

Plasmacytoid dendritic cells during infancy are inversely associated with childhood respiratory tract infections and wheezing

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Background: It has been proposed that immune dysfunction during early childhood plays an important role in asthma pathogenesis. However, it is not known specifically whether changes in dendritic cells (DCs) during infancy antedate the development of respiratory tract infections, asthma, and related clinical phenotypes.

Objectives: We sought to assess the association between the level of blood DCs during the first year and the subsequent development of respiratory tract infections, wheezing, and allergic sensitization.

Methods: A community-based cohort of children with a family history of atopy was followed to age 5 years. Children were monitored intensively for respiratory tract infections. History of wheeze and asthma was collected annually, atopy was documented at 5 years, and flow cytometry was used to identify DC subsets in blood samples collected when children were well.

Results: Levels of plasmacytoid DCs (pDCs) during infancy were inversely correlated with symptoms of lower respiratory tract infections, parent-reported wheezing, and the cumulative rate of physician-diagnosed asthma up to age 5 years. These relationships were independent of atopy, as determined by allergy skin test results and total and specific IgE levels. In contrast, levels of myeloid DCs were not associated with respiratory tract infections, asthma, or wheezing but were associated with total IgE levels at age 5 years.

Conclusion: In children with a family history of atopy, relative deficiency of circulating pDCs during infancy appears to be a risk factor for more frequent and more severe respiratory tract infections, wheezing, and a diagnosis of asthma. Infants with higher numbers of pDCs are protected against these outcomes. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Dendritic cells, respiratory infections, wheezing, childhood asthma, atopy

Abbreviations used

ARI: Acute respiratory tract infection
BDCA: Blood dendritic cell antigen
DC: Dendritic cell
LRI: Lower respiratory tract infection
mDC: Myeloid dendritic cell
pDC: Plasmacytoid dendritic cell
SPT: Skin prick test

In children with asthma and allergic sensitization, particular alterations in immune function have been described during infancy, well before clinical manifestations become apparent.¹ Most reports have focused on T-cell dysfunction, with longitudinal studies showing that deficient IFN- γ synthesis during the first year of life is associated with a greater risk of subsequent wheezing,²⁻⁴ atopy,⁵⁻⁸ and respiratory tract viral infections.⁹ Immune dysfunction might therefore play a key role in the initiation of asthma and atopy.

However, little attention has been paid to antigen-presenting cell function in this context. Dendritic cells (DCs) might be especially relevant to asthma pathogenesis, given their ability to initiate immune responses to common allergens and viruses. DCs are a family of bone marrow-derived cells that can be separated into 2 major subgroups: conventional or myeloid DCs (mDCs) and plasmacytoid DCs (pDC). Animal models have highlighted the importance of mDCs in the pathogenesis of allergic airway inflammation,^{10,11} whereas pDCs appear to play a specialized role in host defense against viral infections at mucosal surfaces¹² and in some circumstances might mediate immune tolerance.¹³ In adults with established asthma, changes in the number and function of mDCs are reported in the airway mucosa,¹⁴⁻¹⁶ although little information is available on lung pDCs in asthma. In the peripheral blood a number of reports indicate that asthma in adults is associated with changes in the relative proportions of mDCs and pDCs.¹⁷⁻¹⁹

Less is known about DC subsets in children with asthma. Hagendorens et al²⁰ reported data from a cross-sectional study showing that blood pDCs were less frequent in atopic or asthmatic children and adolescents (aged from 1-18 years) compared with healthy subjects. Silver et al²¹ enrolled a large cohort of children with severe respiratory syncytial virus-induced bronchiolitis during infancy and followed them to the age of 6 years. In those children who had asthma, the frequency of blood pDCs at 6 years was less than seen in children who did not have asthma. In neither of these studies was it possible to determine whether these reductions in blood pDCs first emerged before or after allergic disease became apparent. This is an important issue and, to our knowledge, has not been addressed.

