

ORIGINAL ARTICLE

Basic Mechanisms in Allergic Disease

The association of allergic sensitization patterns in early childhood with disease manifestations and immunological reactivity at 10 years of age

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Abstract

Background: Allergy to German cockroach (CR) is common in urban environments and is an important allergen in children with asthma.

Objective: We hypothesize that the evolution of allergic sensitization and clinical disease is associated with distinct patterns of allergen-specific T cell reactivity. To test this hypothesis, a subset of high-risk inner-city children participating in the URECA (Urban Environment and Childhood Asthma) birth cohort were selected to evaluate CR-specific T cell reactivity from three distinct groups based on acquisition of aeroallergen sensitivity from ages 2 to 10: low atopy with minimal to no sensitivity (n = 26), early-onset allergic sensitization (n = 25) and late-onset allergic sensitization (n = 25).

Methods: Using pools of previously identified CR-derived T cell epitopes, we characterized the allergen-specific T cell response in these 76 subjects from blood samples obtained at age 10. CR-specific production of IL-5, IFN γ and IL-10 was measured by ELISPOT following two-week in vitro culture with CR extract.

Results: T cell responses were significantly higher in the early-onset atopy group compared to low atopy ($P = 0.01$), and a trend for higher cytokine production in the late onset compared to the low atopy cohort was also observed ($P = 0.06$). T cell responses were similar between early- and late-onset cohorts. Furthermore, a comparison of T cell reactivity between asthmatic and non-asthmatic individuals revealed significantly higher cytokine production in asthmatics compared to non-asthmatics ($P = 0.02$) within both the CR-allergic and non-allergic cohorts.

Conclusions and clinical relevance: In conclusion, the present study reports that higher T cell reactivity is associated with allergen sensitization and asthma. Interestingly, no significant difference in T cell reactivity was observed in allergic children with early-onset versus late-onset atopy.

KEYWORDS

allergens and epitopes, asthma, IgE, T cells

1 | INTRODUCTION

Allergic asthma is a chronic condition associated with substantial morbidity and high healthcare costs.¹ It is a heterogeneous disease manifesting as multiple clinical phenotypes with different pathophysiologic bases^{2,3} and is strongly related to allergy in children.⁴ In contrast to the substantial amount of data available at the level of IgE reactivity, data characterizing T cell responses as a function of different clinical phenotypes are very limited and most studies date back over a decade.⁵⁻⁷ Furthermore, very limited data are available regarding the characterization of T cell reactivity of exposed non-allergic individuals, despite the fact that non-allergic, exposed individuals do exhibit detectable T cell responses against environmental allergens, and despite the potential interest in understanding the tolerant state of these individuals.⁸

Cockroach sensitization and exposure is an important risk factor for asthma in low income, urban populations, previously identified by the work of the NIAID-funded inner-city asthma initiatives.⁹ In pursuing an in-depth understanding of the mechanisms of cockroach-induced allergic sensitization and asthma, we have mapped T cell epitopes recognized by adults sensitized to CR allergens (IgE titres range ≥ 0.35 to 100 kU/L) or non-sensitized (IgE < 0.35 kU/L) and as a function of allergic disease (asthma versus rhinitis vs non-allergic controls).¹⁰ In the case of CR allergy, this study in adults was the first to report that allergic T cell responses were highly polarized towards the production of Th2 cytokines, and of significantly higher magnitude in adult asthmatics compared to non-allergic controls.¹⁰ T cell reactivity in adults with allergic rhinitis but not asthma was higher than in non-allergic controls but lower than in allergic asthmatics. No significant differences in polarization between allergic asthmatic and allergic non-asthmatic but rhinitic donors were noted.¹⁰ By contrast, no characterization is available to date on the correlation of allergen-specific T cell reactivity with clinical phenotypes and previous exposure history in children, in general and for cockroach allergens in particular.

A recent study reported that the type of pertussis vaccine received in infancy (whole cell versus acellular pertussis) induces a long-lasting immunological imprint, resulting in differences in T cell response functionality and proliferation between the two vaccine modalities, detectable well into adulthood.¹¹ Similar imprinting has been reported following early exposure to peanut.¹² In the context of respiratory allergy, we hypothesized that early onset of allergic sensitization may imprint an immune signature that is reflected in both immune responses and asthma later in life. Furthermore, we set out to investigate the extent of allergen-specific T cell reactivity in non-allergic children to evaluate the state of immune tolerance for the first time in a paediatric cohort. The URECA birth cohort provides a unique opportunity to address these issues.

