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Early-onset atopy is associated with enhanced lymphocyte cytokine responses in 11-year-old children

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Summary

Background Early age at onset of atopy is associated with more severe asthma and increased airway responsiveness (AR); the underlying mechanism is unclear but may involve T cell responses.

Objective To test the hypothesis that enhanced T cell responses may be associated with early-onset atopy.

Methods In a longitudinal study, atopy was determined in infancy and at 6 and 11 years of age. Individuals were categorized as persistent infant-onset atopy (PIOA), early childhood-onset atopy (ECOA) and later childhood-onset atopy (LCOA). At 11 years of age, peripheral blood T cell cytokine responses, AR, exhaled nitric oxide (FE_{NO}) and forced expiratory volume in 1 s were determined.

Results The age at onset of atopy was determined for 60 children, of whom 15 had PIOA, 24 had ECOA and 21 had LCOA. An additional 76 children who were never atopic were also included. T cell responses to house dust mite, including interleukin-5, -9, -10 and tumour necrosis factor α , were higher among children with PIA and ECOA, and lower in children with LCOA, $P < 0.05$. In contrast, those children with LCOA or who were not atopic had the highest IL-10 response to PHA ($P = 0.014$). Children with PIOA and ECOA, but not LCOA, had higher AR and FE_{NO} compared with non-atopic children ($P < 0.05$). The group with PIOA were more likely among the atopic children to be admitted to hospital for asthma ($P < 0.05$) and also had lower %FEV₁ compared with non-atopic children ($P = 0.023$).

Conclusions Early age at sensitization is associated with enhanced T cell cytokine responses and indices of adverse asthma outcome. T cell cytokine responses might be programmed at the time of initial atopic sensitization.

Keywords airway responsiveness, asthma, cytokines, longitudinal studies, skin tests

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Introduction

Childhood asthma is a common condition that affects over a quarter of children in Australia, New Zealand and the United Kingdom [1]. The underlying mechanism for asthma is not well understood, and the consistently reported association between atopy and childhood asthma [2, 3] suggests a causal relationship.

The relationship between asthma and atopy is not exclusive [2, 3], with population studies showing that atopy, defined by a positive skin prick test (SPT) to

aeroallergens, is much more common than asthma. Data from several studies suggest that individuals who develop persistent atopy at an early age are more likely to develop asthma compared with those in whom the onset of atopy is delayed [4–7]. Further, early-onset childhood atopy has been associated with more severe asthma outcome in adulthood [6]. The mechanism whereby early onset for persistent atopy is associated with worse asthma outcome is unclear. A study in mice assessed bronchoalveolar lavage fluid (BALF) cytokine concentrations in response

to ovalbumen sensitization and found that early sensitization was associated with increased IL-4 and 5 and later sensitization was associated with higher concentrations of IFN- γ and IL-10 [8]. Cytokine responses have not been related previously to age at onset of atopy in a human population.

Our birth cohort has undergone longitudinal assessments of skin prick reactivity, beginning in infancy, permitting age at onset of atopy to be determined [9]. Peripheral blood T cell cytokine responses have also been measured in these children at 11 years of age and patterns for atopy, bronchial responsiveness and different wheezing phenotypes have been reported [10]. The primary aim of the present study was to relate age at onset of atopy to peripheral blood T cell cytokine responses at 11 years of age. We predicted that early age at onset of persistent atopy would be associated with increased antigen-specific cytokine production *in vitro*. The secondary aim of the study was to relate age at onset of atopy to asthma and spirometric measurements, airway responsiveness (AR) to inhaled histamine and exhaled nitric oxide (FE_{NO}).

Methods

Study subjects

Participants were recruited antenatally as part of a longitudinal study of atopy and asthma; enrolment is described in detail elsewhere [11]. There was no selection for parental asthma or allergic disease in this Caucasian population. Exclusion criteria included premature delivery (i.e. before 36 weeks of gestation) and respiratory infection in the first month of life. The study was approved by the Human Ethics Committee of Princess Margaret Hospital for Children and the Confidentiality into Health Information Committee. Written parental consent was obtained for each assessment and verbal assent from children at 6 and 11 years of age.

Protocol

Assessments were undertaken at ages 1, 6 and 12 months, 6 and 11 years. An SPT was performed at each assessment. A detailed assessment was completed at 11 years of age including respiratory questionnaire, spirometry, AR to histamine, FE_{NO}, cellular studies and *in vitro* studies. Assessments of 11-year-olds were either completed at the hospital or at the child's home, and FE_{NO} was only measured in those who attended the hospital.

