Distinguishing benign from pathologic Th2-immunity in atopic children

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ABSTRACT

**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:** To identify endogenous control mechanism(s) that attenuate expression of IgE-associated responsiveness to aeroallergens in sensitized children.

**Methods:** In three independent population samples we analysed relationships between aeroallergen specific (s)IgE and corresponding sIgG and associated immunophenotypes in atopic children and susceptibility to asthma and rhinitis, focussing on responses to house-dust mite (HDM) and grass.

**Results:** Amongst mite-sensitized children across all populations and at different ages, HDM-specific IgG:IgE ratios (but not IgG4:IgE) were significantly lower in children with asthma compared to those without, and lowest amongst the most severely symptomatic. This finding was mirrored by relationships between rhinitis and antibody responses to grass. Depending on age/allergen-specificity, 20-40% of children with sIgE ≥0.35kU/L were skin test negative, and these 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+ Th-memory responses revealed positive associations between sIgG:sIgE ratios and IL-10-dependent gene signatures, and significantly higher IL-10/Th2-cytokine(protein) ratios amongst non symptomatic children.

**Conclusion:** In addition to its role in blocking Th2-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 via promoting IgG1-mediated damping of the FcεR1-dependent acute phase reaction. sIgG1:sIgE “balance” may represent a readily accessible therapeutic target for asthma/rhinitis control.
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Key Messages:

• dissociation between allergic symptomatology and allergen specific (s)IgE status in children is associated with high level co-production of slgG1 viz. high slgG1:slgE ratios;
• an important exemplar is failure to respond to skin prick testing despite ≥0.35kU/L slgE in serum, which is observed in up to 40% of children depending on age/allergen specificity;
• this atopic immunophenotype manifests in vitro as slgG1-mediated inhibition of FcεR-dependent basophil activation, and an accompanying strong IL-10-dependent gene signature within corresponding aeroallergen-specific CD4+ Th2-memory responses from the serum donors;
• IL-10 may have the dual function of attenuating activation of late phase-dependent Th2-effector memory cells, and driving production of slgG1 which modulates the acute phase response

Capsule Summary:

In the majority of sensitized children endogenous humoral and cellular regulatory mechanisms operative within allergen-specific memory responses act in concert to successfully maintain levels of expression of Th2-associated inflammation below clinically relevant thresholds.
Aeroallergen sensitization is a common feature in childhood asthma, and IgE-mediated inflammation may play a causal role in the disease process. However, observations that only a minority of atopic children have airway symptoms indicate that 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 may point towards novel therapeutic targets.

Current concepts relating to immunoregulation in allergic diseases are derived principally by extrapolation from studies of symptomatic atopic patients undergoing courses of high-dose allergen immunotherapy, where clinical improvement occurs in the absence of significant reductions in specific (s)IgE titres. 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, which target the subsequent activation of allergen-specific Th-memory cells secreting Th2-effector cytokines associated with the late-phase allergic response. These high-dose exposures also selectively boost the production of slgG4 antibodies, resulting in large increases in slgG4:sIgE ratios. Similar findings have been reported in relation to the remission of occupational allergy in the context of very high environmental exposure, which is also associated with high level allergen-specific IL-10 production and high slgG4:sIgE ratios. It has been hypothesized that such induced slgG4 acts to attenuate allergy via competing for binding of the allergen responsible for triggering IgE-loaded Fc-receptors on granulocytes.

A crucial question that remains unanswered is the relevance of these mechanisms induced by ultra-high exposure to those that control baseline allergenic reactivity amongst sensitized subjects at the community-wide level, where allergen exposures are within a log-scale lower range, and slgG4 is generally only a minor component of allergen-specific IgG responses. The contribution of humoral regulatory mechanisms in this context is generally considered minor. However, the abundant evidence that "antigen dose selectivity" may 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 Th1 versus Th2 immunity\textsuperscript{1}), 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 titres of aeroallergen-specific IgE\textsuperscript{3,12}, and obtained evidence that at population-wide level cat-specific total IgG (but not corresponding slgG4) could modify the association between cat-specific IgE and asthma\textsuperscript{13}. In the current study, we hypothesized that at community level, aeroallergen sensitization may be benign or pathologic, and sought to investigate whether the balance between allergen-specific (s)IgE and corresponding slgG1 may 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 slgG levels to HDM and grass as archetypal examples of allergens linked to expression of asthma and rhinitis. In a series of studies, we examined the allergen-specific IgG:IgE balance amongst sensitized children in relation to (i) the presence, severity and persistence of airway symptoms, (ii) their immediate hypersensitivity skin test responses, (iii) the capacity of their sera to arm basophils \textit{in vitro} for IgE-dependent allergen-induced degranulation; and (iv) the activation profile of Th2-memory cell populations which secrete cytokines that control both aeroallergen-specific antibody production and the late-phase allergic response.

