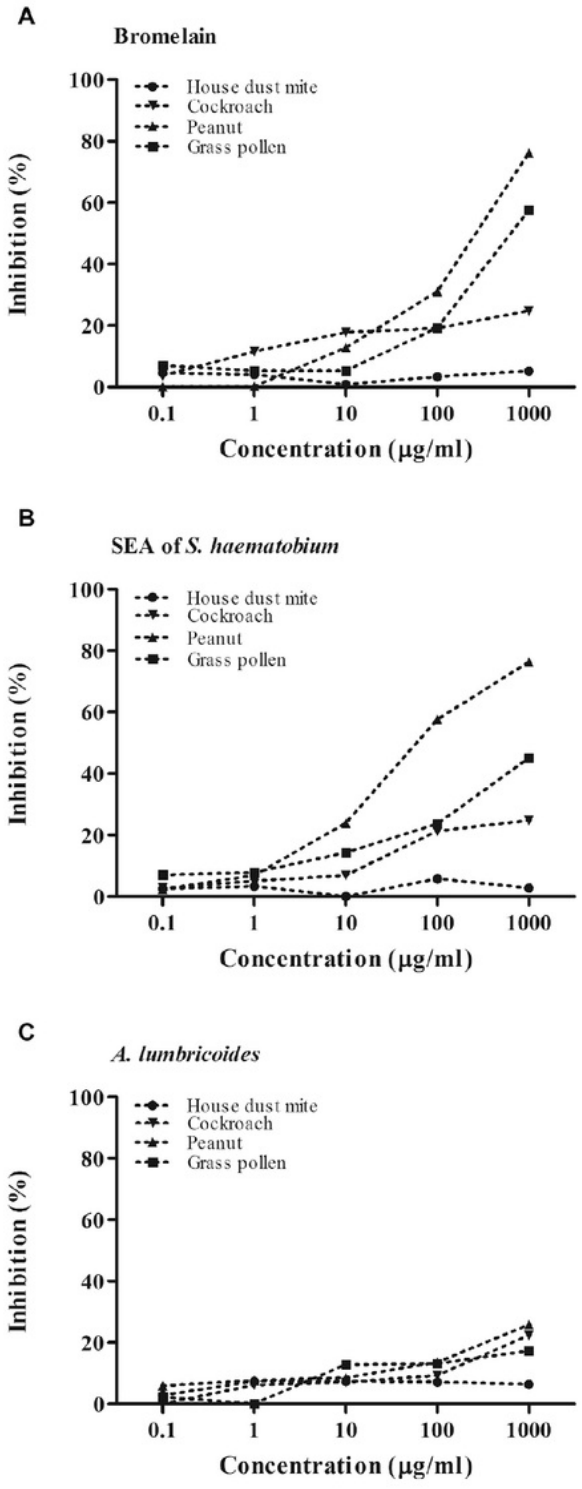

FIG E3. IgE inhibition assay. IgE cross-reactivity of HDM (*D pteronyssinus*), cockroach, peanut, and grass pollen extract tested by using an IgE ImmunoCAP inhibition assay. Bromelain **(A)**, soluble egg antigens (SEAs) of *S haematobium* **(B)**, and *A lumbricoides* **(C)** were used as inhibitors and values were expressed as the percentage of IgE inhibition.

TABLE E1. Characteristics of the study population

Characteristic	N	
Age (y), mean ± SD	150	10.6 ± 2.9
Total IgE (IU/mL), geometric mean (95% CI)	133	2816 (2257-3515)
		n (%)
Sex: female	150	83 (55.3)
Specific IgE (cutoff ≥ 0.35 kU/L)		
HDM (*D pteronyssinus)*	150	111 (74.0)
Cockroach	150	130 (86.7)
Peanut	150	97 (64.7)
Shrimp	150	119 (79.3)
Grass pollen*	30	27 (90)
Bromelain*	30	26 (86.7)
SPT positive		
HDM (*D pteronyssinus and/or D farinae*)†	150	40 (26.7)
Cockroach	150	36 (24.0)
Peanut	150	8 (5.3)
Shrimp	150	7 (4.7)
Helminth infection		
Any helminth	74	69 (93.2)
Hookworms‡	76	58 (76.3)
Ascaris lumbricoides‡	76	32 (42.1)
Trichuris trichiura§	104	30 (28.8)

*Sufficient sera was available from 30 subjects only.
†Considered positive if reaction to either *D pteronyssinus* and/or *D farinae*.
‡Helminth infection was diagnosed by PCR.
§Helminth infection was diagnosed by microscopy.

TABLE E2. The prevalence of sensitization on ImmunoCAP-ISAC

Component allergens	n (%)
Food components	
Shrimp (nPen m 2)	2 (1.3)
Shrimp (nPen m 4)	3 (2.0)
Walnut (nJug r 2)	48 (32.0)
Soybean (nGly m 5)	2 (1.3)
Soybean (nGly m 6)	1 (0.7)
Wheat (rTri a 19.0101)	1 (0.7)
Grass pollen	
Bermuda grass (nCyn d 1)	76 (50.7)
Timothy grass (rPhl p 1)	1 (0.7)
Timothy grass (nPhl p 4)	80 (53.3)
Tree pollen	
Japanese cedar (nCry j 1)	28 (18.7)
Cypress (nCup a 1)	32 (21.3)
Olive pollen (nOle e 1)	13 (8.7)
Olive pollen (rOle e 9)	1 (0.7)
Plane tree (rPla a 1)	19 (12.7)
Plane tree (nPla a 2)	46 (30.7)
Weed pollen	
Plantain (rPla l 1)	1 (0.7)
Saltwort (nSal k 1)	1 (0.7)
Animal	
Dog (rCanf2)	1 (0.7)
Mold	
Alternaria (rAlt a 6)	2 (1.3)
Aspergillus (rAsp f 1)	1 (0.7)
Aspergillus (rAsp f 3)	3 (2.0)
Cladosporium (rCla h 8)	1 (0.7)
HDM	
Blomia tropicalis (rBlo t 5)	3 (2.0)
D farinae (nDer f 1)	7 (4.7)
D farinae (rDer f 2)	2 (1.3)
D pteronyssinus (nDer p 1)	6 (4.0)
D pteronyssinus (rDer p 2)	1 (0.7)
Lepidoglyphus destructor (rLep d 2)	2 (1.3)
Cockroach	
Cockroach (rBla g 1)	1 (0.7)
Venom	
Honey bee (rApi m 1)	28 (18.7)
Paper wasp (rPol d 5)	55 (36.7)
Common wasp (rVes v 5)	51 (34.0)
Latex	
Latex (rHev b 5)	2 (1.3)
Cross-reactive	
Tropomyosin	
Anisakis (rAni s 3)	1 (0.7)
Cockroach (nBla g 7)	2 (1.3)
D pteronyssinus (rDer p 10)	3 (2.0)
Shrimp (nPen m 1)	2 (1.3)
nsLTP	
Walnut (nJug r 3)	2 (1.3)
Peach (rPru p 3)	2 (1.3)
Plane tree (rPla a 3)	1 (0.7)
PR-10 protein	
Hazel pollen (rCor a 1.0101)	3 (2.0)
Hazelnut (rCor a 1.0401)	4 (2.7)
Apple (rMal d 1)	4 (2.7)
Peach (rPru p 1)	5 (3.3)
Kiwi (rAct d 8)	2 (1.3)
CCD	
CCD (nMUXF3)	32 (21.3)

Natural (n) and recombinant (r) allergen components.
The number of positives, n out of 150 subjects examined is given as (%).
nsLTP, Nonspecific lipid transfer protein.

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