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Atopic Dermatitis Phenotypes Impact Expression of Atopic Diseases Despite Similar Mononuclear Cell Cytokine Responses

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Abstract

Background: The atopic march refers to the co-expression and progression of atopic diseases in childhood, often beginning with atopic dermatitis, although children may not "progress" through each atopic disease.

Objective: We hypothesized that future atopic disease expression is modified by atopic dermatitis phenotype, and that these differences result from underlying dysregulation of cytokine signaling.

Methods: Children (n=285) were enrolled into the Childhood Origins of ASThma birth cohort and followed prospectively. Rates of atopic dermatitis, food allergy, allergic rhinitis, and asthma were assessed longitudinally from birth to 18 years of age. Associations between atopic dermatitis phenotype and food allergy, allergic rhinitis, asthma, allergic sensitization, exhaled nitric oxide, and lung function were determined. Peripheral blood mononuclear cell responses (IL-5, IL-10, IL-13, IFN-γ) to dust mite, phytohemagglutinin, Staphylococcus aureus Cowan I, and tetanus toxoid were compared among atopic dermatitis phenotypes.

Results: Atopic dermatitis at year 1 was associated with an increased risk of food allergy ($p=0.004$). Both persistent and late-onset atopic dermatitis were associated with an increased risk of asthma ($p=<0.001$), rhinitis ($p<0.001$), elevated total IgE ($p=<0.001$), percentage of aeroallergens with detectable IgE ($p<0.001$), and elevated exhaled nitric oxide ($p=0.002$). Longitudinal analyses did not reveal consistent differences in PBMC responses among dermatitis phenotypes.

Conclusion: Atopic dermatitis phenotype is associated with differential expression of other atopic diseases. Our findings suggest peripheral blood cytokine dysregulation is not a mechanism

underlying this process, and immune dysregulation may be mediated at mucosal surfaces or in secondary lymphoid organs.

Clinical Implications: Identifying clinical factors and immune mechanisms that underlie the association between atopic dermatitis in early childhood and subsequent allergic disease may lead to personalized strategies towards allergic disease prevention.

Capsule Summary Early persistent atopic dermatitis is associated with risk for food allergy, rhinitis, and asthma. AD onset after age 3 years is associated with asthma and rhinitis risk. These findings suggest that timing of skin disease is important for allergic disease expression.

Key Words: Atopic dermatitis, atopic march, atopic dermatitis phenotypes, allergic sensitization, progression of atopic disease

Abbreviations: Atopic dermatitis (AD), interleukin (IL), peripheral blood mononuclear cell (PBMC)

INTRODUCTION

Atopic dermatitis (AD) is the most common chronic pediatric inflammatory skin condition, affecting approximately 11-20% of children in the United States(1–4). Prevalence of childhood AD is increasing worldwide(5). Most cases of AD manifest before 5 years of age, with a variable natural course that is difficult to predict. In some children, symptoms improve over time, but approximately 50% of children with AD will have persistent symptoms into adulthood(6).

AD is associated with allergic sensitization and the development of further atopic diseases in childhood. AD, food allergy, asthma, and allergic rhinitis are traditionally considered to be atopic diseases. These diseases occur together more often than would be expected if there was no association between them, and they share the common feature of atopy. However, the age of onset, organ involvement, clinical presentation, and comorbidities of allergic diseases vary widely between individuals.

This co-expression and progression of AD to other allergic diseases has been termed the atopic march. The atopic march posits a sequential progression of allergic diseases, starting with AD in childhood and culminating in development of asthma and rhinitis later in childhood. Temporally, food allergy is often co-expressed with AD, most often in early life. Asthma and allergic rhinitis are considered later manifestations of the “atopic march” process. Allergic sensitization to aeroallergens tends to develop after food allergens, and the delayed timing of sensitization to inhaled allergens and subsequent allergic airway inflammation may contribute to the later age of onset for these diseases(7–9). AD is a well-established risk factor for development of asthma and rhinitis later in childhood, and multiple longitudinal and cross-sectional investigations have confirmed the association between childhood AD and asthma(10–

14). Additionally, AD manifesting before 2 years of age has been associated with more severe and persistent asthma(15,16).