The Urban Environment and Childhood Asthma (URECA) study was established as an observational prospective study focused on a birth cohort of children from low-income urban areas of Baltimore, Boston, New York City and St. Louis.¹³ The main objective of the URECA study was to identify immunologic and environmental risk

factors for asthma in children in these medically disadvantaged areas. In URECA, sensitization patterns and clinical outcomes were prospectively measured from the first year of life onwards. Information on sensitization was analysed to determine temporal patterns of allergic sensitization from birth through age 10. Blood samples from children were obtained at 10 years of age. Here, we examine whether patterns of CR-specific T cell reactivity measured at age 10 were distinct in children with early sensitization, including sensitization to CR, or in children with asthma.

2 | METHODS

2.1 | Study design and participant selection

URECA is a birth cohort study initiated in 2005 in inner-city Baltimore, Boston, New York City, and St. Louis; details of the study design have been described elsewhere.¹³ In brief, pregnant mothers were recruited with selection criteria including a history of asthma, allergic rhinitis or eczema in the mother or father. Between February 2005 and March 2007, 1850 families were screened, 776 met eligibility criteria and 560 newborns were enrolled at birth. Informed consent was obtained from the parent or legal guardian of the infant.

Maternal questionnaires were administered prenatally and postnatally, every three months through age 10, to ascertain wheezing illnesses and rhinitis symptoms. Allergen-specific IgE (ImmunoCAP, Phadia) for German cockroach, House dust mite (Der p and Der f), Cat, Dog, Mouse, Alternaria, Aspergillus, Ragweed, Maple box elder, Oak and Timothy grass were measured in serum samples at 2, 3, 5, 7 and 10 years of age, and samples with specific IgE ≥ 0.35 U/mL were considered as positive. Skin prick testing for German cockroach, House dust mite (Der p and Der f), Rat, Cat, Dog, Mouse, Alternaria, Aspergillus, Ragweed, Tree pollen mix, Penicillin and Timothy grass was also performed at ages 3, 5, 7 and 10 years, considering a weal ≥ 3 mm larger than the saline control as positive. Table S1 shows a summary of IgE titres and skin tests performed at year 10.

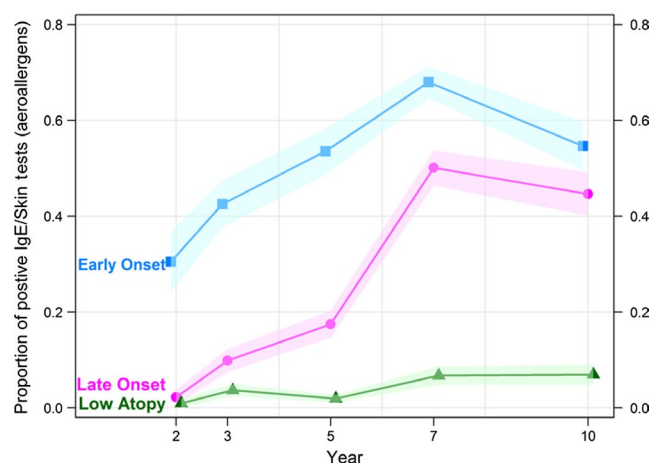


FIGURE 1 Trajectories (solid lines) with standard errors (indicated by shading) based on IgE titres and aeroallergen skin tests among URECA participants through age 10 are depicted demonstrating three groups by latent class mixed models

Asthma at age 10 years was defined using a prespecified algorithm that included parent-reported physician asthma diagnosis, asthma symptoms, healthcare use for asthma, use of asthma medications in the previous year, spirometry with reversibility and bronchial hyper-responsiveness assessed by methacholine inhalation challenge.¹⁴

A nested case-control design was used to select samples from three distinct groups of URECA participants, defined previously¹⁵ on the basis of their trajectories of aeroallergen sensitization identified using latent mixture modelling among the 442 children with follow-up data through age 7: early-onset atopy, late-onset atopy and low atopy (Figure 1). In the current study, a subset of 76 children was selected from these 3 trajectory groups (early onset $n = 25$, late onset $n = 25$, low atopy $n = 26$) identified in the earlier study. In addition to the onset of sensitization trajectory, early- and late-onset children were required to be sensitized to CR, while the low atopy cohort was required not to be CR sensitized, as determined by skin prick test (≥ 3 mm considered positive) and IgE titres (≥ 0.35 kU/L considered positive).

Blood samples were collected from these 76 children recruited at three participating centres (Baltimore, New York City, and St. Louis). Protocol approval was obtained by the Western Institutional review board (IRB study protocol numbers: St. Louis-1153225, NYC-1153193, and Baltimore-1153064). Informed consent was obtained from the parent or legal guardian of the child, and oral assent was obtained from the participants themselves.