\section*{METHODS}

\subsection*{Study design, setting and participants}

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

\textbf{Data sources}

Children were followed prospectively, with clinical assessments and blood collection at ages six and 14 years (Yr6, Yr14) in RAINE, five and 11 (Yr5, Yr11) in MAAS, and three and five (Yr3, Yr5) in CAS. Comparable validated questionnaires were administered to collect information on parentally-reported symptoms, and children were skin-prick tested.

\textbf{Antibody measurements:}

Antibody assays in the Australian studies were (except where specified) performed in the authors’ institution employing reagents supplied gratis by ThermoFisher (Uppsala, Sweden), whereas those for the UK studies were performed in the ThermoFisher laboratories. In Australian birth cohorts, we measured sIgE against whole HDM and Rye allergen, and corresponding sIgG (total and IgG4) against major components Der p 1 and Phloem p 1 by ImmunoCAP\textsuperscript{\textregistered} (ThermoFisher, Uppsala, Sweden); levels were expressed in µg/L, from which we derived sIgG:sIgE ratios. We used the same methodology to measure HDM-specific antibodies in MAAS Yr5. In MAAS Yr11, we measured sIgE/sIgG to \textit{major allergen components} Der p 1 \textit{plus} Der p 2 and Phl p 1 using multiplex Solid-phase Allergen Chip (ImmunoCAP ISAC\textsuperscript{\textregistered}, ThermoFisher)\textsuperscript{17}. In a cross-sectional study on asthma hospitalizations, we employed dissociation-enhanced immunofluorescence assay to measure IgE and IgG1 against the combined major mite components Der p 1 \textit{plus} Der p 2\textsuperscript{16,18}.

Both the latter assay platforms employ 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 risk for expression of specific clinical phenotypes) involve internal comparisons between subgroups within individual cohorts assayed with the same methodology.

\textbf{Definition of variable}

\textit{Current wheeze:} Positive response to the question "Has your child had wheezing or whistling in the chest in the last 12 months?"
Current asthma: All three of the following: 1) Current wheeze; 2) Current use of asthma medication; 3) Physician-diagnosed asthma.

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

Rhinitis: Positive response to “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 (skin prick tests-SPT and sIgE): We defined SPT as positive if the wheal diameter was ≥3mm, and positive sIgE if the titre was >0.35 kU/L. SPTs employed 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 employed the basophil activation assay. Briefly, donor basophils in PBMC were acid stripped to remove FcεR1-bound IgE, and passively re-sensitized by incubation for 1hr at 37°C in buffer containing serum that had been pre-assayed to determine levels of Der p 1-sIgG and HDM-sIgE. Discrete sets of experiments utilized either a series of individual sera with comparable (high level) HDM-sIgE but varying levels of corresponding sIgG, or a series of pooled pre-assayed sera premixed to achieve a range of sIgG:sIgE ratios against a background of the same concentration of sIgE. The basophils were activated by addition of Der p 1 to a final concentration of 0.1ug/ml and incubated for a further 30 minutes, and the response stopped by addition of cold 20mM EDTA. Activation was assessed flow cytometrically via surface expression of CD3, CD19-, CD14-, HLADR-, CD123hi, FcεR1hi, CD11c-. As a follow up to one series of experiments detailed in the text, the sera employed were re-assayed for Der p 1-specific IgE in order 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 which control sIgG:sIgE balance, we performed genome-wide expression profiling of HDM-induced CD4+ Th-memory responses in 45 HDM-sensitized, RAINEx RAINE 7.
Yr14 subjects selected on the basis of cell availability and moderate-high level sensitization (IgE > 24 ug/L) using established methods. Cryobanked PBMC from cohort subjects were cultured for 24 hrs with HDM allergen to activate Th-memory responses, and affinity purified CD4+ T-cells were harvested from the cultures for RNA extraction employing DYNAbeads, and subsequent expression profiling on Affymetrix microarrays, as detailed previously. 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