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The purpose of this prospective, community-based cohort study was to assess the relationship between the relative frequencies of circulating DC subsets in early childhood and the incidence of respiratory tract infections, wheezing, and asthma up to the age of 5 years. Blood samples were taken when the children were 6 months, 12 months, and 5 years old at times when they were clinically well. Blood DC subsets were identified by means of flow cytometry. Because allergic sensitization and recurrent respiratory tract infections in early life are key risk factors for the development of asthma,^{1,22} we also examined the relationships between circulating DC subsets and the rate of acute respiratory tract infections (ARIs) and allergic sensitization.

METHODS

For additional information on the monitoring of respiratory tract infections, blood processing, identification of DC subsets by means of flow cytometry, and statistical analysis, see the [Methods](#) section in this article's Online Repository at www.jacionline.org.

Study design

The Childhood Asthma Study is a prospective birth cohort study that has been described in detail elsewhere.^{22,23} The Ethics Committee at Princess Margaret Hospital for Children approved the study, and informed written consent was obtained from parents before recruitment. Briefly, children with at least 1 first-degree relative with atopy were recruited prenatally between July 1996 and June 1998 and followed to age 5 years. A single study doctor interviewed parents and examined all children at 6 and 12 months of age and then annually, with specific attention paid to the presence of wheezing and doctor-diagnosed asthma. At 5 years of age, the children underwent allergy skin prick tests (SPTs).²² Children were intensively monitored for symptoms of ARIs during the first 5 years by using a combination of symptom diaries, telephone calls, and home visits, as outlined in the [Methods](#) section of this article's Online Repository and as reported previously.²² ARIs were classified as described in [Table I](#).

Blood sampling and enumeration of DCs

Venipuncture was performed at routine scheduled visits. All children had to be clinically well and free of symptoms of infection for at least 2 weeks prior. If children were unwell, the blood sample was deferred at least 2 weeks. PBMCs were isolated, stained, and examined by means of flow cytometry, as detailed in the [Methods](#) section of this article's Online Repository. pDC and mDC subsets were each expressed as the number of cells per 100 PBMCs. All samples were processed and analyzed without knowledge of the children's clinical status.

Statistical analysis

DC numbers had a skewed distribution, and therefore natural log transformation was conducted before further analyses. Group data are presented as geometric means and 95% CIs. In those children with paired DC measurements at both 6 and 12 months of age, bivariate correlation and paired-sample *t* tests showed no significant difference between values obtained at 6 and 12 months. Therefore the variables of "infant pDCs" or "infant mDCs" refer to blood DC numbers at age 6 or 12 months or their mean when the child had measurements at both times. The independent-sample *t* test was used to analyze variations in blood DCs in relation to age, asthma phenotypes, and atopy. Poisson regression was used with the number of ARIs as the dependent variable and *z* scores of the log value of numbers of blood DCs as the independent variables in the model to analyze the association between blood DCs in infancy and ARIs. Incidence rate ratios and their 95% CIs (equivalent to odds ratios in logistic regression analysis) were estimated for every 1-unit increase in the *z* score of blood DC numbers. Finally, the Spearman method was used to evaluate associations between blood DCs and total or specific IgE levels. *P* values of less than .05 were considered significant. Further details of study methodology and statistical analysis are outlined in the [Methods](#) section of this article's Online Repository.

TABLE I. Classification of ARIs

	Abbreviation	Definition
Upper respiratory tract infection	URI	Runny/blocked nose or cough in the absence of tachypnea, difficulty breathing, wheeze,* or rattling chest
Lower respiratory tract infection	LRI	Runny/blocked nose or cough in the presence of tachypnea, difficulty breathing, wheeze,* or rattling chest

*Wheeze as reported by the parent or family doctor.