2.2 | Latent mixture modelling

A latent class mixed model (LCMM) was used to characterize the trajectory of aeroallergen sensitization by either positive (≥ 0.35 kU/L)-specific IgE or skin test (≥ 3 mm) over time as previously described.¹⁶ LCMM combines a latent class model to identify homogenous latent groups of participants and a mixed model to describe the overall trajectory over time in each latent group while accounting for the individual correlation between repeated measures in the URECA longitudinal data set. The R-package LCMM, version 1.7.8¹⁶ was used to fit these models. The optimal numbers of latent classes were determined by sequentially increasing the number of classes (one to three latent classes) with an unconstructed random-effect covariance matrix. The Akaike information criterion (AIC) was used to select the best fit model.

2.3 | PBMC isolation

Whole blood samples were processed, and PBMC were isolated by FICOLL gradient centrifugation using SepMate tubes (StemCell Technologies) following the manufacturer's guidelines. After isolation, cells were washed, counted, cryopreserved in fetal bovine serum (FBS) + 10% DMSO and stored in liquid nitrogen for further future analysis.

2.4 | Cockroach extract and peptide pools

German cockroach extract was obtained from Greer Labs. A total of 228 peptides representing previously validated dominant T cell

epitopes from different disease cohorts¹⁰ were pooled into seven pools. A full list of individual peptides is shown in Table S2. Peptides were synthesized as crude material on a 1-mg scale (purity > 70%).

2.5 | In vitro expansion culture

For in vitro cultures, PBMCs of the 76 study participants were stimulated with German cockroach extract at 10 $\mu\text{g/mL}$ (Greer Labs). Cells were cultured at a density of 2×10^6 cells per mL of RPMI 1640 (Omega Scientific) supplemented with 5% human AB serum (GemCell; Gemini) in a 24-well plate. Cells were incubated at 37°C with 5% CO₂, and IL-2 (10 U/mL; ThermoFisher) was added every 3 days after initial stimulation. After 14 days, cells were harvested, washed and assessed for IL-5, IFN γ and IL-10 production by ELISPOT in response to CR extract or CR antigen-derived peptide pools.

2.6 | ELISPOT assays

IL-5, IFN γ and IL-10 production were assessed after stimulation with either cockroach extract (10 $\mu\text{g/mL}$) or CR allergen-derived peptide pools (5 $\mu\text{g/mL}$). Flat-bottom 96-well plates with a PVDF membrane (Millipore) were prepared and coated with 5 $\mu\text{g/mL}$ anti-human IL-5 (clone TRFK5; Mabtech, Cincinnati, OH), 5 $\mu\text{g/mL}$ anti-human IFN γ (clone 1-D1K; Mabtech) and 5 $\mu\text{g/mL}$ anti-human IL-10 (clone 9D7; Mabtech) according to manufacturer's instructions. Cells from the in vitro culture were harvested and plated at a density of 1×10^5 cells/well. Stimulus containing either cockroach extract, CR allergen-derived peptide pools, PHA (10 $\mu\text{g/mL}$) or medium as a control was added. After 24 hours of stimulation at 37°C, cells were removed and the plates were incubated at room temperature with detection antibodies for IL-5 (2 $\mu\text{g/mL}$) (mAb IL-5 biotin; Mabtech), IFN γ diluted at 1:200 (mAb IFN γ -HRP; Mabtech) and IL-10 (2 $\mu\text{g/mL}$) (mAb IL-10 biotin; Mabtech). After 2 hours, plates were washed, and alkaline-phosphatase complex was added (Vector Laboratories) for 1 hour at room temperature. Peroxidase-conjugated spots were developed with 3-amino-9-ethylcarbazole solution (Sigma-Aldrich). Alkaline phosphatase spots were developed with Vector Blue Substrate Kit (Vector Labs). Spot-forming cells (SFC) were counted by computer-assisted image analysis (AID iSpot ELR07IFL reader; Strasberg, Germany). Each test was performed in triplicate, and criteria for positivity were ≥ 100 SFCs per 10^6 PBMCs, $P < 0.05$, and a stimulation index ≥ 2 .

2.7 | Statistical analysis

Statistical comparisons among the 3 atopy groups were performed using a two-tailed, unpaired, nonparametric Mann-Whitney test. In secondary analyses that classified children by asthma and cockroach sensitivity, we used inverse probability weighting to correct for the inherent bias of the case-control selection process and make the nested case-control population representative of the entire URECA study population.

3 | RESULTS

3.1 | Cohort definition based on clinical phenotypes observed in the 2- to 10-year interval

Within the subset of 76 children selected from the URECA birth cohort previously described^{13,14} latent mixture modelling identified three distinct groups of children: minimal to no allergic sensitization (low atopy, $n = 26$), early-onset allergic sensitization (early-onset atopy, $n = 25$), and late-onset allergic sensitization (late-onset atopy, $n = 25$) (Figure 1) based on the proportion of positive allergen tests (panel of 12), using specific serum IgE and/or skin prick tests at each age of testing (years 2, 3, 5, 7 and 10).¹⁵ The low atopy group had either no positive allergy tests or minimal reactivity (<0.1) throughout the 2- to 10-year period. The late-onset atopy group had no or minimal allergic sensitization at year 2, but a higher proportion with positive tests (~ 0.45) by age 10. Finally, the early-onset atopy group had early-onset sensitization (0.3 proportion with positive tests) at year 2, which progressively increased to 0.55 by year 10.

