

Distinguishing benign from pathologic T_H2 immunity in atopic children

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Background: Although most children with asthma and rhinitis are sensitized to aeroallergens, only a minority of sensitized children are symptomatic, implying the underlying operation of efficient anti-inflammatory control mechanisms.

Objective: We sought to identify endogenous control mechanisms that attenuate expression of IgE-associated responsiveness to aeroallergens in sensitized children.

Methods: In 3 independent population samples we analyzed relationships between aeroallergen-specific IgE and corresponding allergen-specific IgG (sIgG) and associated immunophenotypes in atopic children and susceptibility to asthma and rhinitis, focusing on responses to house dust mite and grass.

Results: Among mite-sensitized children across all populations and at different ages, house dust mite-specific IgG/IgE ratios (but not IgG₄/IgE ratios) were significantly lower in children with asthma compared with ratios in those without asthma and lowest among the most severely symptomatic. This finding was

mirrored by relationships between rhinitis and antibody responses to grass. Depending on age/allergen specificity, 20% to 40% of children with allergen-specific IgE (sIgE) of 0.35 kU/L or greater had negative skin test responses, and these children also expressed the high sIgG/sIgE immunophenotype. sIgG₁ from these children inhibited allergen-induced IgE-dependent basophil activation in a dose-dependent fashion. Profiling of aeroallergen-specific CD4⁺ T_H memory responses revealed positive associations between sIgG/sIgE ratios and IL-10–dependent gene signatures and significantly higher IL-10/T_H2 cytokine (protein) ratios among nonsymptomatic children. **Conclusion:** In addition to its role in blocking T_H2 effector activation in the late-phase allergic response, IL-10 is a known IgG₁ switch factor. We posit that its production during allergen-induced memory responses contributes significantly to attenuation of inflammation through promoting IgG₁-mediated damping of the FcεRI-dependent acute-phase reaction. sIgG₁/sIgE balance might represent a readily accessible therapeutic target for asthma/rhinitis control. (J Allergy Clin Immunol 2015;■■■:■■■-■■■.)

Key words: Asthma, rhinitis, IgE, IgG₁, basophil activation, symptoms, regulatory T cells, IL-10

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The Australian studies were supported principally by a series of grants from the National Health and Medical Research Council of Australia. The United Kingdom studies were funded by UK Medical Research Council (MRC) grants G0601361 and MR/K002449/1 and the JP Moulton Charitable Foundation.

Disclosure of potential conflict of interest: D. Belgrave is employed by GlaxoSmithKline. B. Hales has received grants from the National Health Medical Research Council and the Telethon New Children's Hospital Research Grant, is employed by Telethon Kids Institute, and has received travel support from the National Health Medical Research Council grant review panel. A. Simpson has received grants from the Medical Research Council, the JP Moulton Charitable Foundation, the North West Lung Centre Charity, the National Institute of Health Research, and the European Union 7th Framework Programme; has received a consulting fee from ThermoFisher Scientific (Phadia; ISAC measurements in MAAS for this study were performed by ThermoFisher Scientific); has received payment for lectures from GlaxoSmithKline and Chiesi; and has received travel support from GlaxoSmithKline. A. Custovic has received grants from the Medical Research Council, the JP Moulton Charitable Foundation, the North West Lung Research Centre Charity, the European Union 7th Framework Programme, and the National Institute of Health Research; has received speakers fees from Novartis, ThermoFisher, AstraZeneca, ALK-Abelló, and GlaxoSmithKline; and has consultant arrangements with Regeneron/Sanofi. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication May 21, 2015; revised August 12, 2015; accepted for publication August 14, 2015.

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0091-6749/\$36.00

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<http://dx.doi.org/10.1016/j.jaci.2015.08.044>

Aeroallergen sensitization is a common feature in childhood asthma,¹ and IgE-mediated inflammation might play a causal role in the disease process.² However, observations that only a minority of atopic children have airway symptoms^{3,4} indicate that the pathogenic effects of IgE are usually attenuated by as yet poorly defined endogenous control mechanisms. A thorough understanding of these mechanisms and how they attenuate the pathologic effects of atopy in sensitized but asymptomatic subjects might point toward novel therapeutic targets.

Current concepts relating to immunoregulation in patients with allergic diseases are derived principally by extrapolation from studies of symptomatic atopic patients undergoing courses of high-dose allergen immunotherapy in whom clinical improvement occurs in the absence of significant reductions in specific IgE titers. A variety of evidence is consistent with the hypothesis that these repeated high-dose treatments stimulate the generation of specialized cell populations expressing “regulatory” phenotypes, in particular those producing high levels of IL-10,^{5,6} which targets the subsequent activation of allergen-specific T_H memory cells secreting T_H2 effector cytokines associated with the late-phase allergic response. These high-dose exposures also selectively boost production of allergen-specific IgG₄ (sIgG₄) antibodies,⁷ resulting in large increases in sIgG₄/allergen-specific IgE (sIgE) ratios. Similar findings have been reported in relation to the remission of occupational allergy in the context of very high

Abbreviations used

CAS: Childhood Asthma Study
 HDM: House dust mite
 MAAS: Manchester Asthma and Allergy Study
 RAINE: Western Australia Pregnancy Cohort
 SAM: Significance Analysis of Microarrays
 sIgE: Allergen-specific IgE
 sIgG: Allergen-specific IgG
 SPT: Skin prick test
 URA: Upstream Regulator Analysis

environmental exposure, which is also associated with high-level allergen-specific IL-10 production⁸ and high sIgG₄/sIgE ratios.⁹ It has been hypothesized that such induced sIgG₄ acts to attenuate allergy through competing for binding of the allergen responsible for triggering IgE-loaded Fcε receptors on granulocytes.¹⁰

A crucial question that remains unanswered is the relevance of these mechanisms induced by ultra-high exposure to those that control baseline allergic reactivity among sensitized subjects at the community-wide level, where allergen exposures are within a log-scale lower range and sIgG₄ is generally only a minor component of the sIgG response. The contribution of humoral regulatory mechanisms in this context is generally considered minor. However, the abundant evidence that “antigen dose selectivity” might be a key determinant of immune phenotype selection during immune induction (an archetypal example being the strikingly different dose-response curves for preferential triggering of T_H1 vs T_H2 immunity¹¹) argues that caution should be exercised before extrapolation of findings relating to immunoregulatory mechanisms between high- and low-dose exposure settings.

In our previous population-based studies we have demonstrated a strong relationship between the risk of asthma and titers of aeroallergen-specific IgE^{3,12} and obtained evidence that cat-specific total IgG (but not corresponding sIgG₄) could modify the association between cat-specific IgE and asthma at the population-wide level.¹³ In the current study we hypothesized that aeroallergen sensitization might be benign or pathologic at the community level and sought to investigate whether the balance between sIgE and corresponding sIgG₁ might be a component of the regulatory process that determines the pathologic potential of IgE-mediated sensitization. We addressed our hypothesis by bringing together comprehensive data on aeroallergen-specific humoral immunity from a series of cohorts. We have focused exclusively on atopic children and included the assessment of sIgG levels to house dust mite (HDM) and grass as archetypal examples of allergens linked to expression of asthma and rhinitis. In a series of studies, we examined the sIgG/sIgE balance among sensitized children in relation to (1) the presence, severity, and persistence of airway symptoms; (2) their immediate hypersensitivity skin test responses; (3) the capacity of their sera to arm basophils *in vitro* for IgE-dependent allergen-induced degranulation; and (4) the activation profile of T_H2 memory cell populations that secrete cytokines that control both aeroallergen-specific antibody production and the late-phase allergic response.

METHODS

Study design, setting, and participants

We studied 3 population samples from Perth (Australia) and Manchester (United Kingdom): the Western Australia Pregnancy Cohort (RAINE)^{3,13} and

the Manchester Asthma and Allergy Study (MAAS)¹⁴ are population-based birth cohorts, and the Childhood Asthma Study (CAS; Perth)¹⁵ enrolled children at high risk of atopy. We further investigated observations relating to asthma severity in a cross-sectional case-control study of school-age children with or without a history of severe asthma exacerbation who were equivalently highly HDM sensitized.¹⁶ All study populations are described in detail elsewhere,^{3,4,13-16} and studies were approved by research ethics committees. Informed consent was obtained from parents.