RESULTS

Two hundred sixty-three children with a family history of atopy were recruited into the study, of which 198 were followed for the full 5 years of the study. The clinical features of this high-risk cohort have been described in more detail elsewhere.²²

Changes in DC subsets with age

Circulating pDCs and mDCs were identified by using flow cytometry and expressed as the number of cells per 100 PBMCs. These measurements were performed in 32 children at age 6 months (29 of whom were followed to age 5 years) and 48 children at age 12 months (41 of whom were followed to age 5 years). Thus 60 children had at least 1 measurement of blood DC subsets during infancy, either at 6 months, 12 months, or both. In the other infants insufficient cells were available to perform flow cytometry. DCs were measured in 140 children at age 2 years and in 160 children at age 5 years. Flow cytometry was not performed on blood samples collected at 3 and 4 years of age.

Twenty children had circulating DC subsets measured at both 6 and 12 months of age; in these children there was a significant association between the frequency of DC subsets at both ages with correlation coefficients of 0.69 (*P* = .001) and 0.54 (*P* = .014) for pDCs and mDCs, respectively.

As outlined in [Fig 1](#), the frequencies of both pDCs and mDCs at age 5 years were significantly higher than at any younger age. The frequencies of pDCs and mDCs at age 2 years were slightly but significantly higher than at 1 year.

Circulating DC subsets and ARIs

The incidence rates of ARIs, expressed as episodes per person per year, were 3.24 (95% CI, 3.13-3.34) for ARIs, 0.87 (95% CI, 0.82-0.93) for febrile ARIs, 0.94 (95% CI, 0.88-0.99) for lower respiratory tract infections (LRIs), and 0.46 (95% CI, 0.42-0.50) for wheezy LRIs.

[Table II](#) shows the relationship between the frequencies of circulating pDCs and mDCs and the rate of ARIs during the first 5 years. Because DC measurements did not vary significantly between 6 and 12 months, we analyzed the relationship between the frequency of DCs taken at any time during infancy and the rate of ARIs during the first 5 years, taking into account the 20 subjects in whom we had measurements at both 6 and 12 months. Two approaches were used to analyze these relationships, as described in the [Methods](#) section of this article's Online Repository. First, when pooled infant blood pDC values were fitted as an independent variable in a Poisson regression analysis (method 1), the frequency of pDCs during infancy was inversely correlated with the rates of ARIs, febrile ARIs, LRIs, and wheezy LRIs in the first 5 years (*P* ≤ .03 for all correlations, [Table II](#)). Second, when the frequency of blood pDCs at age 6 and 12 months was treated as a single value for those who had measurements at

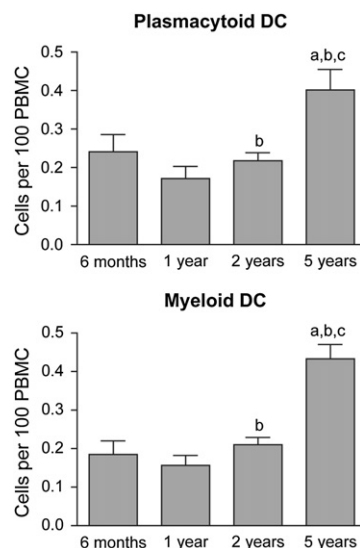


FIG 1. Variations in DC subsets with age. pDCs and mDCs are expressed as the number of cells per 100 PBMCs at age 6 months ($n = 32$), 12 months ($n = 48$), 2 years ($n = 140$), and 5 years ($n = 160$). Data are plotted as means + 95% CIs with statistical significance as indicated (a: $P < .05$ relative to 6 months, b: $P < .05$ relative to 1 year, or c: $P < .05$ relative to 2 years).

both age 6 and 12 months, after adjusting for internal correlation within individual subjects (method 2), the inverse correlations between the frequency of pDCs during infancy and the rates of LRIs and wheezy LRIs in the first 5 years remained significant ($P = .001$ and $P = .004$, respectively). Thus greater frequency of pDCs during infancy was strongly associated with a lower incidence of LRIs and wheezy LRIs, whereas a lower frequency of pDCs during infancy was strongly associated with a higher incidence of LRIs and wheezy LRIs. Numbers of pDCs at 5 years were also inversely correlated with the rate of LRIs and wheezy LRIs in the first 5 years of life ($P = .008$ for both correlations).