As expected given the study design, there were highly significant differences across the 3 atopy groups in several parameters measured at seven years of age, including CR-specific and total IgE titres, as well as CR skin testing and the number of allergic sensitizations (out of 12 allergens tested). The early-onset atopy group children

had significantly greater CR-specific IgE, total IgE and were sensitized to more allergens compared to the other groups (Figure 2).

3.2 | Correlation of early sensitization patterns with allergen-specific T cell reactivity later in life

We analysed T cell responses in 76 URECA children categorized into the 3 clinically distinct groups (early-onset atopy $n = 25$, late-onset atopy $n = 25$ and low atopy $n = 26$) using PBMC samples collected at age 10.

The total number of cytokine spot-forming cells in response to CR epitope pools was significantly higher in the early-onset atopy group (Geomean 425 SFC) compared to the low atopy group (Geomean 63 SFC) ($P = 0.01$) (Figure 3A). Furthermore, a non-significant trend for higher numbers of cytokine-producing cells in the late-onset atopy group (Geomean 228 SFC) compared to the low atopy group was also observed ($P = 0.06$). No differences were observed in response to whole extract (Geomeans: early onset—65 SFC; late onset—131 SFC; low atopy—107 SFC) (Figure 3A). The IL-5 response to CR epitope pools was higher in the late-onset atopy group (Geomean 160 SFC) compared to the low atopy group (Geomean 38 SFC) ($P = 0.04$), and there was a similar trend for the early-onset atopy group (Geomean 139 SFC) ($P = 0.07$) (Figure 3B). IFN γ responses to the CR epitope pools were higher in the early-onset atopy group (Geomean 61 SFC) than the low atopy group