Data sources

Children were followed prospectively, with clinical assessments and blood collection at ages 6 and 14 years (Yr6 and Yr14) in RAINE, 5 and 11 years (Yr5 and Yr11) in MAAS, and 3 and 5 years (Yr3 and Yr5) in CAS. Comparable validated questionnaires were administered to collect information on parentally reported symptoms, and children underwent skin prick tests (SPTs).

Antibody measurements

Antibody assays in the Australian studies were (except where specified) performed in the authors' institutions by using reagents supplied gratis by ThermoFisher (Uppsala, Sweden), whereas those for the United Kingdom studies were performed in the ThermoFisher laboratories. In Australian birth cohorts we measured sIgE levels against whole HDM and rye allergen and corresponding sIgG levels (both total and IgG₄) against the major components Der p 1 and Phl p 1 by means of ImmunoCAP (ThermoFisher); levels were expressed in micrograms per liter, from which we derived sIgG/sIgE ratios. We used the same methodology to measure HDM-specific antibodies in the MAAS Yr5 population. In the MAAS Yr11 population we measured sIgE/sIgG ratios to the major allergen components Der p 1 plus Der p 2 and Phl p 1 using multiplex Solid-phase Allergen Chip (ImmunoCAP ISAC, ThermoFisher).¹⁷ In a cross-sectional study on asthma hospitalizations, we used a dissociation-enhanced immunofluorescence assay to measure IgE and IgG₁ levels against the combined major mite components Der p 1 and Der p 2.^{16,18} Both the latter assay platforms use log(s) fold lower allergen coating concentrations than ImmunoCAP and accordingly select for higher-affinity antibodies. Such differences militate against making direct quantitative comparisons of absolute antibody concentrations across all the cohorts, but the key analyses (those relating IgG/IgE ratios to the risk for expression of specific clinical phenotypes) involve internal comparisons between subgroups within individual cohorts assayed by using the same methodology.

Definition of variable

Current wheeze. Current wheeze was defined as a positive response to the following question: “Has your child had wheezing or whistling in the chest in the last 12 months?”

Current asthma. Current asthma was defined as the presence of all 3 of the following: (1) current wheeze, (2) current use of asthma medication, and (3) physician-diagnosed asthma.

Asthma severity. We used an asthma severity scale (mild/moderate/severe) developed from the Australian asthma management guidelines (www.nationalasthma.org.au).

Rhinitis. Rhinitis was defined as a positive response to the following question: “In the past 12 months, has your child had a problem with sneezing, or a runny nose, or a blocked nose when he/she did not have a cold or the flu?”

Sensitization status (SPTs and sIgE measurements).

We defined SPT responses as positive if the wheal diameter was 3 mm or greater, and sIgE levels as positive if the titer was greater than 0.35 kU/L. SPTs used whole mite extracts (both sites), mixed grass extract (Manchester), and whole rye grass extract (Perth).

In vitro cell studies

To investigate the potential inhibitory effect of sIgG on sIgE-mediated acute inflammatory responses, we used the basophil activation assay.¹⁹ Briefly,

donor basophils in PBMCs were acid stripped to remove FcεRI-bound IgE and passively re-sensitized by means of incubation for 1 hour at 37°C in buffer containing serum that had been preassayed to determine levels of Der p 1 sIgG and HDM sIgE. Discrete sets of experiments used either a series of individual sera with comparable (high-level) HDM sIgE titers but varying levels of corresponding sIgG or a series of pooled preassayed sera premixed to achieve a range of sIgG/sIgE ratios against a background of the same sIgE concentration. The basophils were activated by addition of Der p 1 to a final concentration of 0.1 μg/mL and incubated for a further 30 minutes, and the response was stopped by addition of 20 mmol/L cold EDTA. Activation was assessed flow cytometrically through surface expression (above low background levels) of CD63, CD203c, or both on basophils²⁰ that were gated as CD3⁺CD19⁺CD14⁺HLA-DR⁺CD123^{hi}FcεRI^{hi}CD11c⁺.

As a follow-up to one series of experiments detailed in the text, the sera used were reassayed for Der p 1 IgE to determine Der p 1 IgG/Der p 1 IgE ratios, and relevant basophil activation data were re-expressed in these terms.

To investigate potential mechanisms that control sIgG/sIgE balance, we performed genome-wide expression profiling of HDM-induced CD4⁺ T_H memory responses in 45 HDM-sensitized RAINE Yr14 subjects selected on the basis of cell availability and moderate- to high-level sensitization (IgE >24 μg/L) by using established methods. Cryobanked PBMCs from cohort subjects were cultured for 24 hours with HDM to activate T_H memory responses, and affinity-purified CD4⁺ T cells were harvested from cultures for RNA extraction by using Dynabeads (ThermoFisher) and subsequent expression profiling on Affymetrix microarrays (Affymetrix, Santa Clara, Calif), as detailed previously.^{3,21,22} We examined associations between HDM sIgG/sIgE ratios as continuous traits and differentially expressed genes activated during the *in vitro* HDM-specific recall responses.

Microarray data processing and analyses

Raw microarray data are available through the Gene Expression Omnibus repository (accession no. GSE70760; <http://www.ncbi.nlm.nih.gov/geo/>). Raw microarray data were preprocessed in R software by using the RMA algorithm and a custom mapping of probe sets to genes.^{23,24} Batch effects were identified by using principal components analysis and removed by using ComBat.²⁵ Noisy probe sets were filtered out of the analysis by using the PVAC algorithm.²⁶ Quantitative associations between gene expression patterns and logE ratios were identified by using the Significance Analysis of Microarrays (SAM) algorithm.²⁷ SAM computes a test statistic derived from the linear regression coefficient of each gene on the outcome divided by the sum of the SE and the square root of the residual error. Statistical significance is assessed by means of repeated permutations of the data. Quantitative associations between expression levels of the T_H2 module and logE ratios were assessed by using Gene Set Analysis.²² Upstream Regulator Analysis (URA) was used to identify signaling molecules that have the capacity to drive the observed downstream gene expression changes.²⁸ URA computes an overlap *P* value based on enrichment of known target genes of each regulator in the data. An activation *z* score was also calculated to determine whether the direction of the observed gene expression changes for each regulator are consistent with the predictable pattern based on prior studies.

Statistical analyses

We assessed differences in antibody titers and ratios thereof by using the Mann-Whitney *U* or Kruskal-Wallis tests and categorical differences between groups by using the χ² test (SPSS [SPSS, Chicago, Ill] and STATA [StataCorp, College Station, Tex]).

Role of funding sources

The funding sources had no role in study design, data collection, analysis, interpretation, writing of the report, or decision to submit the manuscript.

RESULTS

Table E1 in this article's Online Repository at www.jacionline.org summarizes the characteristics of cohort participants included in the current study.

Association studies

Immune response phenotypes underlying symptomatic versus asymptomatic atopy. We carried out initial analyses among RAINE Yr14 atopic children, and the findings were then replicated in other populations and at different ages. Table I focuses on asthmatic patients and their responsiveness to HDM. Among the mite-sensitized children in the cohort, HDM sIgE titers were significantly higher in those with asthma compared with titers in those without asthma, and asthma prevalence increased steeply across ascending HDM sIgE quartiles (11%, 16%, 24%, and 41%, respectively, vs 5% in those not sensitized). The higher average sIgE titers in asthmatic patients were mirrored by higher sIgG₄ titers, whereas corresponding total sIgG (predominantly IgG₁) titers were not increased among asthmatic patients. A comparable relationship was observed among rye grass-sensitized children between grass sIgE titers and rhinitis prevalence, with corresponding sIgG₄ titers again higher among symptomatic children (Table I). In both the HDM and grass antibody responses, the contribution of sIgG₄ to overall sIgG titers was very low (in the order of 10%, Table I), and we found no association between sIgG₄/sIgE ratios with asthma and rhinitis; all subsequent analyses involving IgG were thus focused on total sIgG or the dominant IgG₁ isotype.

Expressing these data as sIgG/sIgE ratios revealed a sharp inverse relationship to clinical symptoms in that among mite-sensitized children, HDM sIgG/sIgE ratios were significantly lower in RAINE 14Yr children with asthma compared with ratios in those without asthma (Table I), and corresponding findings relating to grass sIgG/sIgE ratios and rhinitis susceptibility (Table I) were comparable. This inverse relationship was replicated across all cohorts and at different ages for both wheezing-associated and rhinitis symptoms (Fig 1 and see Table E2 in this article's Online Repository at www.jacionline.org).