Skin prick testing

Skin reactivity was assessed using the SPT [12]. Reactivity to the following allergens was ascertained during infancy: cows' milk, egg white, rye grass and *Dermatophagoides*

farinae. In childhood, reactivity to the following additional six allergens was also assessed: mixed grass (Kentucky Bluegrass, Orchard grass, Redtop, Timothy, Sweet Vernalgrass, Meadow Fescue and Perennial Ryegrass.); *Dermatophagoides pteronyssinus* [house dust mite, (HDM)]; cat dander; dog dander; *Alternaria alternans*; and *Aspergillus fumigatus*. All allergens were supplied by Hollister-Stier, Elkhart, IN, USA. Histamine sulphate (10 mg/mL) was used as the positive control and 0.9% saline as the negative control. The positive control was read after 10 min; all other tests were read after 15 min. At all ages, a positive SPT was defined as a weal to any allergen of at least 3 mm in its longest dimension.

Spirometry and airway responsiveness to histamine

A portable spirometer (Pneumocheck Spirometer 6100; Welch-Allyn, Skaneateles Falls, NY, USA) was used to measure forced expiratory volume in 1 s (FEV₁), forced vital capacity (FVC), FEF₂₅₋₇₅ and peak expiratory flow (PEF) in accordance with international guidelines [13]. FEV₁ was expressed as percent predicted using a previously published equation [14] adjusting for height, gender, parental smoking and increased AR (PD₂₀ < 7.8 μ M) (micromoles histamine). The rapid technique was used to determine childhood AR [15]. Briefly, increasing doses of inhaled histamine were administered from a hand-held dosimeter until either a 20% reduction in FEV₁ occurred or the maximal cumulative dose had been administered (7.8 μ M). The dose-response slope (DRS) to histamine was defined as the % reduction in FEV₁ divided by the cumulative dose of histamine delivered. The DRS was log transformed to achieve a near-normal distribution (a constant of three was added to the DRS in order to permit inclusion of values of zero or less).

Exhaled nitric oxide

On-line measurements of FE_{NO} were made using a chemiluminescence analyser (NOA 280, Sievers Instruments Inc, Boulder, CO, USA) as described previously [16]. Measurements were taken before spirometry and histamine challenge. This study commenced before publication of the first international recommendations for FE_{NO} measurements [17] and the children maintained an expiratory flow of 35 mL/s.

Cellular studies

The techniques used have been described in detail previously [10]. *In vitro* studies on peripheral blood mononuclear cell (PBMC) responses were performed on cryobanked samples, using the methods detailed previously [18, 19]. Following thawing, the PBMC were cultured for 48 h in serum-free AIM-V medium alone or

stimulated by culture for 48 h with 10 µg/mL HDM extract or 1 µg/mL PHA (Murex, Dartford, UK). Cell pellets were resuspended in RNALater (Ambion, Austin, TX, USA) and stored at -20 °C.

In vitro analyses

The IgE assay was performed as described previously; total IgE and HDM-specific IgE are reported [20]. IL-5, IL-10, IL-13, TNF- α and IFN- γ protein in the supernatants were assayed using ELISA or time-resolved fluorescence (TRF) [18]. Quantification of IL-4 and IL-9-specific mRNA was performed by Taqman PCR. Details of primer pairs, probes and reference gene are reported elsewhere [10]. Blood eosinophil count was measured using a flow cytometer (Coulter Maxm™, Beckman-Coulter Inc., Fullerton, CA, USA) and expressed as absolute cell count.

Definitions

Infant atopy was defined as a positive SPT on at least one occasion during infancy. The definitions for age at onset of atopy were persistent infant onset atopy (positive SPT in infancy and at 6 and 11 years of age); early childhood-onset atopy (negative SPT in infancy and positive SPT at 6 and 11 years of age); and late childhood-onset atopy (negative SPT in infancy and at 6 years and positive SPT at 11 years of age). Two additional groups were also apparent when classifying never atopic (negative SPT in infancy and at 6 and 11 years of age); and atopy resolved (positive SPT in infancy and/or at 6 years but negative at 11 years). For those individuals not assessed at 6 years, those found to be non-atopic as infants and 11-year-olds were classified as never atopic, those found to be atopic both as infants and 11-year-olds were classified as having persistent infant-onset atopy and those atopic as infants but not as 11-year-olds were classified as atopy resolved. Doctor-diagnosed asthma (DDA) was defined as an affirmative response to the question 'Does your child currently have asthma diagnosed by a doctor?' Current hayfever symptoms were defined as an affirmative response to the question 'Does your child have hayfever symptoms including episodes of sneezing and/or an itchy or runny nose?' Individuals admitted to hospital with a primary diagnosis of asthma were identified from the Maternal and Child Health Data base of Western Australia, which records details of admissions to all hospitals in the state, with the exception of individuals discharged from emergency departments.

Statistics

Comparisons between groups were analysed using the χ^2 test, Student's *t*-test, the Mann-Whitney *U*-test or the Kruskal-Wallis test, where appropriate. As previously

[10], T cell cytokine response values below the limits of detection (including 0 values) were ascribed a value equivalent to the $0.5 \times$ limit of detection. Multi-nomial logistic regression was then used to determine which log-transformed variables were acting independently, after adjustment for parental asthma and being first born. Age at onset of atopy was coded as a class variable: 0, never atopic; 1, atopy resolved; 2, later childhood-onset atopy; 3, early childhood onset; and 4, persistent infant-onset atopy. The variables identified in univariate analyses were introduced in a forward step-wise manner, the variable with the smallest *P*-value being introduced first. The Pearson goodness-of-fit test was used to measure the precision of the multi-variate model. Significance was assumed at 5%. Analyses were performed using a standard statistical software package (SPSS release 10.0.7, SPSS, Chicago, IL, USA).