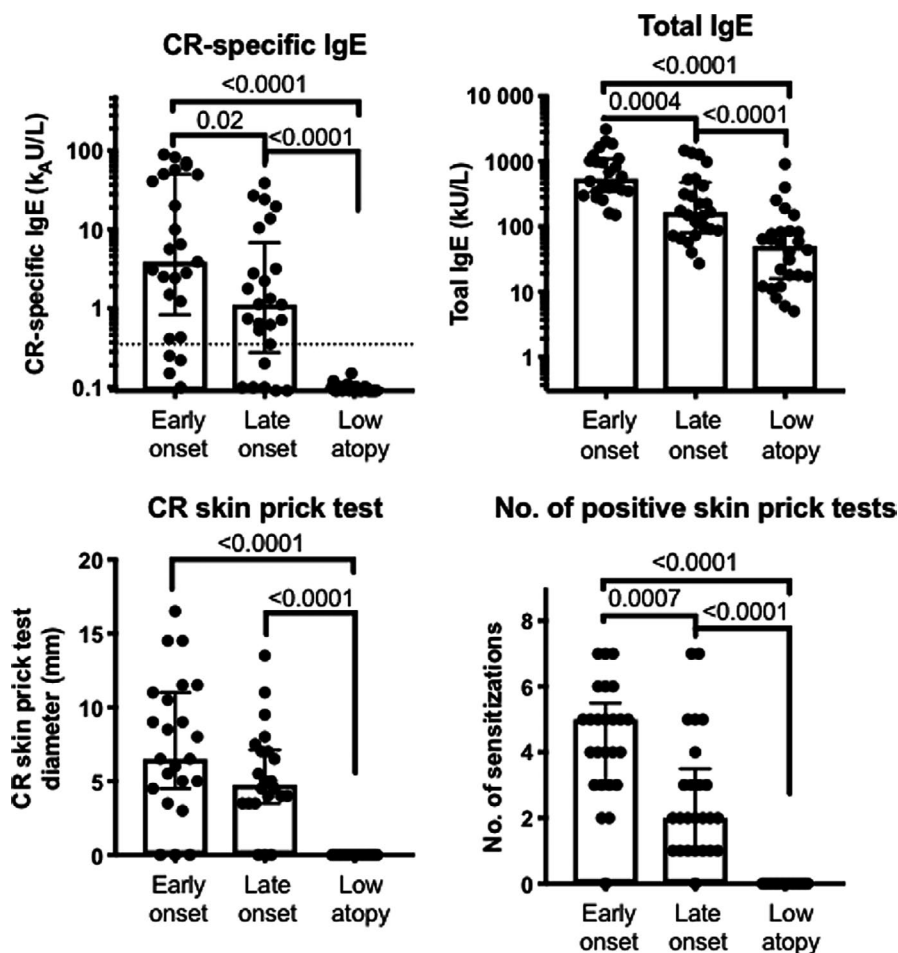


FIGURE 2 Allergic sensitization parameters at age 7 in three defined groups defined as low atopy ($N = 26$), early-onset atopy ($N = 25$) and late-onset atopy ($N = 25$). Graphs show (A) CR-specific IgE, (B) total IgE, (C) CR-skin prick test reactivity (weal diameter), and (D) number of positive skin prick tests. Bars represent Geomean, with 95% CI indicated by error bars. Statistics were performed by Mann-Whitney (two-tailed), and P -values < 0.05 are considered significant

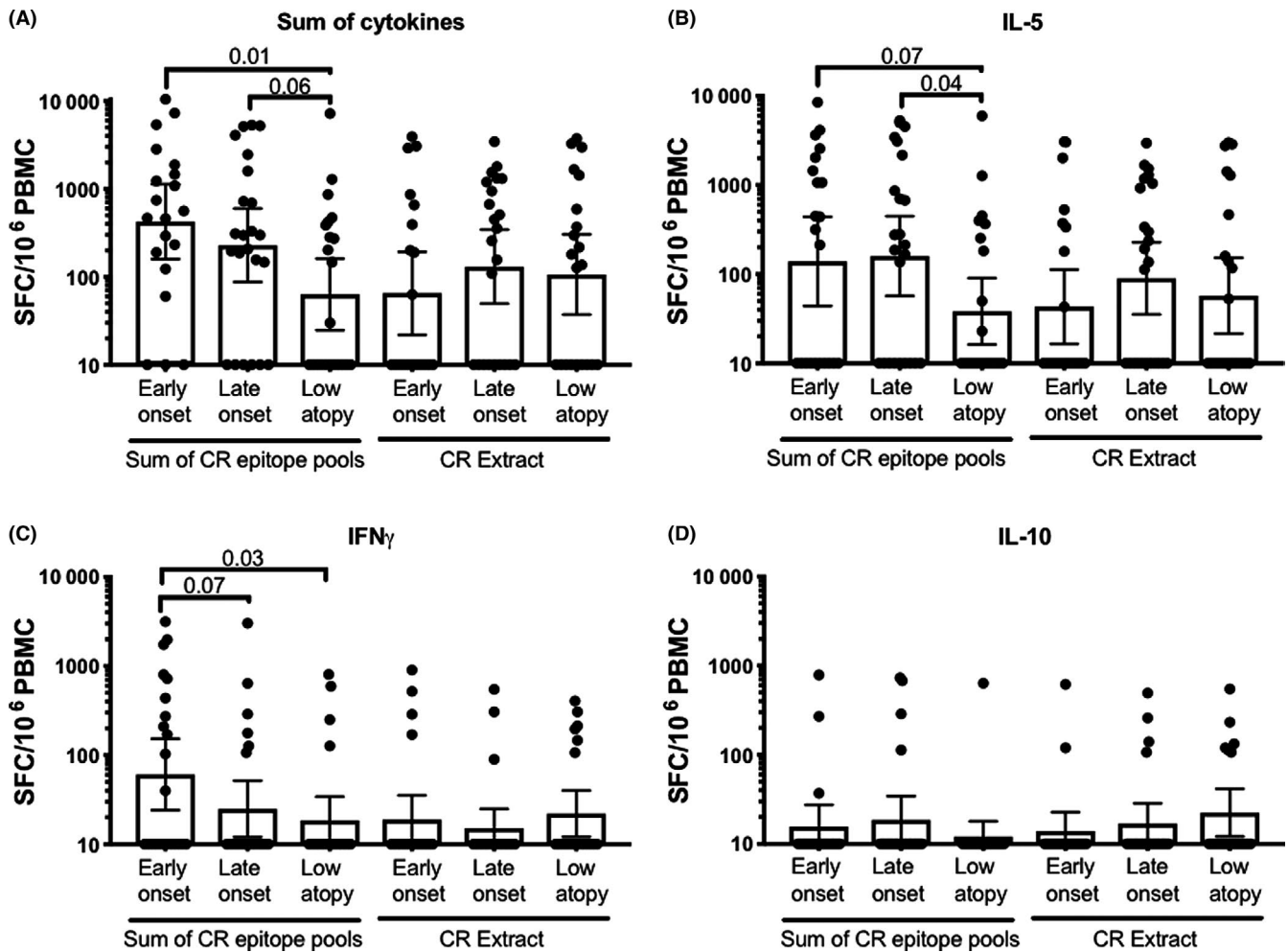


FIGURE 3 Year 10 T cell reactivity in response to CR epitope pools and CR extract in groups defined on the basis of the year 2-7 trajectory group assignment. Bar graphs show (A) sum of all tested cytokines (IL-5, IFN γ and IL-10) and (B) IL-5 (C) IFN γ and (D) IL-10 production as measured by ELISPOT in response to 24-h restimulation with CR epitope pools or extract following 14 d of in vitro expansion culture with CR extract. Bars represent Geomean, with 95% CI indicated by error bars. Statistics performed using ANOVA test (nonparametric, two-tailed). P -values < 0.05 are considered significant. $N = 25$ for early and late onset, $N = 26$ for low atopy. Note—data points of non-responders are shown on the X-axis