Asthma severity and persistence. Analysis among HDM-sensitized children with current asthma demonstrated a downward trend of sIgG/sIgE ratios with increasing asthma severity against a background of high sIgE titers, which were significant in the RAINE cohort but not in MAAS (see Table E3 in this article's Online Repository at www.jacionline.org).

In the RAINE Yr14 population, in addition to patients with current active asthma (physician-diagnosed asthma, current asthma medication, and current wheeze), we identified a subset of 49 children with well-controlled asthma (physician-diagnosed asthma and current asthma medication but no wheeze in the previous year). Patients with well-controlled asthma in whom symptoms had waned had significantly higher sIgG/sIgE ratios, with minimal differences in sIgE levels (see Table E4 in this article's Online Repository at www.jacionline.org). There were insufficient numbers expressing this phenotype for meaningful analyses in the younger age groups and other cohorts.

We have previously reported low HDM sIgG₁ levels in HDM-sensitized children with susceptibility to hospitalization for severe asthma exacerbations in a case-control study.¹⁷ Reanalysis of these data with addition of further subjects confirmed that a

TABLE I. Allergen-specific antibody response profiles and respiratory symptoms among HDM- and rye grass-sensitized children from the 14-year follow-up of the RAINE community cohort

RAINE Yr14 (HDM sensitized)	Asthma	No.	Mean $\pm$ SE	P value
HDM-specific IgE	No	429	70.8 $\pm$ 79	<.001
	Yes	92	187.5 $\pm$ 34.4	
HDM-specific IgG	No	429	1850.9 $\pm$ 81.4	.160
	Yes	92	2126.6 $\pm$ 187.4	
HDM-specific IgG ₄	No	429	195.3 $\pm$ 14.4	<.001
	Yes	42	332.8 $\pm$ 37.7	
HDM-specific IgG/IgE ratio	No	429	316.4 $\pm$ 29.2	<.001
	Yes	92	147.1 $\pm$ 37.4	
HDM-specific IgG ₄ /IgE ratio	No	429	21.0 $\pm$ 3.4	.90
	Yes	92	20.0 $\pm$ 8.1	
RAINE Yr14 (grass sensitized)	Rhinitis	No.	Mean $\pm$ SE	P value
Grass-specific IgE	No	243	36.7 $\pm$ 6.3	<.001
	Yes	300	88.2 $\pm$ 10.1	
Grass-specific IgG	No	243	1465.2 $\pm$ 103.6	.13
	Yes	300	1518.6 $\pm$ 78.8	
Grass-specific IgG ₄	No	243	83.3 $\pm$ 10.8	.001
	Yes	300	148.0 $\pm$ 14.5	
Grass-specific IgG ₄ /IgE ratio	No	243	32.0 $\pm$ 9.5	.11
	Yes	300	17.5 $\pm$ 3.76	
Grass-specific IgG/IgE ratio	No	243	422.6 $\pm$ 45.7	<.001
	Yes	300	204.9 $\pm$ 22.4	

The study population comprised 521 14-year-olds sensitized to HDM and 543 sensitized to grass, as defined by a specific IgE level of 0.35 kU/L or greater. Data shown are group means/SEs of HDM-specific IgE, IgG (total), and IgG₄ levels assayed by using ImmunoCAP and re-expressed in a common unit (micrograms per liter). IgG/IgE ratios were initially computed for individual children, and group mean ratios were then calculated from these. *P* values were derived from the Mann-Whitney *U* test comparing the 2 clinical phenotypes.

low sIgG/sIgE ratio in serum collected at the time of exacerbation is a marker of this clinical phenotype (see Table E5, A, in this article's Online Repository at www.jacionline.org). Reduced sIgG/sIgE ratios were also observed among HDM-sensitized MAAS Yr11 children who had been hospitalized with asthma exacerbations (confirmed through transcription of medical records data; see Table E5, B).²⁹

Mechanistic studies

Immediate hypersensitivity responses.

(a) *sIgG-mediated attenuation of SPT reactivity in vivo.* If sIgG attenuated sIgE-mediated responses in the airways, it was likely that SPT responses would also be affected. To test this, we examined the relationship between sensitization determined based on sIgE measurement versus SPT response across the cohorts, which revealed a nonrandom disparity in a major subgroup of atopic subjects. For example, 27% of RAINE Yr14 children with HDM sIgE levels of greater than 0.35 kU/L (designated IgE⁺) and 44% of those who were IgE⁺ to grass had negative skin test responses to the respective allergens (IgE⁺/SPT⁻). Of note, the frequencies of SPT⁺ children among

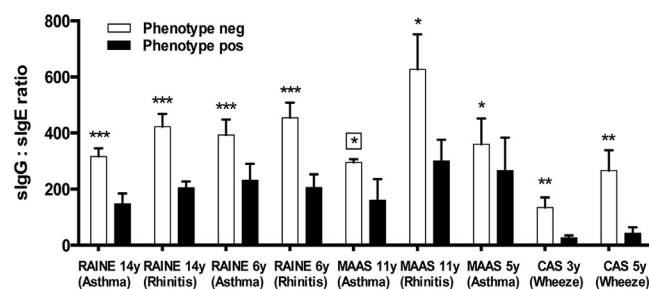


FIG 1. Relationship between HDM- and grass-specific IgG/IgE ratios among sensitized children and expression of asthma/wheezing or rhinitis. Children with HDM- or grass-specific serum IgE levels of greater than 0.35 kU/L were recruited in the Australian RAINE and CAS birth cohorts and the UK MAAS cohort, as specified. **P* < .05, ***P* < .01, and ****P* < .001. [*]*P* = .08.

the IgE⁻ groups were only 3% and 1% for HDM and grass, respectively. A comparable dissociation between sIgE levels and SPT responses was observed across all ages/cohorts (Fig 2, A). Mean sIgE levels in the IgE⁺/SPT⁺ children were significantly higher than in those who were IgE⁺/SPT⁻ (see Table E6 in this article's Online Repository at www.jacionline.org), but multiple IgE⁺/SPT⁻ children had sIgE titers within the 20 to 30 kU/L range. The IgE⁺/SPT⁻ phenotype was consistently associated with higher sIgG/sIgE ratios (Fig 2, B, and see Table E6). Note also the higher prevalence of asthma and rhinitis among the IgE⁺/SPT⁺ relative to IgE⁺/SPT⁻ children (Fig 2, C).

(b) *IgG-mediated attenuation of sIgE-dependent basophil activation in vitro.* We next used the stripped basophil activation assay to test whether sIgG could attenuate allergen-triggered basophil activation *in vitro*. Initially, we selected a series of individual sera from strongly HDM-sensitized RAINE Yr14 participants who were within a narrow band of HDM-specific IgE titers but spanning a broad (approximately 40-fold) range of Der p 1 sIgG titers. Single donor basophils were prearmed with these sera for 1 hour at 37°C before triggering by addition of Der p 1, and basophil activation was quantified 30 minutes later. A strong trend toward IgG dose-related inhibition of basophil activation is evident in these data (representative experiment in Fig 3, A).

We reasoned also that some of the noise in this plot was likely due to intersubject variations in antibody affinity, allergen component specificity, and IgG subclass ratios, for example. To reduce this variability, we first repeated the titration using preassayed serum pools, producing a spectrum of HDM-specific IgE/IgG ratios against the background of precisely standardized HDM-specific IgE. Der p 1 triggering of donor basophils prearmed with these serum pools provided stronger evidence of IgG dose-dependent inhibition of basophil activation: the titration in Fig 3, B, was performed in the presence of Der p 1 at 0.1 μ g/mL, and comparable results were obtained in titrations with Der p 1 at 0.01, 1.0, and 10.0 μ g/mL (data not shown). Second, we re-assayed all these sera for IgE by using a modified ImmunoCAP conjugated with Der p 1 as opposed to whole HDM; we recomputed sIgG/sIgE ratios as Der p 1 sIgG/sIgE ratios and used these for reanalysis of the basophil activation data from Fig 3, with identical conclusions (see Fig E1 in this article's Online Repository at www.jacionline.org).