The raw microarray data are available via the Gene Expression Omnibus repository (accession: GSE70760; http://www.ncbi.nlm.nih.gov/geo/). Raw microarray data was pre-processed in R employing the RMA algorithm and a custom mapping of probe sets to gene. Batch effects were identified using principal components analysis and removed using ComBat. Noisy probe sets were filtered out of the analysis employing the pvac algorithm. Quantitative associations between gene expression patterns and logE ratios were identified employing the Significance Analysis of Microarrays (SAM) algorithm. SAM computes a test statistic derived from the linear regression coefficient of each gene on the outcome divided by the sum of the standard error and the square root of the residual error. Statistical significance is assessed by repeated permutations of the data. Quantitative associations between expression levels of the Th2 module and logGE ratios were assessed using Gene Set Analysis. Upstream Regulator Analysis (URA) was employed to identify signalling molecules that have the capacity to drive the observed downstream gene expression changes. 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 if the direction of the observed gene expression changes for each regulator is consistent with the predictable pattern based on prior studies.

Statistical analyses

We assessed differences in antibody titres and ratios thereof by Mann-Whitney U test or Kruskal-Wallis test, and categorical differences between groups using Chi-squared test (SPSS and STATA).
Role of funding sources

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 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 1 (top) focuses on asthma and their responsiveness to HDM. Amongst the mite-sensitized children in the cohort, HDM sIgE titres were significantly higher in those with asthma compared to non-asthmatics, and asthma prevalence increased steeply across ascending HDM sIgE quartiles (11%, 16%, 24% and 41% respectively, versus 5% in those not sensitized). The higher average sIgE titres in asthmatics were mirrored by higher sIgG4 titres, whereas corresponding total sIgG (predominantly IgG1) was not increased among asthmatics. A comparable relationship was observed amongst rye grass sensitized children between grass sIgE titres and rhinitis prevalence, with corresponding sIgG4 titres again higher amongst symptomatic children (Table 1 bottom). In both the HDM and grass antibody responses, the contribution of sIgG4 to overall sIgG titres was very low (in the order of 10%; Table 1), 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 amongst mite-sensitized children the HDM sIgG:sIgE ratios were significantly lower in Raine 14Yr children with asthma compared to those without (Table 1 top), and corresponding findings relating to grass sIgG:sIgE ratios and rhinitis susceptibility (Table 1 bottom) were similarly strikingly and negatively associated.
bottom) were comparable. This inverse relationship was replicated across all cohorts and at different ages for both wheezing-associated and rhinitis symptoms (Figure 1 and Table E2).

**Asthma severity and persistence**

The analysis amongst HDM-sensitized children with current asthma demonstrated a downward trend of slgG:slgE ratios with increasing asthma severity, against a background of high slgE which was significant in the RAINE cohort but not in MAAS (Table E3).

In RAINE Yr14, in addition to subjects with current active asthma (physician-diagnosed asthma, current asthma medication, current wheeze), we identified a subset of 49 children with well-controlled asthma (physician-diagnosed asthma, current asthma medication, but no wheeze in the previous year). Well-controlled asthmatics in whom symptoms had waned had significantly higher slgG:slgE ratios, with minimal differences in slgE (Table E4). There were insufficient numbers expressing this phenotype for meaningful analyses in the younger age groups and other cohorts.

We have previously reported low HDM-slgG1 in HDM-sensitized children with susceptibility to hospitalization for severe asthma exacerbations in a case-control study\(^\text{17}\) re-analysis of these data with addition of further subjects confirmed that low slgG:slgE ratio in serum collected at the time of exacerbation is a marker of this clinical phenotype (Table E5A). Reduced slgG:slgE ratios were also observed amongst HDM-sensitized MAAS Yr11 children who had been hospitalised with asthma exacerbations (confirmed via transcription of medical records data\(^\text{26}\)) (Table E5B).