(Geomean 19 SFC) ($P = 0.03$) with a non-significant difference ($P = 0.07$) between the late-onset atopy group (Geomean 25 SFC) and the low atopy group (Figure 3C). An analysis to assess any potential differences between the IL-5:IFN γ ratio revealed no significant differences between any of the groups (Figure S1). Only data from donors with positive response for either IL-5 or IFN γ were considered for this analysis. Overall, few participants had detectable IL-10 responses, and no differences between groups were observed (Figure 3D). None of the single cytokine measurements revealed any significant differences between groups in response to whole CR extract (Figure 3A-B).

3.3 | Patterns of CR-specific T cell reactivity and their correlation with clinical manifestations at age 10

Next, we investigated whether asthma and atopy status at age 10 were associated with a different pattern of T cell reactivity. For this

purpose, the same 76 subjects were reclassified on the basis of CR sensitization (IgE ≥ 0.35 kU/L and/or skin test wheal ≥ 3 mm) and asthma assessed at age 10. Consistent with our previous study in adults,¹⁰ the 76 children in this study were re-categorized as CR-allergic and asthmatic (CR⁺/asthmatic, $n = 30$), CR-allergic and non-asthmatic (CR⁺/non-asthmatic, $n = 16$), CR non-allergic with asthma (CR⁻/asthmatics, $n = 19$) and CR non-allergic without asthma (CR⁻/non-asthmatics, $n = 11$). A graph showing eosinophil counts in the four groups is presented in Figure S2.

We then examined whether these four groups demonstrated differences in T cell reactivity. In response to CR epitope pools, the number of cytokine secreting cells was significantly higher in the CR⁺/asthmatics (Geomean 554 SFC) compared to either CR⁺/non-asthmatics (Geomean 165 SFC) ($P = 0.02$), CR⁻/asthmatics (Geomean 75 SFC) ($P < 0.01$) or the CR⁻/non-asthmatic group (Geomean 27 SFC) ($P < 0.01$). Furthermore, responses in CR⁺/non-asthmatics were also significantly higher as compared to CR⁻/non-asthmatics ($P = 0.01$) (Figure 4A). Lastly, a significant difference was also seen in