Allergen-specific T_H memory responses and sIgE/sIgG balance. Specific T_H memory cells play a major role in control of antibody production by B cells, including the balance

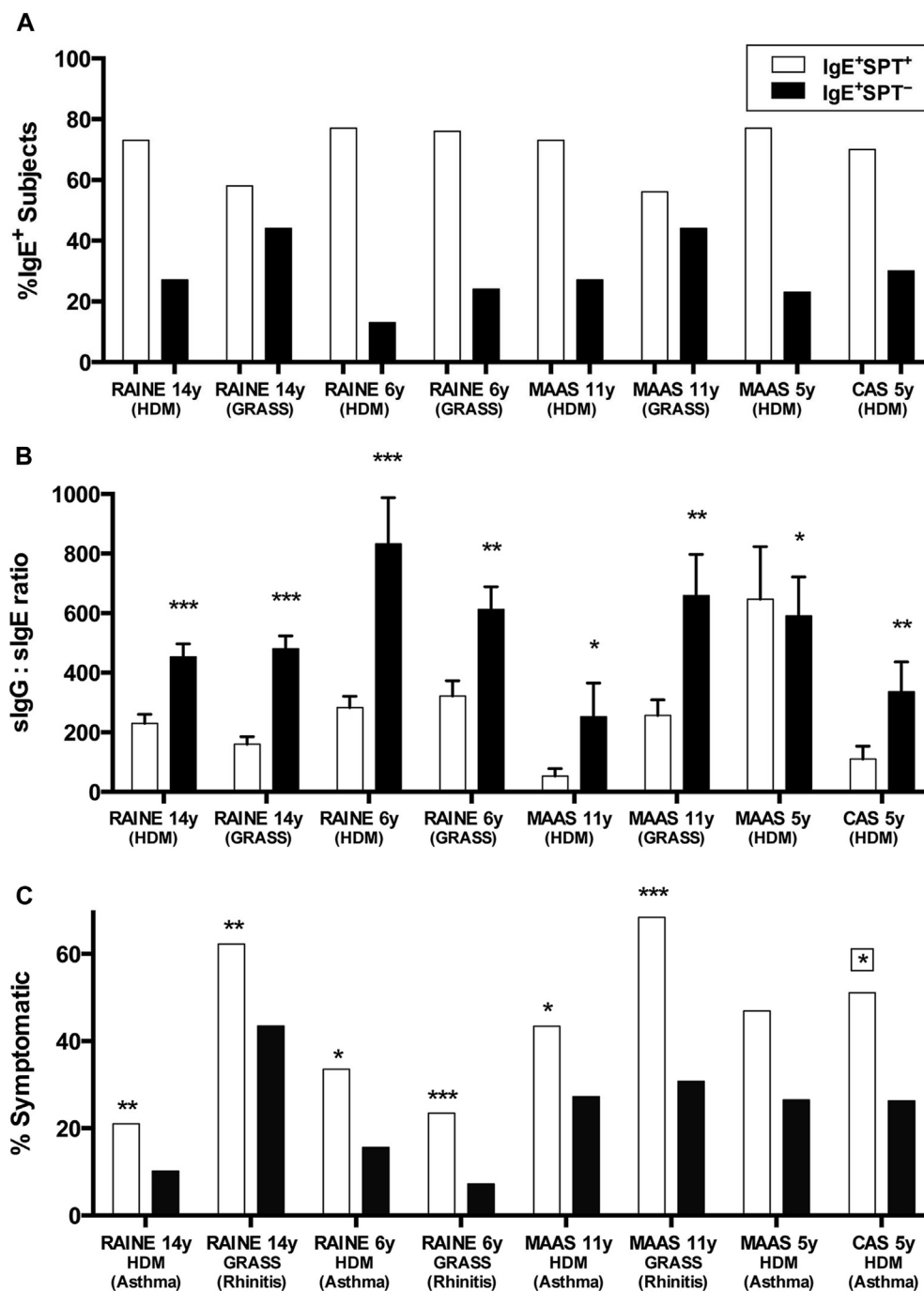


FIG 2. Dissociation between sensitization status determined based on sIgE levels versus SPT responses (A) and its relationship to sIgG/sIgE ratios (B) and clinical symptoms of asthma and rhinitis (C) in 3 cohorts at different ages. Children were selected on the basis of HDM- or grass-specific IgE titers of greater than 0.35 kU/L (IgE⁺) and then stratified on the basis of positive or negative responses to the relevant allergen into dichotomous IgE⁺SPT⁺ and IgE⁺SPT⁻ groups. Fig 2, A, Relative frequency of each of the phenotypes within each cohort. Analyses in 5-year-olds were restricted to HDM because the frequency of grass responders was very low. Fig 2, B, sIgG/sIgE ratios in each group. Fig 2, C, Relative frequency of subjects expressing symptoms of asthma or rhinitis, respectively, in the HDM- and grass-sensitized groups. **P* < .05, ***P* < .01, and ****P* < .001. [*]*P* = .078.

between antibody isotypes. We proceeded to investigate whether the expression of the high sIgG/sIgE immunophenotype among atopic subjects is reflected by characteristic patterns of gene expression in allergen-triggered T_H memory recall responses.

Fig 4, A, is a quantile-quantile plot derived from the SAM algorithm,²⁷ illustrating associations between expression levels of individual HDM-induced genes and HDM-specific IgG/IgE ratios in the T-cell donors; the differentially expressed genes falling

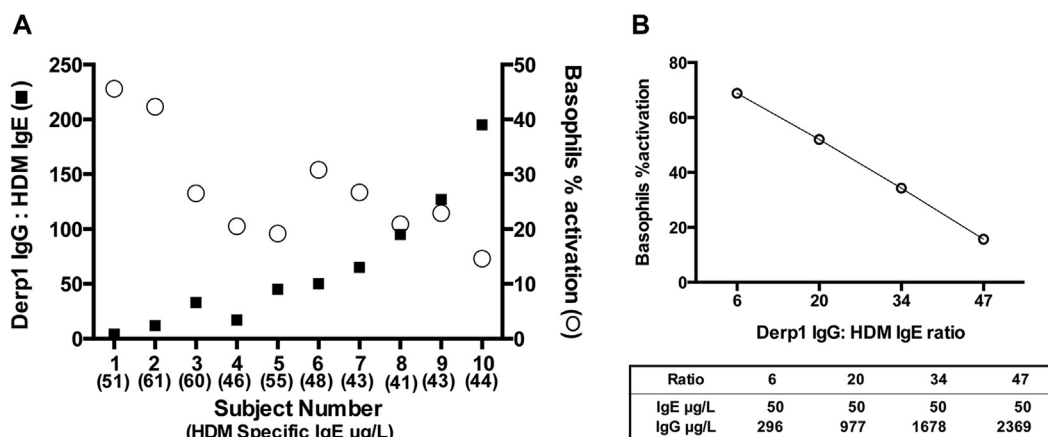


FIG 3. HDM-induced basophil activation after basophil arming with HDM-specific IgE-rich sera against a background of increasingly high levels of Der p 1-specific IgG. **A**, Individual sera from 10 Yr14 RAINE participants strongly sensitized to HDM (HDM-specific IgE titers = 41–61 µg/L as shown) were used to arm stripped donor basophils before activation by means of incubation with HDM-derived Der p 1. Resultant basophil activation levels achieved with each serum were plotted in rank order, as determined by individual specific IgG/IgE ratios. **B**, Preassayed sera from HDM-sensitized Yr14 RAINE participants were used to generate a series of pools standardized for HDM-specific IgE levels (50 µg/L) but with corresponding Der p 1-specific IgG levels varying across a log-fold range. Data illustrated show levels of basophil activation achieved at each specific IgG/IgE ratio.

outside the null distribution are negatively associated with sIgG/sIgE ratios. The majority of these (listed in Table E7 in this article's Online Repository at www.jacionline.org) are key components of the atopy-associated T_H2 module, which we have previously characterized using network analysis of aeroallergen-specific T_H memory responses,^{21,22} and overall expression of this module was inversely related to sIgG/sIgE ratios (false discovery rate < 0.001, Gene Set Analysis²²). URA²⁸ confirmed that increasing sIgG/sIgE ratios are associated with reduced signaling of multiple pathways (particularly those denoting T_H2 immunity/T-cell activation) coupled with increased IL-10 signaling (Fig 4, B, and see Table E8 in this article's Online Repository at www.jacionline.org). Moreover, re-expression of our published HDM-induced PBMC cytokine response data from the whole population of HDM-sensitized RAINE cohort subjects³ and stratification by clinical phenotype demonstrated a reciprocal relationship between IL-10 and T_H2 cytokine production and asthma risk (Fig 4, C). Finally, across the same sensitized population, T_H2 cytokine/IL-10 response ratios also correlated negatively with HDM sIgG/sIgE ratios in serum from respective donors (Spearman correlations, $n = 527$ subjects: IL-5/IL-10, $\rho = -0.448$, $P = .001$; IL-13/IL-10, $\rho = -0.390$, $P = .001$; IL-4/IL-10, $\rho = -0.203$, $P = .01$).