Results

Study subjects

The original cohort numbered 253 infants. Thirty-two of the 253 infants were SPT positive (22 to ingested allergens, eight to inhaled allergens and two to both ingested and inhaled allergens), 39 of the 112 six years olds tested were SPT positive (38 to any aeroallergen, 21 to HDM and one to both ingested and inhaled allergens) and 94 of the 182 eleven year olds were SPT positive (75 to any aeroallergen, 66 to HDM and 19 to both ingested and inhaled allergens). The age at onset of atopy could be determined in 150 children, and data from these children were used in this study; Table 1 shows that these were mostly representative of the whole cohort. Of these 150 children, AR and FEV₁ were determined in 144, cytokines and IgE were determined in 125 and FE_{NO} in 72. There were 15 children with persistent infant-onset atopy, 24 with early childhood-onset atopy, 21 with later childhood-onset atopy, 12 whose atopy resolved and 76 who were never atopic, Fig. 1. Additionally, there were two children who were atopic in infancy and at 11 years of age but who were not atopic at 6 years of age. Table 2 compares asthma-related outcomes at 11 years of age for these five groups. Figs 2 and 3 display the dose-response slope and exhaled nitric oxide values across the five groups.

Association between age at onset of atopy and immune parameters for all groups

Cytokine responses and IgE were determined in 125 children including 14 with persistent infant-onset atopy, 20 with early childhood-onset atopy, 20 with later childhood-onset atopy, 10 whose atopy resolved and 61 who were never atopic. Univariate analysis across the five groups demonstrated higher IL-5 and IL-9 responses to

Table 1. This table compares details of the children where age at onset of atopy was ascertained with the whole cohort

	Included in the present study (total = 150 unless stated)	Whole cohort
Male	57% (85)	58% (142/253)
Parental asthma at enrolment	27% (39/146)	31% (75/242)
First born	31% (47)	38% (96/251)*
Atopic as infant	18% (27)	13% (32/253)*
Atopic at 6 years	33% (34/103)	35% (39/112)
Atopic at 11 years	41% (62)	52% (94/182)*
DDA at 11 years	17% (19)	15% (29/194)
Admitted for asthma ever	6% (9)	6% (11/193)
Hayfever symptoms at 11 years	18% (27)	18% (35/194)
Mean %FEV ₁ at 11 years (SD)	100 (11) (<i>n</i> = 144)	100 (11) (<i>n</i> = 176)
Median dose-response slope at 11 years (IQR)	1.7 (0.6, 2.9) (<i>n</i> = 144)	1.7 (0.8, 3.4) (<i>n</i> = 176)
Median FE _{NO} at 11 years (IQR)	9.8 (5.4, 19.6) (<i>n</i> = 72)	10.3 (6.3, 20.6)* (<i>n</i> = 93)

**P* < 0.05 for comparison between groups.

DDA, doctor-diagnosed asthma; FEV₁, forced expiratory volume in 1 s; SD, standard deviation; IQR, interquartile range; FE_{NO}, exhaled nitric oxide.

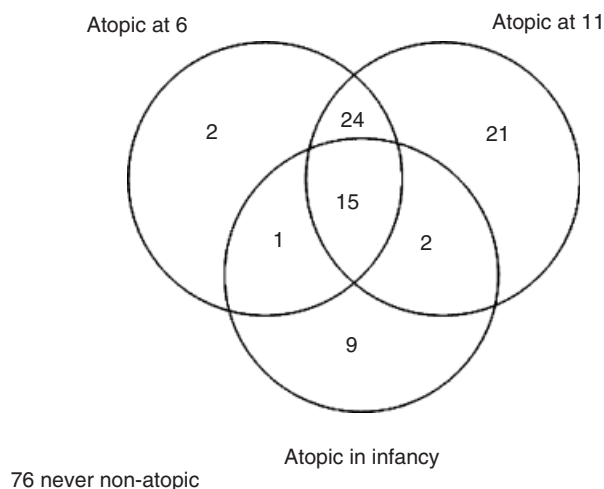


Fig. 1. Venn diagram demonstrating overlap between atopy, as evidenced by skin prick positivity, in infancy and at ages 6 and 11 years. There were five additional atopic infants lost to follow up. Seven children defined as atopic at all three assessments who were not assessed aged six years.