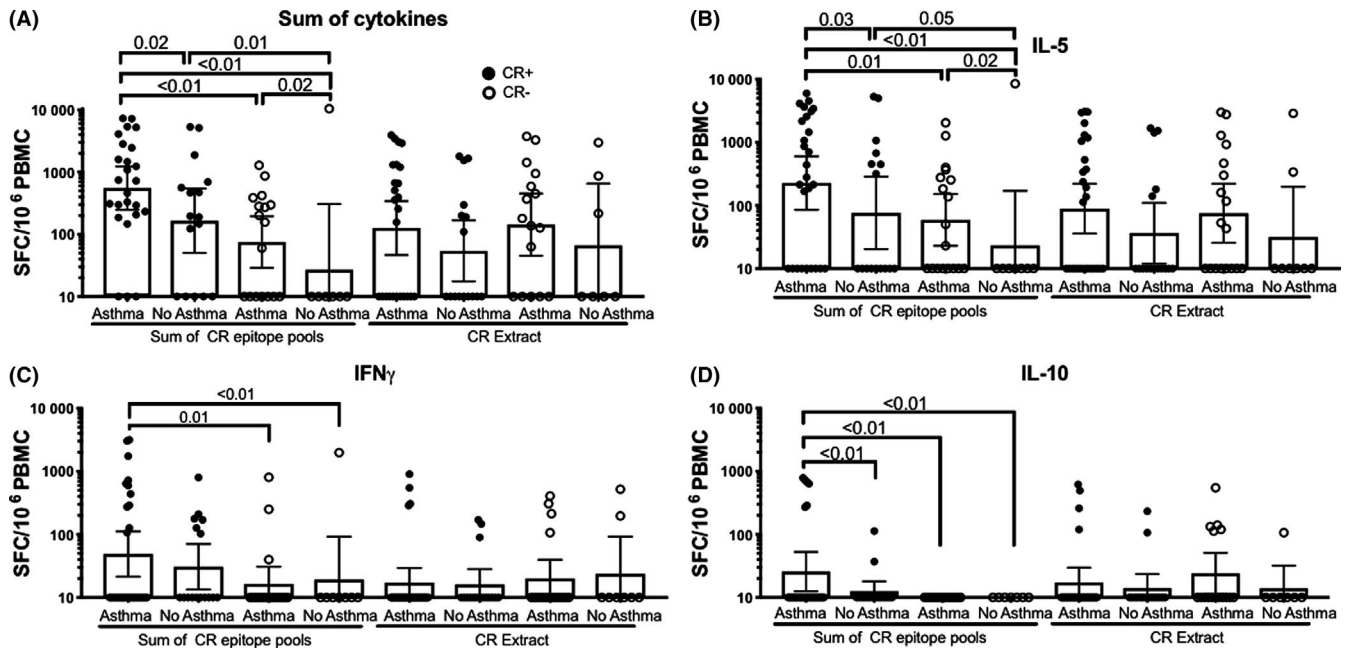


FIGURE 4 T cell reactivity in response to CR epitope pools and CR extract in CR-sensitized (CR IgE+ and or CR-skin test positive, black dots) patients with (N = 27) or without (N = 16) asthma and CR IgE- donors (open circles) with (N = 21) and without (N = 12) asthma. Bar graphs show (A) sum of all tested cytokines, (B) IL-5, (C) IFN_γ, and (D) IL-10 production as measured by ELISPOT in response to 24-h restimulation with CR epitope pools or extract following 14 d of in vitro expansion culture with CR-extract. Bars represent Geomean, with 95% CI indicated by error bars. Statistics were performed using ANOVA (nonparametric, two-tailed); $P < 0.05$ is considered significant

CR-/asthmatics compared to CR-/non-asthmatics ($P = 0.02$). These differences and trends were observed for all three cytokines (IL-5, IFN_γ and IL-10), but were most pronounced for IL-5 (Figure 4B-D). Analysis of the IL-5:IFN_γ ratio revealed no statistically significant differences between the groups (Figure S3), suggesting that it is the absolute increase in IL-5 rather than a shift in the ratio, that drives the allergic T cell response. Only data from donors with positive response for either IL-5 or IFN_γ were considered for this analysis.

In contrast to findings using CR peptide pools as a stimulant, no differences between clinical phenotype groups in the numbers of cytokine spot-forming cells in response to CR extract were observed (Figure 4A-D). These data suggest that differences in T cell reactivity are associated with both CR sensitization as well as the presence of asthma.

4 | DISCUSSION

This study evaluated the potential association between evolving clinical phenotypes of allergic sensitization and allergen-specific T cell responses. We report three major findings. First, we find that CR-specific T cell responses measured at age 10 differ between individuals classified on the basis of CR sensitization. The most striking observation in this analysis is that low atopy individuals exhibited lower CR-specific T cell reactivity as compared to early-onset and late-onset atopy. Comparisons between early- and late-onset atopy cohorts revealed non-significant trends for differences and IFN_γ responses in the former were numerically greater. Second, our data

show that in addition to detecting differences in T cell responses between children with and without CR allergy, we further observed significantly higher T cell reactivity in CR-allergic and non-allergic individuals with asthma compared to those without asthma. Third, we found that the differences at the level of T cell responses are revealed by the use of sets of epitopes that we have previously defined through a comprehensive analysis¹⁰ but not when CR extract responses are considered.

Evaluation of CR-specific T cell responses revealed that at age 10, differences are primarily observed between the early-/late-onset atopy groups compared to the low atopy group, respectively. Only non-significant trends are observed between early and late atopy groups. Reduced CR-specific T cell reactivity in non-sensitized donors is perhaps considered unsurprising. However, we have shown in previous studies^{10,17} that healthy people exhibit T cell reactivity due to allergen exposure. The low atopy cohort has a similar exposure to cockroach allergen as the early and late atopy groups and we do detect T cell responses, albeit of lower magnitude. Therefore, it is unlikely that the reduced response is simply due to the absence of memory T cells. In a study conducted in adults exposed to grass pollen, we have reported an active down-regulation of T cell responses during grass pollen season, which was associated with a suppressive transcriptomic signature.¹⁷ It is conceivable that a similar mechanism is responsible for the reduced T cell responses in the low atopy cohort, where CR-specific T cells do exist but their responses are detected at much lower levels, potentially due to an active suppression. Due to the limitations in sample availability from a paediatric cohort, this hypothesis could