DISCUSSION

Our findings indicate that a consistent feature of children who are moderately or highly sensitized but who do not have asthma and rhinitis is an increased aeroallergen-specific IgG/IgE antibody ratio relative to equivalently sensitized but symptomatic subjects. This association is robust and reproducible across different populations and at different ages, it holds for sensitization-associated risk for asthma and rhinitis in relation to sensitization against perennial and seasonal aeroallergens (mite and grass, respectively), and is demonstrable with different assay

platforms. This relationship holds for asthma severity and exacerbations and appears to hold in regard to asthma remission.

We identified a subset of children with positive sIgE results who had negative skin test responses; these children expressed the same high sIgG/sIgE immunophenotype and were also differentially resistant to asthma and rhinitis despite high-level sIgE sensitization. Skin test reactivity results from allergen triggering of high-affinity IgE receptors on skin mast cells/basophils, and experimental evidence suggests that the presence of a sufficiently high sIgG/sIgE ratio during activation might interfere with this process through mechanisms that include activation of inhibitory Fcγ receptors on mast cells/basophils.^{30–34} Our demonstration of the dose-dependent inhibitory effects of sIgG₁ in sera from these children in the presence of saturating levels of mite allergen is consistent with such a mechanism. However, it is implausible that this alone could account for the overall effects observed in our studies in relation to symptoms. The development of persistent/severe aeroallergen-induced airway inflammation in sensitized children is considered to derive from a cascade involving sequential activation of IgE-dependent acute-phase and T_H memory cell-dependent late-phase reactions (Fig 5).³⁵ The late-phase reaction is driven by T_H2 cytokines, including IL-4 and IL-13, which also control IgE B-cell maturation,³⁶ and repeated cycles of production of these cytokines by aeroallergen-triggered T_H memory cells might account for the preferential expansion of the sIgE component relative to the sIgG₁ component of the humoral response in the symptomatic subgroup.

Our recent studies have established that aeroallergen-specific T_H memory responses in children involve activation of complex networks comprising multiple effector and regulatory genes,^{21,22} which we hypothesized might include those that can influence the balance between the IgE and IgG₁ antibody isotypes. In this regard our T_H memory profiling studies in mite-sensitized children identified a distinctive pattern of gene expression by aeroallergen-specific CD4⁺ T_H memory cells associated with sIgG/sIgE ratios, which is characteristic of IL-10 exposure of these cells during

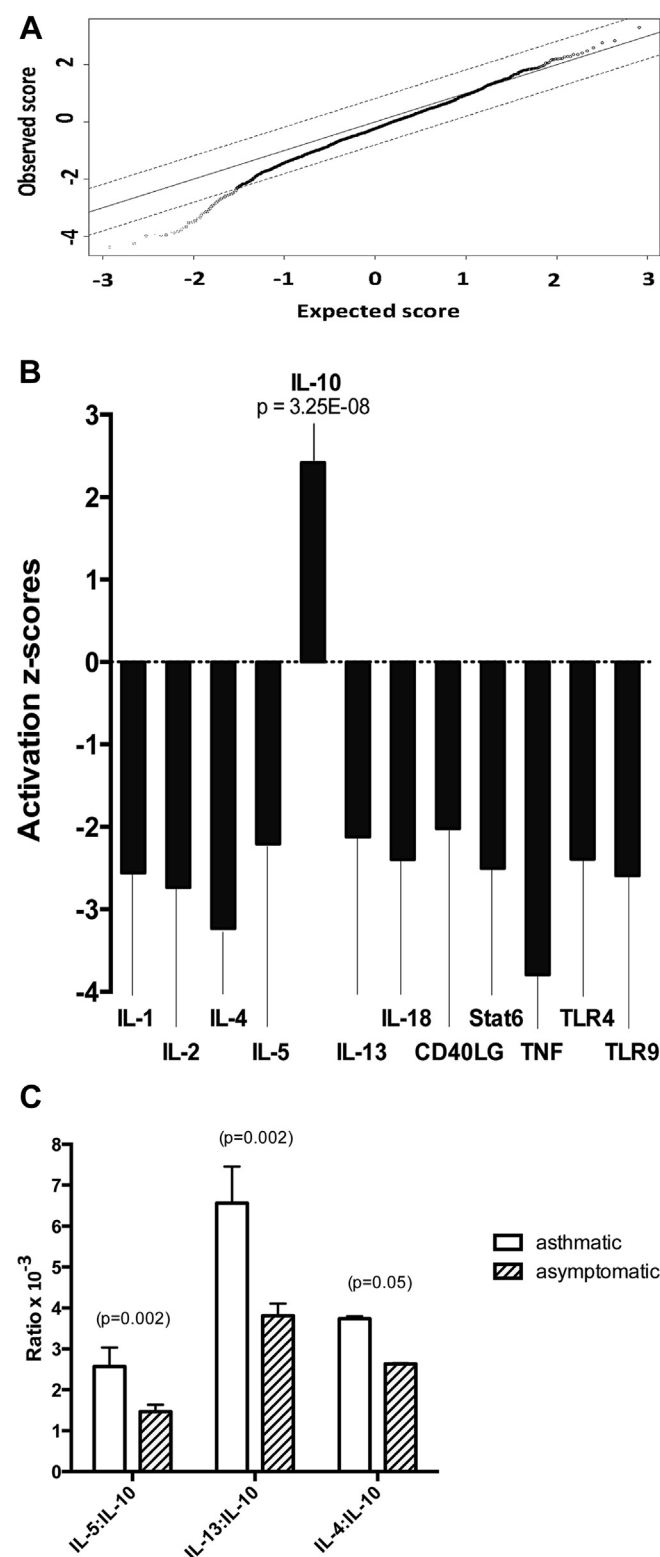


FIG 4. Variations in HDM-specific T_H memory programming as determinants of specific IgG/IgE ratios and asthma susceptibility among RAIN E Yr14 participants. **A**, HDM-specific IgG/IgE ratios in 45 sensitized RAIN E Yr14 participants were tested as a continuous trait against corresponding HDM-induced $CD4^+$ T-cell gene expression profiles by using the SAM algorithm. Data are illustrated as a quantile-quantile plot; differentially expressed genes inversely associated with the trait (negative scores) are shown in green (see Table E7). **B**, Candidate upstream regulators driving

allergen-induced reactivation. The most likely proximal sources of IL-10 in this context are IL-10-producing regulatory cells,^{5,6} which are coactivated to varying degrees in all T_H memory responses. The principal immunologic function ascribed to IL-10 is control of T cell-mediated inflammation; however, it has also been identified as a significant immunoglobulin switch factor driving IgG₁ production.³⁷ Thus its release in sufficient amounts during repeated aeroallergen-induced T_H2 cell activation cycles might contribute to slowing the progressive decrease in sIgG/sIgE ratios as the sIgE response expands over time in chronically exposed sensitized subjects by means of provision of an IgG₁ trophic signal in parallel with those driving sIgE production. The presence of such sIgG levels greater than a critical threshold provides a mechanism for attenuation of allergen-triggered FcεRI-mediated acute-phase inflammation. It is important to note that the mediators released during these acute reactions also play a crucial role in recruiting the myeloid and T_H2 memory cells responsible for the ensuing late-phase response,³⁵ and it is plausible that both these components of the allergic inflammatory cascade might be subject to coregulation through complementary IL-10-dependent mechanisms (proposed mechanism is shown in Fig 5).