HDM and HDM-specific IgE in those with persistent infant-onset and early childhood-onset atopy and lower responses for the remaining groups, $P \leq 0.037$, Table 3 and Fig. 4. The IL-10 and TNF- α responses to HDM were lower for those with later childhood-onset atopy but did not differ between the remaining groups, $P \leq 0.020$ (Table 3 and Fig. 4). Finally, the IL-10 response to PHA was the

lowest for the early childhood-onset atopy group and did not differ among the remaining groups, $P = 0.017$ (Table 3). Multi-variate analysis revealed that IgE specific to HDM ($P < 0.001$) and TNF- α response to HDM ($P = 0.027$) were independently related to age at onset of atopy (Table 4). There were no interactions between these terms. The goodness-of-fit test (Pearson's χ^2) indicated that the model was of acceptable precision ($\chi^2 = 256.4$, degrees of freedom = 296, $P = 0.953$).

Associations between age at onset of atopy and immune parameters for all atopic and only HDM SPT-positive children

When only atopic children were considered, IL-5, IL-10 and TNF- α responses to HDM were higher among infant-onset and early childhood-onset atopy and lower among later childhood-onset atopy, $P \leq 0.037$ (Table 3); similar trends that approached significance were also present for IL-13 and IFN- γ responses to HDM. In contrast, the IL-10 response to PHA was higher for later childhood-onset atopy and lower for the other two atopic groups. The analyses across the three atopic groups were repeated but only for children HDM SPT-positive at 6 and 11 years of age to ascertain whether the relationships were comparable with all atopic children. Of the 66 HDM SPT-positive children, age at onset of atopy could be ascertained in 46 (12 had persistent infant-onset atopy, 19 had early childhood-onset atopy and 15 had later childhood-onset atopy) and cytokine responses were elicited in 40 of the 46. The T cell cytokine responses in HDM SPT-positive children (Table 5) were comparable with those for all SPT-positive children; the differences for IL-10 responses between the 40 HDM SPT children were comparable with those between all SPT-positive children ($n = 54$), but only the latter analysis achieved statistical significance.

Association between cytokine responses and asthma admissions

Positive relationships between HDM-specific IgE, IL-5, -9 and -13 responses to HDM, and DDA, and increased AR at 11 years of age were previously described in this cohort [10]. Nine children were admitted to hospital with acute asthma and admission to hospital was associated with increased mean (SEM) log HDM-specific IgE (2.2 (0.5) vs. -0.7 (0.2) for other children, $P < 0.001$), log IL-5 response to HDM (3.2 (0.5) vs. 2.3 (0.1) for other children, $P = 0.022$) and log IL-13 response to HDM (4.0 (0.5) vs. 2.9 (0.1) for other children, $P = 0.014$).

Relationship between age at onset of atopy, asthma and related outcomes

There was no relationship between atopy group and diagnosed asthma at 11 years; however, persistent infant-

Table 2. Outcomes at 11 years of age in children grouped by age at onset of atopy

	Persistent infant-onset atopy (<i>n</i> = 15)	Early childhood-onset atopy (<i>n</i> = 24)	Later childhood-onset atopy (<i>n</i> = 21)	Atopy resolved (<i>n</i> = 12)	Never atopic (<i>n</i> = 76)	<i>P</i> -value across all groups
Male	53% (8)	63% (15)	62% (12)	42% (5)	55% (42)	0.778
Doctor-diagnosed asthma aged 11	27% (4)	25% (6)	29% (6)	0% (0)	8% (6)	0.019
Admitted for asthma ever	33% (5)	4% (1)	14% (3)	0% (0)	0% (0)	< 0.001**
Hayfever symptoms aged 11	47% (7)	38% (9)	29% (6)	8% (12)	5% (4)	< 0.001
Mean %FEV ₁ aged 11 years (SD)	95 (11)	102 (12)	99 (11)	102 (13)	101 (10)	0.023*
Median dose-response slope at 11 years	2.6	3.3	1.5	0.7	1.4	< 0.001
Median-exhaled nitric oxide at 11 years (p.p.b.)	20.5	23.5	7.8	9.7	7.5	< 0.001
Number of positive skin tests	4 (3–9)	5 (2–9)	2 (1–6)			0.003 [†]

There were no differences in height between the children.

*Comparison between persistent infant-onset atopy and never atopic.

[†]Comparison between three atopic groups.

***P* = 0.046 for comparison across atopic groups.

FEV₁, forced expiratory volume in 1 s; SD, standard deviation.