**Mechanistic studies**

**Immediate hypersensitivity responses**

(a) slgG-mediated attenuation of skin prick test reactivity in vivo

If slgG attenuated slgE-mediated responses in the airways, it was likely that SPT responses may also be affected. To test this, we examined the relationship between sensitization determined by slgE versus SPT across the cohorts, which revealed a non-random disparity in a major subgroup of atopics. For example, 27% of RAINE Yr14 children with HDM-slgE>0.35kU/L (designated IgE\(^+\)) and 44% of those who were IgE\(^+\) to grass, were skin test negative to the respective allergens (IgE\(^+\)SPT\(^-\)). Of note, the frequencies of SPT\(^+\) children amongst the IgE\(^-\) groups were only 3% and 1% for
HDM and grass respectively. A comparable dissociation between sIgE and SPT was observed across all ages/cohorts (Figure 2A). Mean sIgE levels in the IgE+/SPT+ children were significantly higher than in those who were IgE+/SPT- (Table E6), but multiple IgE+/SPT+ children had sIgE titres within the 20-30kU/L range. The IgE+/SPT+ phenotype was consistently associated with higher sIgG:sIgE ratios (Figure 2B; Table E6). Note also the higher prevalence of asthma and rhinitis amongst the IgE+/SPT+ relative to IgE+/SPT- children (Figure 2C).

(b) sIgG-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. We initially selected a series of individual sera from strongly HDM-sensitised RAINE Yr14 participants who were within a narrow band of HDM-specific IgE titres, but spanning a broad (~40-fold) range of Der p 1 sIgG titres. Single donor basophils were pre-armed with these sera for 1 hour at 37°C prior to triggering by addition of Der p 1, and basophil activation quantified 30 minute later. A strong trend towards IgG-dose-related inhibition of basophil activation is evident in these data (representative experiment in Figure 3A).

We reasoned also that some of the noise in this plot was likely due to inter-subject variations in antibody affinity, allergen component specificity, IgG subclass ratios etc. To reduce this variability, we firstly repeated the titration employing pre-assayed 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 pre-armed with these serum pools provided stronger evidence of IgG dose-dependent inhibition of basophil activation: the titration in Figure 3B was performed in the presence of Der p 1 at 0·1ug/ml, and comparable results were obtained in titrations employing Der p 1 at 0·1, 1·0 and 10·0ug/ml (not shown). Secondly, we reassayed all these sera for IgE 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 and used these for reanalysis of the basophil activation data from Figure 3A/B, with identical conclusions (Figure E1).

Allergen-specific Th-memory responses and sIgE/sIgG “balance”

Specific Th-memory cells play a major role in control of antibody production by B-cells, including the balance between antibody isotypes. We proceeded to investigate whether the expression of
"high sIgG:sIgE" immunophenotype amongst atopics is reflected by characteristic pattern(s) of gene expression in allergen-triggered Th-memory recall responses. Figure 4A is a q-q plot derived from the Significance Analysis of Microarrays (SAM) algorithm, 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 outside the null distribution are negatively associated with sIgG:sIgE ratios. The majority of these (listed in Table E7) are key components of the atopy-associated Th2 module which we have previously characterized employing network analysis of aeroallergen-specific Th-memory responses, and overall expression of this module was inversely related to sIgG:sIgE ratios (fdr<0.001, Gene Set Analysis). The Upstream Regulator Analysis confirmed that increasing sIgG:sIgE ratios are associated with reduced signalling of multiple pathways (particularly those denoting Th2 immunity/T-cell activation), coupled with increased IL-10 signalling (Figure 4B; Table E8). Moreover, re-expressing our published HDM-induced PBMC cytokine response data from the whole population of HDM-sensitized RAINE cohort subjects and stratification by clinical phenotype demonstrated a reciprocal relationship between IL-10 and Th2 cytokine production and asthma risk (Figure 4C). Finally, across the same sensitized population, Th2 cytokine:IL-10 response ratios also correlated negatively with HDM-slgG:slgE ratios in serum from respective donors (Spearman’s Correlations, n=527 subjects: IL-5:IL-10, Rho=-.448, p=0.001; IL-13:IL-10, Rho=-.390, p=0.01; IL-4:IL-10, Rho=-.203, p=0.01).