It is possible that the application of higher-resolution technologies that could capture additional measures of IgG antibody response maturation, such as affinity and major/minor allergen component specificity, might further add to its value as a risk assessment tool. The lack of such data represents a limitation of our study that should be addressed in follow-up investigations.

It is of interest to note that children expressing the “asthma-susceptible” phenotype also exhibit deficient IgG₁ antibody production against common respiratory pathogens.^{18,38} This suggests that a generalized deficit in IgG response capacity at the mucosal surfaces might be an integral component of the high-risk phenotype in relation to inflammatory airway diseases,³⁸ further emphasizing the need for increased focus on immunologic mechanisms beyond IgE in the host response to aeroallergens.

Our findings might be relevant to designing improved desensitization strategies. In particular, our observations on direct inhibitory effects of sIgG on acute phase-associated basophil activation point toward an alternative and testable approach: notably, the use of short-course allergen-containing vaccines appropriately adjuvanted to selectively promote sIgG₁ synthesis and affinity maturation, which is conceptually similar to vaccines targeting IgG₁-mediated protection against microbial pathogens.

We thank the RAINE Study participants and their families and the RAINE Study Team for cohort coordination and contribution to data collection.

HDM-induced gene expression patterns associated with IgG/IgE ratios were identified by using the Ingenuity Pathway URA algorithm, as detailed in the Methods section in this article’s Online Repository. Activation z scores illustrated for candidate regulators were calculated based on the pattern match between observed gene expression patterns and predicted patterns based on prior studies. The P value for IL-10 is based on enrichment of known IL-10 target genes identified in the analysis (see Table E8 for P values for candidate negative regulators). *CD40LG*, CD40 ligand; *Stat6*, signal transducer and activator of transcription 6; *TLR*, Toll-like receptor. **C**, Banked data on HDM-induced cytokine secretion by PBMCs from 521 HDM-sensitized children (92 asthmatic and 429 nonasthmatic children) were reanalyzed to derive T_H2 cytokine/IL-10 ratios in the 2 groups.

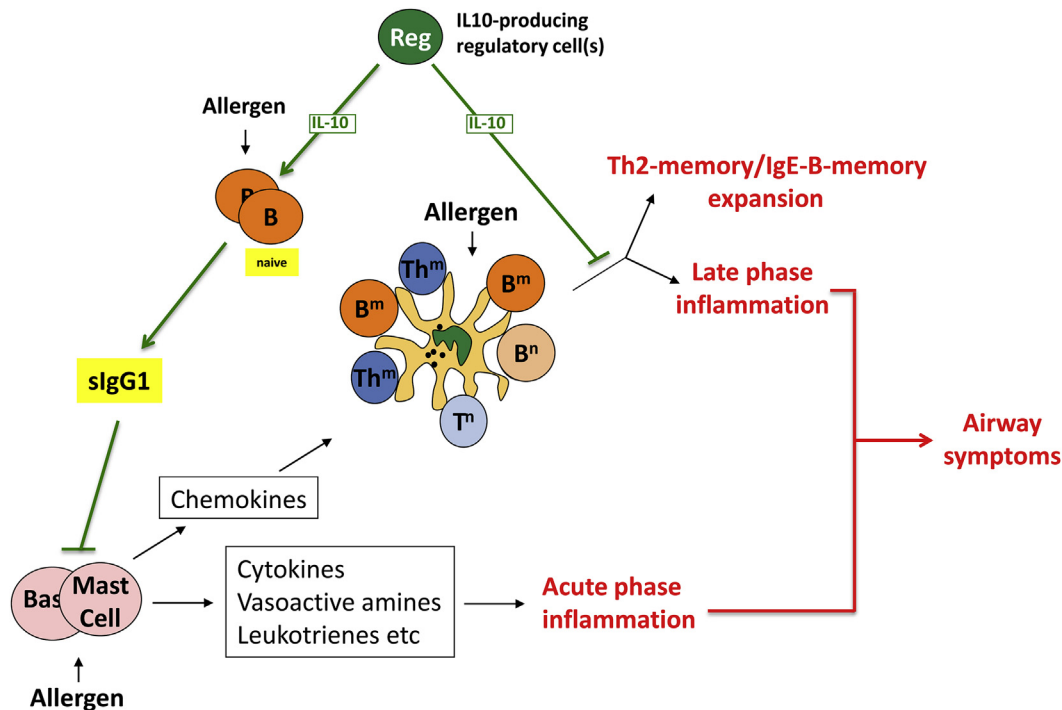


FIG 5. Complementary IL-10-dependent pathways for regulation of allergic inflammation. As detailed in the text, IL-10 might play a dual role in attenuation of the allergic inflammatory cascade by (1) acting indirectly through promotion of specific IgG₁ production, which modulates the FcεRI-dependent acute phase, and (2) acting directly through modulation of T_H2 memory cell activation in the late-phase response.

Key messages

- Dissociation between allergic symptomatology and sIgE status in children is associated with high-level coproduction of sIgG₁ as seen in high sIgG₁/sIgE ratios.
- An important exemplar is failure to respond to SPTs despite 0.35 kU/L sIgE or greater in serum, which is observed in up to 40% of children depending on age/allergen specificity.
- This atopic immunophenotype manifests *in vitro* as sIgG₁-mediated inhibition of Fcε receptor-dependent basophil activation and an accompanying strong IL-10-dependent gene signature within corresponding aeroallergen-specific CD4⁺ T_H2 memory responses from serum donors.
- IL-10 might have the dual function of attenuating activation of late phase-dependent T_H2 effector memory cells and driving production of sIgG₁, which modulates the acute phase response.

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1 **Online Supplement**

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3 **Distinguishing benign from pathologic Th2-immunity in atopic children**

4

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24 **Figure legend**

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26 **Figure E1:** Modulation of HDM-induced IgE-mediated basophil activation by HDM-specific IgG

Table E1: Birth cohort studies and characteristics of subjects contributing data to the present study
Number assessed equates to the number of subjects who provided blood samples for immunology assays.

Cohort	Age of Assessment (yrs)	Number available	Number Assessed	Number Atopic		Number with Disease	
				Mite	Grass	Asthma ^a /wheeze ^b	Rhinitis
RAINE	Yr6	2537	1016	255	247	155	102
	Yr14	2337	1328	514	543	130	300
MAAS	Yr5	1044	603	114	107	127	168
	Yr11	921	461	96	147	83	151
CAS	Yr3	235	209	50	19	61	-
	Yr5	184	169	64	38	50	-

^a RAINE, MAAS; ^b CAS

TABLE E2: Asthma or wheeze amongst HDM sensitized children from the RAINE, MAAS and CAS cohorts.

These analyses utilized a total of 329 additional samples collected at different ages as detailed. Data expression is as detailed in legends of Table 1.

RAINE Yr6	Asthma	N	Mean $\pm$ S.E.	P
HDM-IgE	No Yes	175 79	66.6 $\pm$ 11.6 267.5 $\pm$ 44.8	<0.001
HDM-IgG	No Yes	172 78	1905.2 $\pm$ 129.2 2155.2 $\pm$ 214.2	0.30
HDM-IgG:IgE ratio	No Yes	172 78	393.3 $\pm$ 54.8 231.5 $\pm$ 58.6	<0.001
MAAS Yr11	Asthma			
HDM-IgE	No Yes	62 39	24.1 $\pm$ 3.0 34.2 $\pm$ 5.6	0.018
HDM-IgG	No Yes	56 30	1860.5 $\pm$ 196.1 2212.3 $\pm$ 270.6	0.10
HDM-IgG:IgE ratio	No Yes	56 29	294.7 $\pm$ 89.6 159.7 $\pm$ 404.8	0.08
MAAS Yr5	Asthma			
HDM-IgE	No Yes	69 45	62.9 $\pm$ 13.6 231.7 $\pm$ 52.4	<0.001
HDM-IgG	No Yes	64 34	3422.1 $\pm$ 315.3 3168.1 $\pm$ 339.9	0.69
HDM-IgG:IgE ratio	No Yes	64 33	339.7 $\pm$ 91.7 276.5 $\pm$ 116.4	0.035
CAS Yr5	Wheeze			
HDM-IgE	No Yes	36 28	64.1 $\pm$ 27.5 227.5 $\pm$ 54.6	0.005
HDM-IgG	No Yes	31 25	1602.1 $\pm$ 289.6 2475.8 $\pm$ 431.2	0.09
HDM-IgG:IgE ratio	No Yes	31 25	266.6 $\pm$ 71.6 43.3 $\pm$ 20.4	0.005
CAS Yr3	Wheeze			
HDM-IgE	No Yes	31 19	60.5 $\pm$ 26.6 198.6 $\pm$ 71.5	0.04
HDM-IgG	No Yes	31 19	805.9 $\pm$ 183.7 1375.4 $\pm$ 363.6	0.13
HDM-IgG:IgE ratio	No Yes	31 19	134.6 $\pm$ 36.0 26.1 $\pm$ 8.7	0.006