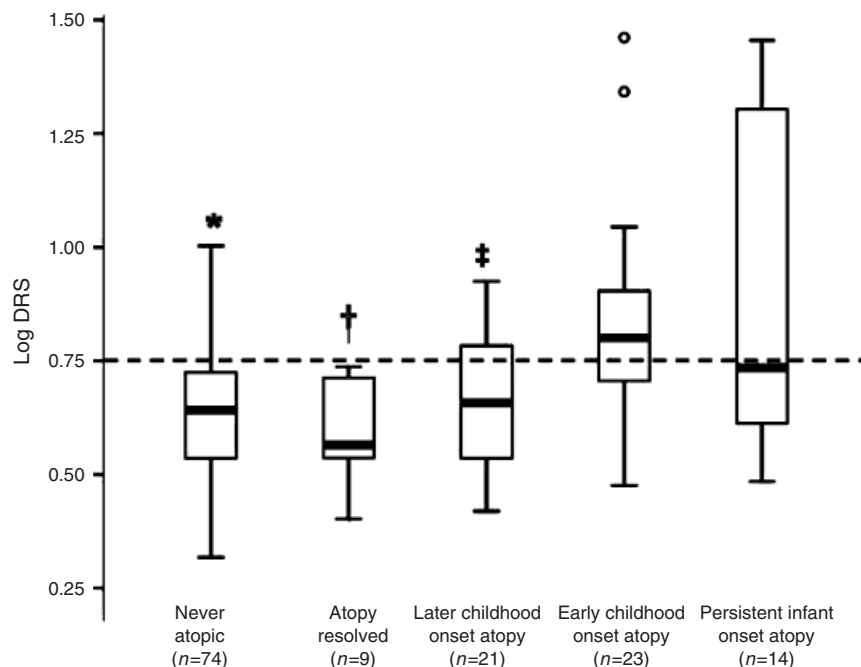


Fig. 2. Box and whiskers plot of the dose-response slope (DRS) to inhaled histamine in 11-year-old children categorized by their atopic status. The dashed horizontal line corresponds with the threshold for bronchial hyper-responsiveness ($PD_{20} < 7.8 \mu M$ histamine). **P* = 0.001 for comparison with persistent infant-onset atopy and *P* = 0.008 for comparison with early childhood-onset atopy, [†]*P* = 0.003 and [‡]*P* = 0.013 for comparison with persistent infant-onset atopy (ANOVA with Bonferroni's correction used for comparisons).

onset atopy was associated with increased risk for asthma admission compared with the other groups (*P* < 0.001, Table 2). The values of FEV₁, AR and FE_{NO} differed across the groups, with the lowest %FEV₁ being present in those with persistent infant-onset atopy (*P* = 0.023, Table 2) and the highest levels of AR (*P* < 0.001, Table 2, Fig. 2) and FE_{NO} (*P* < 0.001, Table 2, Fig. 3) were present in those with persistent infant-onset and early childhood-onset atopy.

Relationship between cytokine response and exhaled nitric oxide and FEV₁

Among the 41 atopic children, there were positive correlations (Spearman's ρ) between FE_{NO} and the following cytokine responses to HDM: IL-4 (0.46, *P* = 0.004), IL-5 (0.63, *P* < 0.001), IL-9 (0.48, *P* = 0.002), IL-13 (0.45, *P* = 0.003), IFN- γ (0.32, *P* = 0.042) and TNF- α (0.50,

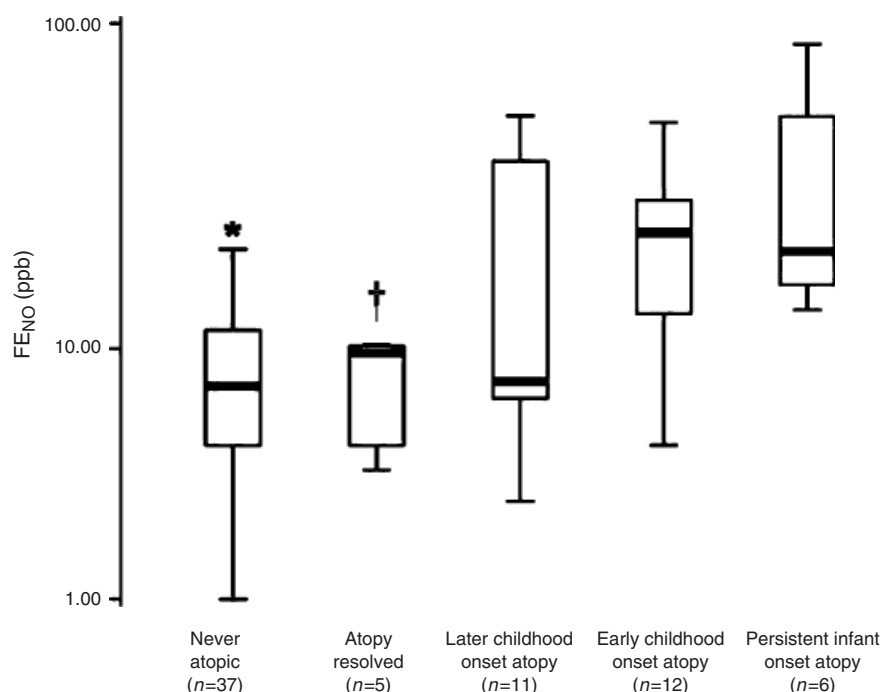


Fig. 3. Box and whiskers plot of exhaled nitric oxide (FE_{NO}) in 11-year-old children categorized by their atopic status. * $P < 0.001$ for comparison with persistent infant onset atopy and early childhood-onset atopy, † $P = 0.045$ for comparison with persistent infant-onset atopy (ANOVA with Bonferroni's correction of log-transformed FE_{NO} values was used for comparisons).