In contrast, no significant correlation was found between the frequencies of mDCs during infancy and ARIs or LRIs in the first 5 years (Table II). There was also no correlation between the frequencies of mDCs at 2 and 5 years of age and the rate of ARIs or LRIs in the first 5 years (data not shown).

Circulating DC subsets and cumulative rates of asthma or wheezing

During infancy, pDCs were significantly less frequent in those children who subsequently had an asthma diagnosis by age 5 years compared with children with no asthma diagnosis ($P = .006$, Fig 2). This significant difference was seen regardless of whether asthma diagnosis by age 5 years was analyzed in relation to the composite variable of infant pDCs, as shown in Fig 2, or whether the 6- and 12-month pDC data were analyzed separately, as shown in Table E1 in this article's Online Repository at www.jacionline.org. The combination of parent-reported wheezing and an asthma diagnosis by age 5 years was also associated with significantly fewer pDCs during infancy (see Table E1). Thus having fewer pDCs during infancy was a risk factor for asthma-related outcomes by the age of 5 years, whereas having more pDCs during infancy was associated with relative protection against asthma-related outcomes.

In contrast, the frequency of mDCs during infancy did not vary significantly in relation to a diagnosis of asthma or parent-

reported wheezing by age 5 years (Fig 2 and see Table E1). The frequencies of pDCs and mDCs at 2 and 5 years did not vary significantly in relation to a diagnosis of asthma or parent-reported wheezing by age 5 years (data not shown).

The rate of asthma diagnosed by a doctor or wheezing up to the age of 5 years did not vary significantly between children whose cells were analyzed by means of flow cytometry during infancy (15/52 [28%]) versus those in whom flow cytometry was not performed (57/144 [37%], $P = .17$). It was not practical to separately analyze subjects with early-onset wheezing (before 3 years of age) versus late-onset wheezing (after 3 years of age) because of the numbers of subjects.

Circulating DC subsets and atopic sensitization at 5 years

We also examined the relationship between the frequencies of circulating pDCs and mDCs during infancy and atopic sensitization at age 5 years. No significant correlations were found between pDCs during infancy and SPT reactivity at 5 years (Table III) or between pDCs during infancy and total or specific IgE levels (Table IV).

No significant correlations were found between mDCs during infancy and either SPT reactivity at 5 years (Table III) or specific IgE levels at 5 years (Table IV). However, there was a weak positive correlation between infant mDCs and total IgE levels at 5 years (Table IV). Both pDCs and mDCs measured at 2 and 5 years were not correlated with SPT reactivity at 5 years or with total or specific IgE levels at 5 years (data not shown).

DISCUSSION

In this prospective cohort study we assessed the relationship between the frequencies of circulating DC subsets during early childhood and the development of respiratory tract infections, wheezing, and allergic sensitization up to the age of 5 years. The key findings to emerge were that the frequency of circulating pDCs was inversely correlated with LRIs, wheezing reported by a parent, and the cumulative rate of asthma diagnosed by a doctor. Thus fewer pDCs at 6 months of age, 12 months of age, or both was a risk factor for LRIs, asthma, and wheezing by age 5 years, whereas more frequent pDCs were correlated with relative protection against these clinical outcomes. The frequency of pDCs appeared unrelated to the incidence of allergic sensitization. In contrast, the frequency of circulating mDCs was not correlated with LRIs or asthma-related outcomes but was correlated with total IgE levels at 5 years.

Animal models suggest that pDCs play a dual role in virus infections, providing important host defense against mucosal viral infections through their capacity to produce type I IFNs, such as IFN- α and IFN- β ,^{12,24} and also by modulating the extent of inflammation and tissue damage induced by viruses.²⁴

Studies in human subjects suggest that lower frequency of blood pDCs is a risk factor for both recurrent viral infections and more severe clinical manifestations of systemic viral infections,^{25,26} although this has not been examined previously in relation to childhood respiratory tract infections, in which viruses are frequently detected.^{22,27} In the study described herein, we found that having fewer blood pDCs was correlated with an increased risk of clinically diagnosed ARIs up to age 5 years. The strongest and most consistent statistical correlations were seen in relation to LRIs and wheezy LRIs, as opposed to ARIs in general (Table II).