RAINE Yr6	Rhinitis			
Grass-IgE	No Yes	201 46	27.1 ± 6.8 41.4 ± 8.9	0.21
Grass-IgG	No Yes	199 45	1518.4 ± 99.4 1370.5 ± 176.0	0.51
Grass-IgG:IgE ratio	No Yes	199 45	454.0 ± 54.5 205.8 ± 47.2	0.001
MAAS Yr11	Rhinitis			
Grass-IgE	No Yes	56 90	22.5 ± 4.6 66.6 ± 8.1	<0.001
Grass-IgG	No Yes	56 89	2166.2 ± 185.7 2911.6 ± 192.9	0.005
Grass-IgG:IgE ratio	No Yes	56 89	626.9 ± 124.9 300.9 ± 74.5	0.009

Table E3: Asthma severity and allergen specific IgG:IgE ratio (Mean $\pm$ S.E) in HDM-sensitized children. Data shown are antibody titres and ratios thereof amongst cohort subjects stratified by asthma severity. Antibody determinations for the MAAS Yr11 were carried out employing the Multiplex Allergen Chip Platform. Values represent Means $\pm$ S.E.

Cohort and Variable	Asthma presence and severity				
	No Asthma	Mild	Moderate	Severe	P Value
RAINE Yr14	(n=428)	(n=35)	(n=33)	(n=24)	
HDM-IgE	70.9 $\pm$ 8.7	164.5 $\pm$ 44.3	260.03 $\pm$ 43.7	121.4 $\pm$ 32.1	<0.001
HDM-IgG	1847.4 $\pm$ 81.3	2380.3 $\pm$ 361.5	2152.6 $\pm$ 278.6	1720.5 $\pm$ 251.3	0.33
HDM-IgG:IgE ratio	319.3 $\pm$ 82.3	230.0 $\pm$ 84.3	68.2 $\pm$ 19.3	134.8 $\pm$ 61.2	<0.001
RAINE Yr6	(n=161)	(n=31)	(n=21)	(n=29)	
HDM-IgE	67.3 $\pm$ 13.3	222.0 $\pm$ 72.3	242.3 $\pm$ 61.3	279.0 $\pm$ 61.2	<0.001
HDM-IgG	1941.9 $\pm$ 141.3	2262.7 $\pm$ 421.3	2507.3 $\pm$ 369.2	1504.8 $\pm$ 253.2	0.19
HDM-IgG:IgE ratio	401.6 $\pm$ 61.2	260.8 $\pm$ 80.3	349.2 $\pm$ 144.7	113.3 $\pm$ 57.3	<0.001
MAAS Yr11	(n=55)	(n=13)	(n=14)	(n=14)	
HDM-IgE	56.0 $\pm$ 8.7	56.1 $\pm$ 10.6	44.6 $\pm$ 7.2	123.4 $\pm$ 24.7	0.010
HDM-IgG	3110.7 $\pm$ 317.8	5035.4 $\pm$ 938.0	3009.3 $\pm$ 373.8	5009.3 $\pm$ 814.1	0.03
HDM-IgG:IgE ratio	192.8 $\pm$ 39.2	134.7 $\pm$ 39.6	97.2 $\pm$ 22.1	72.3 $\pm$ 22.8	0.23
MAAS Yr5	(n=85)	(n=16)	(n=24)	(n=21)	
HDM-IgE	80.5 $\pm$ 16.9	231.9 $\pm$ 87.6	240.0 $\pm$ 53.2	177.5 $\pm$ 75.6	0.007
HDM-IgG	3116.0 $\pm$ 305.4	2898.0 $\pm$ 300.7	3524.9 $\pm$ 444.2	2901.3 $\pm$ 300.0	0.81
HDM-IgG:IgE ratio	491.3 $\pm$ 82.2	246.3 $\pm$ 134.9	130.4 $\pm$ 67.9	191.3 $\pm$ 79.0	0.037

Table E4: HDM-specific immunity in HDM-sensitized children in RAINE YR14
“well-controlled” versus current active asthma. Values represent Means $\pm$ S.E.

Variable	Asthma	N	Mean $\pm$ S.E	P
HDM-IgE	Well controlled Current	49 92	111.67 $\pm$ 29.74 187.49 $\pm$ 34.39	0.07
HDM-IgG	Well controlled Current	49 92	1898.15 $\pm$ 156.20 2126.57 $\pm$ 187.36	0.83
HDM-IgG:IgE ratio	Well controlled Current	49 92	256.77 $\pm$ 65.53 147.13 $\pm$ 37.39	0.035

Table E5: Susceptibility to acute severe exacerbations amongst HDM-sensitized children: a cross-sectional study in Australia and MAAS birth cohort in the UK. Antibody assays used to generate Panel A data employed the dissociation-enhanced immunofluorescence assay platform, whereas Panel B data were derived from the Multiplex Allergen Chip Platform. Values represent Mean $\pm$ S.E.

A: Acute asthma study group Perth	Sensitized controls (n=58)	Hospitalized (n=105)	P
HDM-IgE	128 $\pm$ 27	132 $\pm$ 23	n.s.
HDM-IgG1	13518 $\pm$ 3048	6210 $\pm$ 3012	0.000
HDM-IgG1:IgE ratio	1791 $\pm$ 1562	157 $\pm$ 97	0.000

B: MAAS Yr11	Sensitized controls (n=82)	Hospitalized (n=21)	P
HDM-IgE	42.99 $\pm$ 5.40	103.25 $\pm$ 20.36	<0.001
HDM-IgG	2991.36 $\pm$ 272.21	4201.43 $\pm$ 650.79	0.028
HDM-IgG:IgE ratio	218.35 $\pm$ 31.45	108.75 $\pm$ 32.41	0.04

Table E6: Antibody response phenotypes amongst sensitized children (specific serum IgE to HDM or grass >0.35 kU/L) stratified by skin test positivity
Data derived from use of standard ImmunoCAP assay except for MAAS Yr11 (Multiplex Allergen Chip). Values represent Means $\pm$ S.E.

Cohort/variable	SPT	N	Mean <u>±</u> S.E.	P
RAINE Yr14:HDM				
HDM-IgE	Neg	141	35.5 <u>±</u> 7.9	<0.001
	Pos	373	114.9 <u>±</u> 12.1	
HDM-IgG	Neg	141	1625.7 <u>±</u> 115.7	0.027
	Pos	373	1996.4 <u>±</u> 92.9	
HDM-IgG:IgE Ratio	Neg	141	453.3 <u>±</u> 44.1	<0.001
	Pos	373	229.8 <u>±</u> 30.4	
RAINE Yr14:Grass				
Grass-IgE	Neg	238	14.3 <u>±</u> 2.6	0.001
	Pos	300	104.7 <u>±</u> 10.9	
Grass-IgG	Neg	238	1284.3 <u>±</u> 83.9	0.005
	Pos	300	1653.7 <u>±</u> 94.2	
Grass-IgG:IgE ratio	Neg	238	481.2 <u>±</u> 43.1	0.001
	Pos	300	159.9 <u>±</u> 24.8	
RAINE Yr6:HDM				
HDM-IgE	Neg	35	3.3 <u>±</u> 0.6	0.004
	Pos	226	141.1 <u>±</u> 18.4	
HDM IgG	Neg	34	1486.9 <u>±</u> 213.1	0.07
	Pos	222	2082.7 <u>±</u> 121.2	
HDM-IgG:IgE ratio	Neg	34	843.2 <u>±</u> 175.0	<0.001
	Pos	222	283.3 <u>±</u> 38.3	
RAINE Yr6:Grass				
Grass-IgE	Neg	75	3.5 <u>±</u> 0.5	0.001
	Pos	176	40.8 <u>±</u> 7.9	
Grass-IgG	Neg	75	1164.5 <u>±</u> 113.8	0.008
	Pos	173	1664.0 <u>±</u> 112.4	
Grass-IgG:IgE ratio	Neg	75	612.9 <u>±</u> 77.6	0.002
	Pos	173	322.5 <u>±</u> 50.5	