$P = 0.001$). There were no correlations between FE_{NO} and cytokine responses among the 36 non-atopic children where data were available. There were no correlations between cytokine responses and FEV₁ with and without stratification for atopy.

Discussion

The primary aim of this study was to determine whether early age at onset of atopy was positively associated with T cell cytokine responses. T cell cytokine responses to HDM, including IL-5, IL-9, IL-10, IL-13, IFN- γ and TNF- α , were greater for individuals with early-onset atopy, i.e. atopy persisting from infancy or early childhood, compared with those with later-onset atopy. In contrast, IL-10 response to the non-specific stimulant PHA was the lowest in individuals with persistent infant-onset atopy and the highest in children with later-onset atopy or who were never atopic. Our study also found associations between early age at onset of atopy and a more severe asthma phenotype, characterized by increased AR, FE_{NO} and risk for asthma admission and also reduced FEV₁, suggesting that age at onset of atopy may be an important determinant of asthma severity although not of asthma *per se*. The T cell responses may have been programmed at the time of initial sensitization and could be important to the previously described phenomenon where early-onset atopy is associated with adverse asthma outcomes, including in-

creased asthma severity and bronchial hyper-responsiveness [4–7].

The study finding that an early age at onset of atopy was associated with a more severe asthma phenotype was consistent with previous studies [4–7]. However, contrary to other studies [5, 21], we did not find that early age at onset of atopy was associated with increased asthma prevalence and these differences could be due to variations in sample sizes, ages of the participants and duration of follow-up. Although atopy is important to increased AR and asthma, there are additional factors involved in the development of asthma [22], as evidenced by the occurrence of asthma in non-atopic individuals. Respiratory infections in early life could be one such factor and we have previously reported an association between lower respiratory tract illness in infancy and increased AR at 11 years of age, independent of the onset of atopy in infancy in this cohort [23].

The onset of atopy in infancy was generally associated with a number of adverse outcomes; however, for infants whose atopy subsequently resolves, no differences were found at 11 years of age compared with individuals who had never been atopic. The phenomenon of infantile atopy resolving in some individuals has been described previously [24, 25], although outcomes in later life were not reported in these other studies. The mechanism whereby the early onset of atopy reverses in later life is not completely understood but the progressive development

Table 3. Mean values (SEM) for variables across groups characterized by age at onset of atopy; *P*-values are from Kruskal–Wallis's test

	IL-4 (Taqman units):			IL-5 (pg/mL)			IL-9 (Taqman units):			IL-10 (pg/mL)			IL-13 (pg/mL)			IFN- γ (pg/mL)			TNF- α (pg/mL)			IgE*			Eos ($\times 10^{-12}$ /mL)
	HDM	IL-4		HDM	IL-5		HDM	IL-9		HDM	IL-10		HDM	IL-13		HDM	IFN- γ		HDM	TNF- α		Total	HDM		
Persistent infant-onset atopy (<i>n</i> = 14)	68 (52)	70 (22)	500 (151)	21933 (16138)	21 (9)	144 (25)	101 (32)	1746 (280)	59 (22)	5587 (1282)	67 (20)	784 (186)	294 (60)	29 (9)	0.47 (0.09)										
Early childhood-onset atopy (<i>n</i> = 20)	42 (22)	45 (13)	284 (43)	4166 (1359)	14 (3)	100 (16)	59 (18)	1631 (222)	64 (33)	5624 (1523)	46 (18)	574 (138)	324 (61)	31 (11)	0.55 (0.08)										
Later childhood-onset atopy (<i>n</i> = 20)	22 (9)	30 (16)	557 (118)	1226 (513)	9 (2)	213 (35)	35 (12)	2524 (567)	8 (2)	6436 (1463)	11 (5)	609 (95)	568 (330)	2.4 (0.8)	0.46 (0.05)										
Atopy resolved (<i>n</i> = 10)	9 (4)	7 (2)	325 (71)	579 (314)	22 (9)	184 (27)	39 (18)	1734 (335)	131 (84)	8351 (2162)	94 (73)	987 (244)	24 (6)	0.1 (0.04)	0.25 (0.05)										
Never atopic (<i>n</i> = 61)	21 (6)	9 (1)	351 (48)	1235 (423)	25 (3)	191 (24)	29 (5)	1681 (173)	94 (21)	8568 (1086)	84 (20)	888 (104)	93 (28)	0.7 (0.5)	0.31 (0.03)										
<i>P</i> -value for trend across all groups	NS	< 0.001	NS	0.037	0.016	0.017	0.077	NS	NS	NS	0.020	0.100	< 0.001	< 0.001	0.078										
<i>P</i> -value for trend across atopic groups	NS	0.037	NS	NS	0.016	0.014	0.062	NS	0.064	NS	0.005	NS	NS	0.003	NS										

NS indicates *P* > 0.1.

*The units for total IgE are IU/mL and RAST U/mL for HDM-specific IgE.

HDM, house dust mite; PHA, phytohaemagglutinin.

of allergen-specific immunological tolerance in young children, after an initial burst of transient IgE production in infancy, may be one mechanism [26]. A better understanding of the mechanism whereby atopy in early life is 'turned off' could be important to secondary preventative strategies in sensitized infants and young children.