However, only a small subset of children follow the "classic" atopic march. We have previously described the use of latent class modeling analysis to identify 3 AD phenotypes in the Childhood Origins of Asthma (COAST) study(17), a high-risk birth cohort composed of children with parental histories of asthma and/or allergies. To investigate the role of AD phenotypes in atopic disease co-expression and progression, we conducted longitudinal analyses comparing incidence and expression of atopic diseases and biomarkers of allergic disease from birth until 18 years of age among the 3 AD groups. To determine if systemic immune dysregulation contributes, we also measured and compared stimulated peripheral blood mononuclear cell (PBMC) cytokine response patterns of each AD phenotype group longitudinally. The identification of at-risk infants would provide valuable opportunities for early intervention by evaluating for sensitization, implementing allergen avoidance measures, or initiating controller therapies as indicated.

Methods

The Childhood Origins of ASThma (COAST) cohort is comprised of 285 children enrolled from November 1998 through May 2000(18). To qualify, at least one parent was required to have history of physician-diagnosed asthma and/or respiratory allergies. Participants were enrolled at birth and followed prospectively. Questionnaires for both parents and children were periodically administered and included questions regarding health histories, with a focus on atopic diseases and environmental exposures. Physical examinations were performed at regularly scheduled visits.

AD was defined as having an Eczema Area and Severity Index score (completed by study team) greater than or equal to 1, physician diagnosis of AD at study visit, or parental report of physician-diagnosed AD. Greater than 99% of yearly AD diagnoses were made by EASI score or physician diagnosis at study visit(17). Food allergy was defined by using allergen-specific IgE test results and historical reports of clinical reactions from parents or documentation in the medical record. Asthma was defined as the documented(19,20). Rhinitis was defined as having perennial or seasonal frequent sneezing, itchy nose, and/or rhinorrhea, and was ascertained by affirmative response on historical questionnaires as previously described(21).

Blood was routinely collected and specific IgE to dog, cat, cockroach, ragweed, birch, timothy grass, *Alternaria alternata*, *Dermatophagoides farinae*, and *Dermatophagoides pteronyssinus*, were measured by using automated fluoroenzyme immunoassays as previously described[16]. Allergen-specific IgE values of 0.35 kU/L or greater were considered positive. Additional studies performed at annual routine clinic visits included total IgE level measurement, peripheral blood eosinophil count measurement, exhaled nitric oxide measurement (FeNO), and spirometry.

Blood samples from birth (cord blood), 1, 3, 6, 8, and 11 years of age was used for cytokine analysis. Cord blood mononuclear cells (MNCs) and peripheral blood mononuclear cells (PBMCs) were stimulated with dust mite, PHA, *Staphylococcus aureus cowan*, and tetanus toxoid, and levels of IFN-γ, IL-5, IL-10, and IL-13 in culture supernatants were evaluated by means of ELISA as previously described(22).

Statistical analysis

Latent class analysis (LCA) was previously performed using the observed pattern of AD in a child during the first 6 years of life(17). Briefly, the Schwarz or Bayesian information criterion was used to select the number of latent classes; a 3-class model was selected. After considering co-variates, the 3-class model was selected by Bayesian information criterion. Classifications from the 3-class LCA model with covariates were used for all subsequent analyses.

Comparisons of the demographic characteristics between AD phenotypes were completed using data from 1 year. Categorical variables were compared using Fisher's exact test and continuous measures were compared using a Kruskal-Wallis Rank Sum test.

Repeated measures analyses were performed with the "repeated" statement in SAS GLIMMIX using the link function appropriate for the outcome. All measures that represent presence/absence of the outcome (food allergy, allergic rhinitis, asthma, aeroallergen sensitization) were analyzed with logistic regression (using the logit link) and represented graphically as the percent at each year with the outcome present. All other outcomes were analyzed with linear regression (using the identity link) and represented graphically as the mean at each age. The model included a term for age (up to 18 levels), AD phenotype (3 levels) and

the interaction. The interaction p-value is reported and represents the test of whether there are any ages where the outcome differs by AD status. Where the overall term is significant, we did pairwise comparisons of the AD phenotypes.

Since the numbers in the late-onset AD group are small, we did secondary analyses where those with late-onset AD were grouped either with the none/intermittent AD or with the persistent AD and re-evaluated the models.

Cytokines were analyzed after log transformation. SAS v9.4 (Cary, NC) was used for all analyses.