CAS Yr5:HDM				
HDM-IgE	Neg	19	22.8 $\pm$ 9.8	<0.001
	Pos	45	181.1 $\pm$ 40.7	
HDM-IgG	Neg	14	1693.7 $\pm$ 455.9	0.66
	Pos	42	2091.6 $\pm$ 305.8	
HDM-IgG:IgE Ratio	Neg	14	336.4 $\pm$ 99.6	0.003
	Pos	42	110.4 $\pm$ 44.2	
MAAS Yr5:HDM				
HDM-IgE	Neg	26	110.4 $\pm$ 44.2	0.09
	Pos	88	121.08 $\pm$ 54.93	
HDM-IgG	Neg	19	2388.56 $\pm$ 487.59	0.01
	Pos	78	3594.22 $\pm$ 280.53	
HDM-IgG:IgE Ratio	Neg	19	591.26 $\pm$ 130.76	0.03
	Pos	78	647.36 $\pm$ 176.59	
MAAS Yr11:HDM				
HDM-IgE	Neg	26	27.07 $\pm$ 6.57	0.37
	Pos	69	29.23 $\pm$ 3.22	
HDM-IgG	Neg	26	1897.69 $\pm$ 292.26	0.34
	Pos	69	2045.80 $\pm$ 192.44	
HDM-IgG:IgE ratio	Neg	26	251.5 $\pm$ 112.72	0.11
	Pos	69	53.47 $\pm$ 24.94	
MAAS Yr11:Grass				
Grass-IgE	Neg	63	31.02 $\pm$ 6.61	0.002
	Pos	80	64.66 $\pm$ 8.42	
Grass IgG	Neg	63	2428.71 $\pm$ 233.41	0.129
	Pos	80	2755.75 $\pm$ 176.88	
Grass-IgG:IgE ratio	Neg	63	659.17 $\pm$ 137.88	0.002
	Pos	80	257.41 $\pm$ 52.58	

Table E7: Differentially expressed genes identified by SAM analysis

The gene list are those identified by SAM analysis in Fig 4A as significantly differentially expressed in relation to the phenotype of interest (Log IgG:IgE ratio). The negative scores(d) indicate downregulation of these genes with increasing ratios; q-values shown are analogous to p-values corrected for multiple testing.

Gene Name	Score(d)	Numerator(r)	Denominator (s+s0)	q-value
<i>RASL11A</i>	-4.371	-0.166	0.038	0.001
<i>NSMCE1</i>	-4.257	-0.146	0.034	0.001
<i>PLXDC1</i>	-3.989	-0.232	0.058	0.001
<i>PECAM1</i>	-3.970	-0.177	0.045	0.001
<i>RAB27B</i>	-3.962	-0.243	0.061	0.001
<i>SLC39A8</i>	-3.949	-0.143	0.036	0.001
<i>IL17RB</i>	-3.904	-0.229	0.059	0.001
<i>CAMK2D</i>	-3.883	-0.214	0.055	0.001
<i>DACT1</i>	-3.825	-0.288	0.075	0.001
<i>SLC26A11</i>	-3.767	-0.110	0.029	0.001
<i>IL4R</i>	-3.691	-0.192	0.052	0.001
<i>FCER2</i>	-3.675	-0.179	0.049	0.001
<i>FAM102B</i>	-3.528	-0.170	0.048	0.001
<i>MAL</i>	-3.509	-0.123	0.035	0.001
<i>KIAA1324L</i>	-3.506	-0.134	0.038	0.001
<i>KPNA6</i>	-3.462	-0.089	0.026	0.001
<i>NDFIP2</i>	-3.460	-0.224	0.065	0.001
<i>SLC37A3</i>	-3.358	-0.163	0.049	0.001
<i>CERS6</i>	-3.344	-0.089	0.027	0.001
<i>NFKBIZ</i>	-3.284	-0.078	0.024	0.001
<i>RNF125</i>	-3.182	-0.109	0.034	0.001

Gene Name	Score(d)	Numerator(r)	Denominator (s+s0)	q-value
<i>RASGRP3</i>	-3.171	-0.203	0.064	0.001
<i>CNKS2</i>	-3.153	-0.126	0.040	0.001
<i>RAB30</i>	-3.132	-0.172	0.055	0.001
<i>CD1C</i>	-3.129	-0.145	0.046	0.001
<i>RAB19</i>	-3.060	-0.180	0.059	0.001
<i>IL5</i>	-3.025	-0.205	0.068	0.001
<i>NCOA3</i>	-2.965	-0.079	0.027	0.001
<i>FAM98A</i>	-2.924	-0.067	0.023	0.001
<i>GFI1</i>	-2.901	-0.123	0.042	0.001
<i>SGSH</i>	-2.886	-0.079	0.027	0.001
<i>TIAM1</i>	-2.857	-0.075	0.026	0.001

Table E8: Upstream regulator analysis of differential gene expression patterns associated with increasing IgG:IgE ratios

Candidate upstream regulators positively (IL-10; bold) and negatively (all other genes) associated with IgG:IgE, as per Figure 4B. P-values reflect levels of enrichment of known target genes relevant to individual candidate regulators

Upstream Regulator	Predicted Activation State	Activation z-score	p-value of overlap
IL4	Inhibited	-3.234	4.60E-13
RNASE2	Inhibited	-2.425	6.02E-10
NFkB	Inhibited	-2.906	3.24E-09
IL10	Activated	2.419	3.25E-08
TNF	Inhibited	-3.795	8.37E-08
IL2	Inhibited	-2.735	8.38E-08
CD40LG	Inhibited	-2.022	8.82E-08
TLR8	Inhibited	-2.211	1.31E-07
IL1B	Inhibited	-3.351	1.74E-07
IL3	Inhibited	-2.570	2.67E-07
RNASE1	Inhibited	-2.000	2.95E-07
TLR9	Inhibited	-2.595	3.38E-07
IL5	Inhibited	-2.208	5.54E-07
Jnk	Inhibited	-2.180	5.64E-07
IL33	Inhibited	-2.163	9.52E-07
TLR4	Inhibited	-2.393	1.32E-06
IL1A	Inhibited	-2.547	1.50E-06
IL18	Inhibited	-2.397	1.80E-06
STAT6	Inhibited	-2.504	1.94E-06
TLR2	Inhibited	-2.386	2.63E-06

MYD88	Inhibited	-2.572	2.86E-06
CSF2	Inhibited	-2.502	3.03E-06
IL13	Inhibited	-2.123	7.40E-06
Tlr	Inhibited	-2.203	1.23E-05
IL7	Inhibited	-2.176	2.88E-05
IL1	Inhibited	-2.558	3.00E-05
ERK1/2	Inhibited	-2.141	8.46E-05
JUN	Inhibited	-2.177	9.14E-05

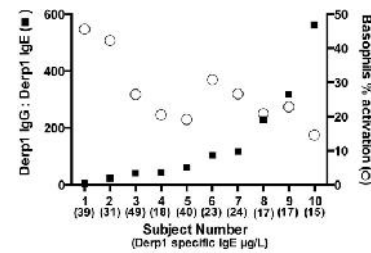


Figure E1 Panel A

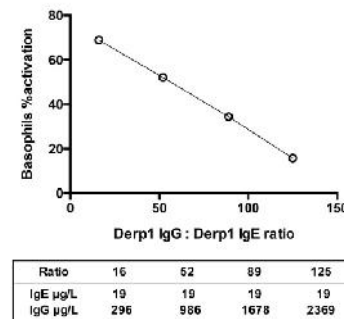


Figure E1 Panel B

Fig E1.

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Journal of Allergy and Clinical Immunology, 2015, Available online 27 October 2015

<http://dx.doi.org/10.1016/j.jaci.2015.08.044>