TABLE II. Circulating DC subsets and ARIs in the first 5 years

Dependent variable	Method	Independent variable	IRR	P value	95% CI	
					Lower	Upper
ARI	1	Infant pDCs	0.93	.028	0.87	0.99
	2	Infant pDCs	0.90	.150	0.77	1.04
		5-y pDCs	0.98	.19	0.94	1.01
	1	Infant mDCs	1.02	.51	0.96	1.09
	2	Infant mDCs	0.97	.73	0.82	1.15
Febrile ARI		5-y mDCs	1.00	.87	0.97	1.04
	1	Infant pDCs	0.86	.03	0.75	0.99
	2	Infant pDCs	0.83	.06	0.68	1.01
		5-y pDCs	1.01	.71	0.94	1.09
	1	Infant mDCs	1.06	.34	0.94	1.20
LRI	2	Infant mDCs	0.98	.87	0.80	1.21
		5-y mDCs	1.07	.054	1.00	1.15
	1	Infant pDCs	0.81	.001	0.71	0.92
	2	Infant pDCs	0.77	.001	0.66	0.90
		5-y pDCs	0.91	.008	0.85	0.98
Wheezy LRI	1	Infant mDCs	0.91	.16	0.81	1.04
	2	Infant mDCs	0.87	.23	0.69	1.09
		5-y mDCs	1.07	.06	1.00	1.15
	1	Infant pDCs	0.76	.014	0.62	0.95
	2	Infant pDCs	0.79	.004	0.67	0.93
		5-y pDCs	0.88	.008	0.80	0.97
	1	Infant mDCs	0.86	.15	0.69	1.06
	2	Infant mDCs	0.88	.39	0.66	1.18
		5-y mDCs	1.05	.35	0.95	1.16

Poisson regression was used to estimate incidence rate ratios (IRRs) for every 1-unit increase in the *z* score of blood DC subsets measured as cells per 100 PBMCs. Data in boldface are significant at the .05 level. *Infant pDCs* and *infant mDCs* refer to the frequency of cells at age 6 or 12 months or their mean when the child had measurements at both times.

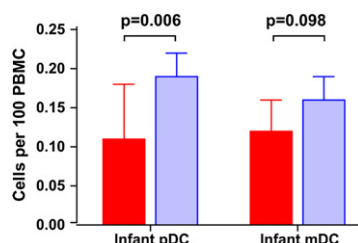


FIG 2. Circulating DC subsets during infancy and asthma diagnosed by a doctor up to age 5 years. DC subsets are expressed as the number of cells per 100 PBMCs relative to the presence (red bars, *n* = 13) or absence (blue bars, *n* = 39) of doctor-diagnosed asthma at age 5 years. *Infant pDC* and *infant mDC* refer to the frequency of cells at age 6 or 12 months or their mean when the child had measurements at both times. Data shown are geometric means + 95% CIs.

Because not all subjects had flow cytometry at both 6 and 12 months, the associations between pDCs and ARIs were analyzed by using 2 different methods. Regardless of the method used, a consistent inverse correlation was noted between the frequency of pDCs and the cumulative incidence of LRIs or wheezy LRIs. There are 2 possible explanations for this correlation: either having fewer blood pDCs predisposes to more frequent LRIs, or perhaps LRIs cause changes in the frequency of blood pDCs. Severe respiratory syncytial virus infection requiring hospitalization might induce a transient decrease in blood pDCs, although this does not appear to occur with other common respiratory viruses.²⁸ In the current community-based study, blood samples were taken from children at routine visits when they were clinically well and at least 2 weeks after any symptomatic ARIs, and therefore it seems unlikely that changes in blood pDCs can be attributed to

acute viral infections. None of the children required admission to the hospital for severe bronchiolitis during the first year of life. Hence we think that the more likely explanation for our findings is that a lower frequency of pDCs predisposes to more ARIs, particularly those that produce lower respiratory tract symptoms (ie, LRIs and wheezy LRIs). Subjects in the study were monitored for a variety of respiratory viruses by means of PCR, as previously reported.²² Although there was a trend for more frequent rhinovirus detection in relation to lower frequency of pDCs during infancy (data not shown), the numbers were too small to draw any statistically valid conclusions.