**DISCUSSION**

Our findings indicate that a consistent feature of children who are moderately or highly sensitized, but who do not develop asthma and rhinitis, is elevated 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 sensitisation-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, who were skin test negative; these expressed the same high sIgG:sIgE immuno-phenotype, 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 may interfere with this process via mechanisms which include activation of inhibitory Fc-gamma receptors on mast cells/basophils. 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 Th-memory-cell-dependent late-phase reactions (25 and Figure 5). The late-phase reaction is driven by Th2 cytokines including IL-4 and IL-13, which also control IgE-B-cells maturation, and repeated cycles of production of these cytokines by aeroallergen-triggered Th-memory cells may account for the preferential expansion of sIgE relative to sIgG1 component of the humoral response in the symptomatic subgroup.

Our recent studies have established that aeroallergen-specific Th-memory responses in children involve activation of complex networks comprising multiple effector and regulatory genes, which we hypothesized may include those that can influence the balance between the IgE and IgG1 antibody isotypes. In this regard, our Th-memory profiling studies in mite-sensitized children identified a distinctive pattern of gene expression by aeroallergen-specific CD4+ Th-memory cells associated with sIgG:sIgE ratios, which is characteristic of IL-10 exposure of these cells during allergen-induced reactivation. The most likely proximal sources of IL-10 in this context are IL-10-producing “regulatory” cells, which are co-activated to varying degrees in all Th-memory responses. The principal immunological 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. Its release in sufficient amounts during repeated aeroallergen-induced Th2-cell activation cycles may thus contribute to slowing the progressive decline in sIgG:sIgE ratios.
as the sIgE response expands over time in chronically exposed sensitized subjects, by provision of an IgG1-trophic signal in parallel with those driving sIgE production. The presence of such sIgG above a critical threshold provides a mechanism for attenuation of allergen-triggered FcεR1-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 Th2-memory cells responsible for the ensuing late-phase response\textsuperscript{35}, and it is plausible that both these components of the allergic inflammatory cascade may be subject to co-regulation via complementary IL-10-dependent mechanisms (proposed mechanism in Figure 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, may 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\textsuperscript{18,38}. This suggests that a generalized deficit in IgG response capacity at mucosal surfaces may be an integral component of the high-risk phenotype in relation to inflammatory airway diseases\textsuperscript{38}, further emphasizing the need for increased focus on immunological mechanisms beyond IgE in the host response to aeroallergens.

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


Table 1: Allergen specific antibody response profiles and respiratory symptoms amongst HDM and rye grass sensitized children from the 14yr follow-up of the RAINE community cohort

**Legend:** The study population comprised 521 14yr olds sensitized to HDM (top panel) and 543 sensitized to grass (bottom panel), as defined by sIgE ≥ 0.35kU/L. Data shown are group mean/standard errors of HDM-specific IgE, IgG (total) and IgG4 levels assayed via ImmunoCAP and re-expressed in a common unit (ug/L); IgG:IgE ratios were initially computed for individual children, and group mean ratios were then calculated from these. P values derived from Mann Whitney U test comparing the two clinical phenotypes.

**Legends for Figures**

**Figure 1:** Relationship between HDM and grass-specific IgG:IgE ratios amongst sensitized children and expression of asthma/wheezing or rhinitis

Children with HDM-specific or grass-specific serum IgE>0.35 kU/L were recruited in the Australian RAINE and CAS birth cohorts, and the UK MAAS cohort as specified.

*** p<0.001; ** p<0.01; * p<0.05; φ p=0.08

**Figure 2:** Dissociation between sensitization status determined by sIgE versus skin prick tests (A) and its relationship to sIgG:sIgE ratios (B) and clinical symptoms of asthma and rhinitis (C) in three cohorts at different ages.

Children were selected on the basis of HDM-IgE or grass-IgE titres>0.35 kU/L ("IgE+") and then stratified on the basis of positive or negative responses to relevant allergen into dichotomous IgE" SPT+ and IgE" SPT- groups.

**Panel A:** The relative frequency of each of the phenotypes within each cohort (analyses in the 5yr olds restricted to HDM as the frequency of grass responders was very low).