not be investigated and further analysis is required to address this issue in more depth. Assessment of the atopy trajectories generated by latent mixture modelling reveals that differences in atopy between the early- and late-onset cohorts decrease over time. At age 10, patterns of atopy, with respect to the number of sensitizations as assessed by the trajectory analysis, for the early- and late-onset groups are similar. Therefore, it is perhaps not surprising that CR-specific T cell reactivity measured at age 10 is similar in these two groups. Interestingly, IgE-related data collected at age 7 still showed significant differences for CR-specific IgE, the number of allergic sensitizations and total IgE, between the two groups. This relative dissociation between T cell reactivity and IgE-mediated reactivity raises the intriguing possibility that children with late-onset atopy are just as likely to benefit from therapeutic interventions modulating T cell reactivity as those with early-onset atopy, whereas differences between these groups with treatments targeting IgE may exist. Furthermore, we see elevated levels of IFN γ in the early-onset cohort compared to the late-onset cohort, indicating a more mixed inflammatory response. Such heterogeneous immune responses have been described for disease such as atopic dermatitis¹⁸ but further investigation is required to evaluate this observation in more depth.

In addition to assessing T cell responses among the 3 atopy trajectory groups, we further report differences in T cell responses between children with and without CR allergy and significant differences in individuals with asthma versus without asthma. This analysis was performed post hoc after re-classification of subjects based on the secondary outcomes of CR sensitization and presence of asthmatic disease at age 10. To overcome any potential bias due to over- or underrepresentation of asthma in the selected cohort subset, we created an inverse probability weighting to make the nested case-control sample representative of the whole URECA study populations, using clinical data from the complete database. Interestingly, we observed significantly higher T cell responses associated with both CR sensitization as well as asthma. In a previous study focused on adults,¹⁰ we reported significant differences in T cell reactivity to CR epitopes between CR-allergic adults with asthma and non-sensitized control donors, but the difference between CR-allergic asthmatics and non-asthmatics did not reach significance. This previous study assessed data from fewer individuals, resulting in less statistical power. In addition, it is possible that differences in allergen-specific T cell reactivity between asthmatics and non-asthmatics are more readily detected at a younger age, perhaps due to fewer comorbidities. Moreover, low levels of IL-10 were also detected. It is possible that regulatory T cells play a role in protection from onset of asthma.

In this study, we see a consistent trend for children with asthma to have stronger T cell reactivity (for IL-5 and for the sum of cytokines) to CR even when we consider the groups that are not allergic to CR (Figure 4, panels A and B). This may be the result of T cell cross-reactivity with other allergens. Alternatively, it is possible that these children exhibit CR-specific T cell responses in the absence of allergen-specific IgE, a phenomenon we have previously reported

for cockroach,¹⁰ house dust mite¹⁹ and Timothy grass²⁰ and/or that asthma status in childhood represents an overall heightened T cell reactivity resulting from a long-standing, systemic, type 2, inflammatory milieu. The clinical importance of T cell reactivity in the absence of conventional allergic sensitization has not been assessed, but it represents an interesting question in the field of allergic diseases, where type 2 inflammation may be present even in the absence of conventional sensitization.

Notably, between-group differences were detected in response to validated T cell epitope pools, but not in response to a commercial CR extract. Several studies have documented that the relative content of different allergens in allergen extracts, including CR extracts, varies substantially.²¹⁻²⁵ In addition, we have reported several CR-derived proteins capable of inducing potent T cell responses, despite not being classified as CR allergens.¹⁰ The content of these antigens in the extract is unknown. In this context, one could speculate that the use of defined pools of synthetic peptides corresponding to validated and published T cell epitopes offers an attractive alternative for this line of research, allowing a consistent, balanced stimulation with accurately characterized molecules.

In conclusion, this is the first assessment of the relationship between allergen-specific T cell responses and evolving clinical phenotypes in allergy research. Further longitudinal analyses are required for the comprehensive exploration of this topic, examining the variability of T cell responses of the same children over time, contrasting such responses to B cell responses and correlating them with further evolution of the clinical phenotype.

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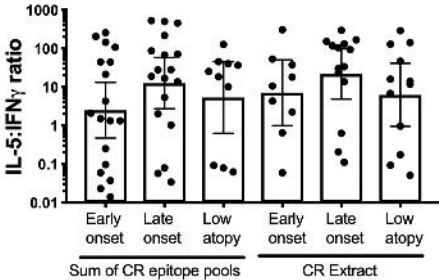
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SUPPORTING INFORMATION

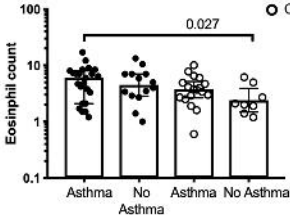
Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Eosinophil count

● CR+
○ CR-



IL-5:IFN γ ratio

1000
100
10
1
0.1
0.01

● CR+
○ CR-

Asthma No Asthma Asthma No Asthma Asthma No Asthma Asthma No Asthma

Sum of CR epitope pools CR Extract