Blood leukocytes from older children with allergic asthma appear to have a reduced capacity to synthesize type I IFN in response to viruses compared with those of healthy subjects,²⁹ although whether this is related to numeric or functional deficits in pDCs has not been examined. Limitations on available cell numbers precluded assessment of type I IFN synthesis in the current study, although it is reasonable to hypothesize that those children with fewer pDCs have a reduced capacity for type I IFN production and that this places them at greater risk of either more frequent viral infections or more severe LRIs in response to viral infections.

Although some alterations in infant immune function have been described in those children who later have asthma or allergic sensitization, most reports have focused on reduced capacity to produce IFN- γ ²⁻⁸ or other aspects of T-cell function.³⁰ Little attention has been paid to antigen-presenting cell function in this context, although we recently showed an association between HLA-DR expression on cord blood monocytes and the subsequent risk of clinical allergic disease.³¹ In relation to DCs and asthma, several cross-sectional studies in adults and older children have described changes in DC subsets in patients with asthma,^{17,18,20}

TABLE III. Circulating DC subsets during infancy and allergen skin test reactivity at 5 years

	SPT reactivity	No.	GM	95% CI		P value
				Lower	Upper	
pDCs at 6 mo	No	19	0.23	0.17	0.31	.41
	Yes	12	0.19	0.13	0.28	
mDCs at 6 mo	No	19	0.16	0.12	0.20	.98
	Yes	12	0.16	0.11	0.22	
pDCs at 12 mo	No	29	0.15	0.12	0.20	.42
	Yes	14	0.13	0.09	0.18	
mDCs at 12 mo	No	29	0.13	0.11	0.16	.37
	Yes	14	0.15	0.11	0.21	
Infant pDCs	No	34	0.17	0.14	0.22	.61
	Yes	20	0.16	0.12	0.21	
Infant mDCs	No	34	0.14	0.11	0.16	.11
	Yes	20	0.17	0.14	0.22	

Infant pDCs and infant mDCs refer to the frequency of cells at age 6 or 12 months or their mean when the child had measurements at both times.

GM, Geometric mean.

although all these studies have been performed well after asthma was first diagnosed. Hence it has not been possible until now to determine whether such changes antedate the onset of airway inflammation, suggesting that DCs might contribute to the initiation of disease, or whether changes in DCs manifest only after asthma becomes clinically apparent and might therefore be a consequence of airway inflammation or its treatment.

Two previous studies have shown links between a reduced frequency of pDCs in childhood and atopy or asthma,^{20,21} although in both of these studies, DC subsets were studied after atopy or asthma had become apparent. Our study is the first to examine numbers of DC subsets in a prospective, community-based cohort and the first to show that changes in blood pDCs during the first year of life are related to the cumulative risk of LRIs, wheezing reported by a parent, or asthma diagnosed by a doctor up to age 5 years.

It is widely thought that pDCs contribute to immune tolerance,¹³ with one recent study suggesting that children with fewer blood pDCs are at risk for autoimmune diseases, such as type I diabetes mellitus.³² Similarly, de Heer et al³³ found in a mouse model that lung pDCs protect against allergic sensitization to inhaled antigens. However, in the current study we found no correlation between the frequency of circulating pDCs and the rate of allergic sensitization up to age 5 years, regardless of whether this was measured based on skin test reactivity or total or specific IgE levels. Limitations on cell numbers meant that we were not able to assess pDC function, and therefore it would be premature to conclude that pDCs are not involved in tolerance or sensitization in human subjects. However, it appears in the current study that the correlations between the frequency of circulating pDCs and LRIs, wheezing, and “asthma” are independent of allergic sensitization.