**Panel B:** sIgG:sIgE ratios in each group
Panel C: Relative frequency of subjects expressing symptoms of asthma or rhinitis respectively in the HDM and grass-sensitized groups.

*** p<0·001; ** p<0·01; * p <0·05; ψ p=0·078

Figure 3: HDM-induced basophil activation following basophil arming with HDM-IgE-rich sera against a background of increasingly high levels of HDM Der p 1-specific IgG.

Panel A: Individual sera from 10 Yr14 RAINE participants strongly sensitized to HDM (HDM-IgE titres 41-61 ug/L as shown) were used to arm stripped donor basophils prior to activation by 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.

Panel B: Preassayed sera from HDM-sensitized Yr14 RAINE participants were used to generate a series of pools standardized for HDM-specific IgE level (50ug/L) but with corresponding Der p 1-specific IgG varying across a logfold range. Data illustrated show levels of basophil activation achieved at each specific IgG:IgE ratio.

Figure 4: Variations in HDM-specific Th-memory programming as determinants of specific IgG:IgE ratios and asthma susceptibility amongst RAINE Yr14 participants

Panel A: HDM-IgG:IgE ratios in 45 sensitized RAINE Yr14 participants were tested as a continuous trait against corresponding HDM-induced CD4+ T-cell gene expression profiles, employing the SAM algorithm. Data are illustrated as a q-q plot; differentially expressed genes inversely associated with the trait (negative scores) are shown in green (see corresponding TableE7).

Panel B: Candidate upstream regulators driving HDM-induced gene expression patterns associated with IgG:IgE ratios were identified utilizing the Ingenuity Pathway URA algorithm, as detailed in online methods. 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 corresponding TableE8 for p-values for candidate negative regulators).
Panel C: Banked data on HDM-induced cytokine secretion by PBMC from 521 HDM-sensitized children (92 asthmatics/429 non asthmatics) was reanalyzed to derive Th2 cytokine:IL-10 ratios in the two groups.

Figure 5: Complementary IL-10-dependent pathways for regulation of allergic inflammation

As detailed in text, IL-10 may play a dual role in attenuation of the allergic inflammatory cascade: (i) acting indirectly via promotion of sIgG1 production which modulates the FcεRI-dependent acute phase, and (ii) acting directly via modulation of Th2-memory cell activation in the late phase response.
<table>
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<tr>
<th>RAINE Yr14 (HDM-sensitized)</th>
<th>Asthma</th>
<th>N</th>
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<td>HDM-IgE</td>
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<td>187.5 ± 34.4</td>
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<td>HDM - IgG</td>
<td>No</td>
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<td>1850.9 ± 81.4</td>
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<td>HDM - IgG4</td>
<td>No</td>
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<td>195.3 ± 14.4</td>
<td>&lt;0.001</td>
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<td>332.8 ± 37.7</td>
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<td>HDM – IgG:IgE ratio</td>
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<th>N</th>
<th>Mean ± S.E</th>
<th>P</th>
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<td>Yes</td>
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<td>204.9 ± 22.4</td>
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</table>
Figure 1

![Graph showing the IgG : sIgE ratio for different phenotypes and ages.](image)

- **RAINE 6y (Asthma)**
- **RAINE 6y (Rhinitis)**
- **RAINE 14y (Asthma)**
- **RAINE 14y (Rhinitis)**
- **MAAS 5y (Asthma)**
- **MAAS 5y (Rhinitis)**
- **MAAS 11y (Asthma)**
- **MAAS 11y (Rhinitis)**
- **CAS 3y (Wheeze)**
- **CAS 5y (Wheeze)**

**Phenotype neg**

**Phenotype pos**

- ***
- **
- *
- *
- φ

**RAINE 14y (Asthma)**

**RAINE 14y (Rhinitis)**

**RAINE 6y (Asthma)**

**RAINE 6y (Rhinitis)**

**MAAS 11y (Asthma)**

**MAAS 11y (Rhinitis)**

**MAAS 5y (Asthma)**

**MAAS 5y (Rhinitis)**

**CAS 3y (Wheeze)**

**CAS 5y (Wheeze)**
Figure 2.
Figure 4

(A) Observed score vs. Expected score

(B) Activation z-scores for different ILs

(C) Ratio x 10^7 for asthmatic vs. asymptomatic