We were not able to determine when the level of T cell responses were first influenced (or 'turned on'), although there are data to support the concept that the immune system of some individuals with allergic disease is already predisposed towards atopy at or shortly after birth and before sensitization. For example, increased cord blood IgE has been associated with atopy [27] and atopic eczema during infancy [28]; however, not all studies have been able to reproduce these results [29, 30] and it is evident that while elevated cord blood IgE is a highly specific predictor of atopic risk it lacks sensitivity. IFN- γ production in early life may be a more meaningful indicator of atopic outcome than IgE. Increased IFN- γ levels in cord blood have been associated with reduced atopy and respiratory symptoms at 6 years of age [31], and reduced IFN- γ at 3 months of age (but not at birth) has been associated with increased wheeze in infancy [32]. Additionally, some studies [19, 33] have demonstrated a relationship between attenuated IFN- γ responses by cord blood T cells and risk for subsequent atopy development. A longitudinal study where T cell cytokine responses and atopy were ascertained on a number of occasions during infancy and childhood would confirm when the enhanced T cell cytokine responses are initiated and then established in atopic individuals.

The cytokine responses for those with early childhood-onset and persistent infant-onset atopy did not differ, suggesting that the critical period when cytokine responses are initiated and ultimately determined may extend beyond the first year of life. In this study and elsewhere [24], atopy in infants was predominantly to ingested allergens, suggesting that the initial site of allergen exposure is within the intestine. Data from other longitudinal studies indicate that sensitization to ingested allergens in infancy may be a surrogate for a high-risk phenotype [34, 35]. The results of the present study support this concept as sensitization to ingested allergens in infancy was associated with a high-risk phenotype in contrast to sensitization to inhaled allergens in later childhood, as evidenced by cytokine responses, AR, FE_{NO} and FEV₁.

The results of the present study extend the previous findings on this longitudinal birth cohort. In earlier cross-sectional analyses of this cohort, we have described associations between enhanced cytokine responses and asthma and bronchial hyper reactivity [10] and also FE_{NO} and atopy [16], and the present longitudinal analysis demonstrated that the age at onset of atopy explained some of the variability in cytokine responses, AR and

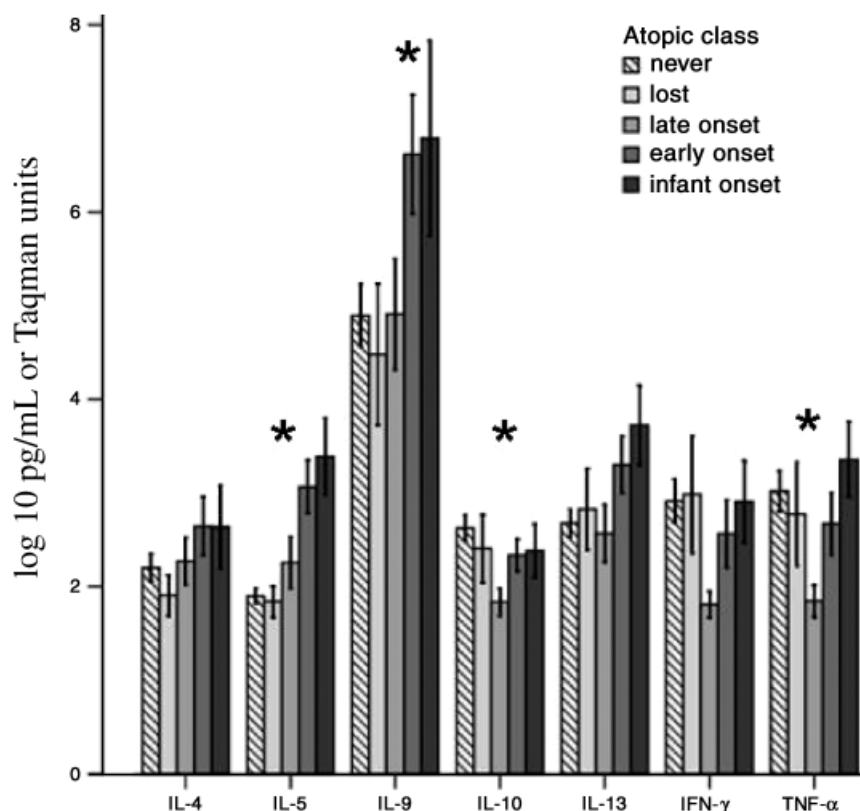


Fig. 4. Data for log-transformed *in vitro* cytokine responses to house dust mite are shown as mean $\pm$ standard error. * $P < 0.05$ for trend across five atopic groups.