Results

Characteristics of study population

Three AD phenotypes were identified as previously described(17) by latent class analysis: (1) “none/intermittent” group was comprised of children who never had AD or had an intermittent course (n=180, 63%); “late onset” group had no AD early in life with onset later in childhood, between 4-6 years of age (n=38, 13%), and (3) “persistent” group developed AD in infancy and had persistent symptoms throughout the observation period (n=67, 24%). Further details regarding the phenotypes have been previously published(17). Participant demographics are shown in Supplement Table 1.

AD phenotype and the association of other atopic diseases in childhood

To evaluate the influence of each AD phenotype on the risk of development of subsequent allergic disease, we first compared rates of food allergy in our cohort longitudinally, stratified by AD phenotype. At 1 year of age, the persistent AD group already has a significantly higher proportion of food allergy (15%) compared to 5% in the none/intermittent and late-onset AD groups (Figure 1A). This association continued thoughout childhood; the persistent AD group consistently 2-3 fold higher incidence of food allergy compared to the none/intermittent and late-onset groups. Overall, the differences were not significant (Table I) despite these apparently consistent differences. Since the late-onset group appeared very similar to the none/intermittent group, we did a secondary analysis where late-onset group was combined with none/intermittent group and compared to persisitent AD. This analysis showed an overall significant association ($p=0.004$).

We next examined rates of asthma in each AD group longitudinally from 6-18 years of age. Both persistent and late-onset AD phenotypes had double the rate of asthma compared to the none/intermittent phenotype at 6 years of age, and quadruple the rate throughout the remainder of childhood (Figure 1B). Compared to children with none/intermittent AD, those with either persistent or late-onset AD had significantly higher rates of asthma longitudinally throughout childhood ($p < 0.001$). There was no significant difference in rates of asthma between the persistent and late-onset AD groups (Table I).

Both persistent and late-onset AD were associated with higher rates of rhinitis compared to none/intermittent AD (Figure 1C). All 3 AD groups had higher rates of rhinitis at 1 year of age that decreased sharply by 2 years of age. Rates of rhinitis then increased steadily throughout

childhood. There was no significant difference in rates of rhinitis between the persistent and late-onset AD groups (Table I). Specific IgE confirmed rhinitis is shown in Supplemental Table 1.

When we compare AD diagnosis at year 1 (persistent) with children that do not have AD at year 1 (none/intermittent and late-onset), food allergy ($p=0.004$), asthma ($p<0.001$) and self-reported rhinitis ($p<0.001$) are more common throughout childhood (Figure 2)

AD phenotypes and biologic markers of atopy

We next compared biologic markers of Type 2 inflammation between the 3 AD groups longitudinally from birth to 18 years of age. Mean total IgE levels for all 3 groups were similar at 1 year of age; by 5 years of age the persistent AD group had significantly higher total IgE levels compared to the other phenotypes, which persisted across childhood (Figure 3A). Total IgE level was not significantly different between the none/intermittent and late-onset groups (Table I).

Both persistent and late-onset AD were associated with higher peripheral eosinophil counts longitudinally throughout the study period compared to the none/intermittent AD phenotype (Figure 3B). There was no significant difference in eosinophil counts between the persistent and late-onset groups (Table I).

Allergic sensitization, defined as detectable specific IgE to at least one of the allergens tested, was not significantly different between the 3 groups, although the persistent AD trended higher from 3-9 years of age (Figure 3C). Quantitative assessment of the degree of allergic sensitization comparing the percentage of allergens tested with detectable IgE revealed a significantly higher percentage of positive allergens in the persistent AD group compared to both late-onset and transient AD phenotypes (Figure 3D). In addition, late-onset AD had significantly higher percentage of positive allergens compared with the none/intermittent group (Table I). When looking at food sensitization, food sensitization to both egg and peanut was more common

with persistent AD, but this was not statically significant (Figure 1). Similar to food allergy diagnosis, when we compare persistent AD compared to the other phenoytes, we see a significant difference in both allergens, peanut =0.05, egg p=0.007) (Figure 2)

AD phenotypes and physiologic indicators of atopy

We measured FeNO (Figure 4A) and spirometry (Figure 4B) from 6 to 17 years of age. Longitudinal analyses revealed an association between both persistent and late-onset AD (but not none/intermittent AD) with higher FeNO (Table I). In contrast, no significant association was found between AD phenotypes and FEV1/FVC ratio (Table I).

AD phenotypes and cytokine responses

To evaluate for evidence of underlying cytokine dysregulation, PBMC cytokine responses after stimulation were compared between AD phenotypes. There was variability in ages with data available; all available data was analyzed. We compared levels of IL-10 and IL-13 after stimulation with dust mite at 1, 3, and 6 years of age; levels of IFN-γ, IL-5, IL-10, and IL-13 after stimulation with PHA at birth, 1, 3, 6, 7, and 11 years of age; levels of IFN-γ after simulation with *Staphylococcus aureus* at birth, 3, and 6 years of age; levels of IL-10 after stimulation with *Staphylococcus aureus* at birth, 1, 3, and 6 years of age.; levels of IFN-γ after stimulation with tetanus toxoid at birth, 1, 3, and 6 years of age; levels of IL-10, IL-13, and IL-5 after stimulation with tetanus toxoid at 1, 3, and 6 years of age. No significant associations between AD phenotype and PBMC cytokine response profiles were identified (Table II).

Discussion

The increasing prevalence of atopic diseases(23–27) has intensified focus on characterizing the mechanisms underlying the development of these disorders in childhood. Additionally, allergic diseases once thought of as single entities actually represent several related endotypes and/or phenotypes, and elucidating these differences is crucial to refine our understanding of allergic disease co-expression and mechanisms of disease. We have previously utilized latent class analysis to identify 3 distinct AD phenotypes in a high-risk birth cohort – none/intermittent, late-onset, and persistent AD. In this study, we found that AD phenotype, based on age of onset and persistence of AD, is differentially associated with other atopic disease expression and progression in childhood.

This study is unique in that we were able to perform a longitudinal analysis with follow-up to 18 years, accompanied by robust clinical, laboratory, and immunologic data. This provides a more complete picture of immunologic development and changes that occur throughout childhood. Although only persistent AD was associated with food allergy, both persistent and late-onset AD were associated with asthma and rhinitis. This likely reflects the different pathogenesis of these disorders, and how age of onset effects disease expression.

Early onset AD was associated with food allergy, while other AD phenotypes were not. The dual-allergen exposure hypothesis(28,29) suggests that allergic sensitization can occur through cutaneous exposure, while early oral consumption of food protein induces tolerance. Our study illustrates that AD that manifests in infancy is most crucial in perturbing the development of oral tolerance, and that associated impairment of the skin barrier *at this time in the lifespan* is most important for food allergy. In food allergy, timing, and balance of cutaneous versus oral

exposure predominantly influence whether food allergy or tolerance occurs. In contrast, asthma and rhinitis have overlapping but differential risk factors.

Sensitization to inhaled allergens is a well-established risk factor for asthma and rhinitis, and is more likely to occur later in childhood compared to food allergen sensitization(7). The ORCA Study characterized the natural history of sensitization during the first 6 years of life of 229 children with moderate-to-severe AD. The results of that study showed food sensitization decreasing over time, from 58% to 34%, while aeroallergen sensitization increased from 17% to 67% over the study period(30). Another study showed that sensitization to food allergens is more common in children from 0-4 years of age, while environmental allergies are more common after 4 years of age(31). This may help explain why late onset AD is associated with an increased risk for asthma and rhinitis, but not food allergy.

We also found associations between persistent AD and increased total IgE, higher percentage of allergens with detectable specific IgE, and higher blood eosinophils. We found no associations between AD and single allergen sensitization. These findings likely reflect the high-risk nature of the cohort, whereby the children in the cohort have a higher risk for allergic sensitization compared to the general population. Thus, there are high levels of sensitization throughout the cohort, regardless of AD phenotype. However, there were significant differences in the percentage of aeroallergens children were sensitized to between the 3 AD phenotypes, with persistent AD having the highest percentage of positive aeroallergens. Indeed, by 11 years of age at least 80% of the cohort was sensitized to at least one allergen. The increased in Type 2 biomarkers in persistent AD is consistent with the understanding that atopic dermatitis is associated with Type 2 inflammation. Th2 polarizaiton is often initiated with increased expression of epithelial cytokines, such as TSLP. Thymic stromal lymphopoietin (TSLP) is an