Table 4. Results from a multinomial logistic regression model showing the relative differences in T cell responses for individuals with infant-or early childhood-onset atopy compared with children with later onset atopy

	Persistent infant-onset atopy	Early childhood-onset atopy	Later childhood-onset atopy	Atopy resolved	Never atopic	P-value for trend
HDM-specific IgE	3.5 [2.1, 5.8]	3.6 [2.2, 5.9]	2.4 [1.5, 3.9]	0.6 [0.1, 2.2]	1	< 0.001
TNF- α response to HDM	0.8 [0.6, 1.8]	0.8 [0.5, 1.3]	0.5 [0.2, 0.9]	0.9 [0.6, 1.4]	1	0.027

The values in the square brackets correspond to 95% confidence intervals. Parental asthma and being first born were included in this model as potential confounders.

HDM, house dust mite.

FE_{NO}. Consistent with our previous report that FE_{NO} was only correlated with peripheral blood eosinophilia among atopic children [16], in the present study, FE_{NO} was only correlated with cytokine responses in atopic children. We have also demonstrated enhanced cytokine responses in children with a history of previous asthma admissions. Our earlier analyses of T cell responses within this cohort suggested that T-helper type 1 (Th1) cytokine production may contribute to clinical symptomatology in atopic children including expression of increased AR [10]. Other studies have also demonstrated that stimulation of airway lymphocytes in asthmatics yields greater production of IFN- γ and TNF- α compared with controls [36]. The finding that early onset atopy was characterized by allergen-

specific immunity that includes a large Th1 component, and also reduced FEV₁, increased AR and asthma admissions is thus consistent with previous work [36] and implicates Th1 cytokines in the pathogenesis of asthma.

There are some possible limitations to this study. Firstly, the number of individuals was relatively small, particularly the 'atopy-resolved' group, and the results should be confirmed elsewhere. Secondly, the follow-up of the cohort was not complete and although we have demonstrated that children on whom the present study was based were more likely to be atopic as infants and less likely to be atopic as 11-year-olds, we do not believe that incomplete ascertainment has substantially affected the results as the differences were relatively small and there

Table 5. Mean values (SEM) for variables across HDM SPT positive 11-year-old children characterized by age at onset of atopy

	IL-4 (Taqman units):		IL-5 (pg/mL)		IL-9 (Taqman units):		IL-10 (pg/mL)		IL-13 (pg/mL)		IFN- γ (pg/mL)		TNF- α (pg/mL)		IgE*		Eos ($\times 10^{-12}/\text{mL}$)
	HDM	PHA	HDM	PHA	HDM	PHA	HDM	PHA	HDM	PHA	HDM	PHA	HDM	PHA	Total	HDM	
Persistent infant-onset atopy ($n = 11$)	87 (69)	87 (25)	590 (183)	28916 (21284)	25 (11)	151 (31)	124 (38)	2015 (300)	73 (27)	5734 (1477)	82 (24)	884 (230)	339 (69)	36 (11)	0.54 (0.10)		
Early childhood-onset atopy ($n = 15$)	49 (31)	56 (17)	310 (53)	5564 (1740)	14 (4)	107 (21)	71 (23)	1555 (207)	82 (44)	6922 (1921)	31 (13)	637 (177)	312 (74)	37 (14)	0.57 (0.10)		
Later childhood-onset atopy ($n = 14$)	87 (69)	40 (23)	636 (164)	16223 (710)	10 (3)	208 (44)	45 (16)	2951 (786)	10 (3)	6632 (1355)	11 (6)	686 (132)	279 (117)	2.4 (1.1)	0.43 (0.06)		
P -value	NS	0.031	NS	0.066	NS	NS	NS	NS	0.075	NS	0.004	NS	NS	< 0.001	NS		

P values are from Kruskal–Wallis's test. NS indicates $P > 0.1$.

*The units for total IgE are IU/mL and RAST units/mL for HDM-specific IgE. HDM, house dust mite; PHA, phytohaemagglutinin; SPT, skin prick test.

is no evidence of enrichment with atopic participants. Thirdly, the associations between early-onset atopy and all of the outcomes measured at 11 years could be due to children with early onset atopy having a longer time to develop these adverse outcomes; thus, further follow-up of this cohort is currently underway to explore whether cytokine responses, AR, FE_{NO} and FEV₁ for the individuals with later-onset atopy (by the criteria in the present study) become comparable with those in individuals with early-onset atopy. Fourthly, the assumptions made when categorizing children where data were missing may not all be correct as we have shown that two of the 17 individuals who were atopic as infants and 11-year-olds were skin prick negative as 6-year-olds. Finally, this study was initially designed to measure the development of pulmonary function, rather than atopy, during childhood and assessments between one and 6 years were not performed as pulmonary function testing in this age range is particularly challenging.

In summary, the present study demonstrated that early age at onset of atopy is associated with enhanced T cell cytokine responses and also increased risk for asthma admission, increased AR and FE_{NO} and reduced FEV₁ in 11-year-old children. These findings add to the evidence from this cohort study and elsewhere that factors present in early life are important to the development of allergic disease. If the T cell responses are programmed at the time of initial atopic sensitization, then strategies aimed at preventing or palliating asthma by immune modulation in older children and adults with established asthma may be too late [37].

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