alarmin that can stimulate Th2 cell polarization and cytokine responses(34). TSLP is undetectable in normal skin, but highly expressed in lesional skin of AD patients(35). Expression of TSLP, as well as IL-33 and IL25 promote Th2 inflammation. Indeed, expression of IL-4, IL-5, and IL-13 is upregulated in the lesional skin in AD patients compared to healthy controls(32,33). CCL17, CCL18, CCL22 and CCL26 are type 2 chemokines and have been found to be overexpressed in AD lesional skin(36). We also found an association between persistent and late-onset AD and elevated FENO, but not with decreased FEV1/FVC ratio. This is likely because FENO is a better marker of allergic inflammation than FEV1/FVC ratio. For example, children with non-allergic asthma would be expected have a decreased FEV1/FVC ratio, but not an elevated FENO.

We hypothesized that patient with persistent AD (with more atopic manifestations), would have evidence of underlying peripheral blood cytokine dysfunction. We found no associations between AD phenotypes and PBMC cytokine signaling patterns, and these negative findings suggest that the signaling networks underlying allergic disease progression are not demonstrated by peripheral cytokine levels. This raises a potential role for barrier function and local immune responses in atopic disease expression patterns. Indeed, epithelial barrier function is a well-known contributor to allergic inflammation. Further investigation in the role of epithelial cytokine levels (such as TSLP, IL-33 and IL-25), and how barrier function relates to these cytokines are needed. Also, mutations in filaggrin, a skin barrier protein, have been associated with both AD and asthma expression(37,38), likely by a variety of mechanisms, such as increased penetrance of allergens and irritants, and promotion of inflammation. Genetic study in a larger sample size can help clarify this further. Epithelial cells and resident immune cells in

the tissue such as dendritic cells may influence cytokine release patterns in regional lymph nodes, and alter the subsequent immune response.(34,39,40)

In conclusion, we have shown that both age of onset and persistence of AD differentially associate with expression of atopic disease, and peripheral blood cytokine dysregulation does not appear to be an underlying mechanism. The growing body of evidence demonstrating the importance of multiple phenotypes and disease variance in allergic disease lends evidence against a relatively simple, linear progression through the atopic march. Although many children with AD do indeed co-express other atopic disease(s), only a subset of patients follow the strict and "classic" path. Further longitudinal studies will provide a more complete picture compared to cross-sectional studies, and enable identification of the mechanisms of disease co-expression. A more complete understanding of risk factors for atopic disease progression will enable development of targeted precise interventions.

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FIGURE LEGENDS

FIG 1. Associations between AD phenotypes and atopic diseases. Persistent AD, but not transient or late-onset AD, was associated with higher rates of food allergy (A). In contrast, both persistent and late-onset AD were associated with higher rates of asthma (B) and rhinitis (C). There was no association of AD phenotype with peanut (D) or Egg (E) sensitization. Blue circles = none/intermittent AD, open green triangles = late onset AD, red triangles = persistent AD

FIG 2. Associations between AD diagnosis at year 1 and atopic diseases. AD at year 1 (persistent AD) was associated with higher rates of food allergy (A), asthma (B), and self-reported rhinitis (C) in childhood compared to children without AD at year 1 (none/intermittent combined with late onset). The association with peanut sensitization (D) was at the level of significance, and egg sensitization was associated with AD at year 1 (E). Red triangles = AD present at age 1, Blue circles = no AD at year 1.

FIG 3. Associations between AD phenotypes and biologic markers of atopy. Persistent AD group had a significantly higher mean total IgE compared to the other AD groups (A). Persistent and late-onset AD had significantly higher peripheral eosinophil count compared to transient group (B). No significant differences were seen when sensitization rates to at least one aeroallergen were compared (C); when the percentage of allergens with detectable specific IgE was compared, significant differences were seen between all 3 AD groups. Blue circles = none/intermittent AD, open green triangles = late onset AD, red triangles = persistent AD

FIG 4. Associations between AD phenotypes and lung function. Persistent and late-onset AD were associated with an elevated FeNO (A), while no significant differences were seen with FEV1/FVC ratio (B). Blue circles = none/intermittent AD, open green triangles = late onset AD, red triangles = persistent AD