A weak but statistically significant correlation was noted between the frequency of blood mDCs during infancy and total IgE levels at 5 years (Table IV). Although this is consistent with a role for mDCs in the pathogenesis of allergic sensitization, the lack of a correlation between levels of mDCs and SPT reactivity and specific IgE levels at age 5 years suggests that these findings should be viewed with some caution at this stage.

There are a number of mechanisms by which the frequency of blood pDCs might be reduced in some children, including

TABLE IV. Circulating DC subsets during infancy and total or specific IgE levels at 5 years

	No.	Total IgE level		No.	Specific IgE level (Phadia)*	
		R	P value		R	P value
pDCs at 6 mo	29	−0.06	.76	29	−0.15	.44
mDCs at 6 mo	29	0.03	.86	29	−0.21	.27
pDCs at 12 mo	40	0.16	.33	40	0.02	.93
mDCs at 12 mo	40	0.30	.06	40	0.09	.60
Infant pDCs	50	0.10	.49	50	−0.05	.73
Infant mDCs	50	0.31	.03	50	0.08	.56

Spearman correlation coefficients (R) and P values are shown. Data in boldface are significant at the .05 level. Infant pDCs and infant mDCs refer to the frequency of cells at age 6 or 12 months or their mean when the child had measurements at both times.

*Phadia, Pharmacia, Uppsala, Sweden.

impaired differentiation from bone marrow precursors, poor cell survival, and increased homing to peripheral tissues. Recent studies in experimental animals suggest that aberrant bowel flora can lead to systemic reductions in pDCs,³⁴ and it is conceivable that changes in blood pDCs might partly account for the reported association between the composition of the gut flora and the differing risks of allergic sensitization in resource-rich and resource-poor regions of the world.³⁵

The findings from the current study do not allow us to conclude whether pDCs *per se* are directly involved in the pathogenesis of wheezing and LRIs or whether having fewer pDCs is a surrogate for other deficits in immune function. However, it is noteworthy that blood pDCs during infancy, but not at later time points, were associated with wheezing and “asthma.” In this context we have previously demonstrated that interactions between LRIs and atopy are major determinants of risk for development of persistent asthma by age 5 to 6 years^{22,36} and moreover that risk is maximal if these interactions occur simultaneously during a critical early period during infancy.²²

There are 3 limitations of the current study that should be borne in mind. First, small blood volumes and limitations in available cell numbers meant that we were only able to perform flow cytometry on a proportion of children at 6 and 12 months of age. However, we have no reason to believe that this has biased the findings of the study because the key outcome measures of LRIs, wheezing and asthma diagnosed by a doctor up to the age of 5 years, did not vary significantly between children who had flow cytometry during infancy versus those who did not.

Second, the frequency of DC subsets was expressed relative to numbers of PBMCs. The results obtained with this method can potentially be skewed by changes in other leukocyte subpopulations. However, if increases in other leukocytes were responsible for a reduced frequency of pDCs in children with LRIs or wheezing, then one would also expect a similar reduction in the frequency of mDCs, and this was not the case. Moreover, the frequency of basophils relative to PBMCs during infancy was not significantly associated with LRIs, wheezing, or allergic sensitization (data not shown). This argues against a dilution effect as an explanation for the findings of this study. Although measuring absolute numbers of DCs per milliliter of blood is the gold standard in this situation, the current cohort study was established in such a way that this was not possible. Since the study was commenced, additional methods for identifying pDCs by means of flow cytometry have become readily available, including the BDCA family of antibodies.³⁷ Although some authors have found

that BDCA-2 is exclusively expressed by CD123⁺ pDCs,³⁷ others claim that BDCA-2⁺ pDCs and CD123⁺ pDCs are slightly distinct populations,³⁸ and therefore future studies might need to use a variety of antibodies to define pDCs in childhood.

Finally, we acknowledge that the diagnosis of asthma in young children is not exact: many infants and preschool children with transient virus-induced wheezing do not have persistent asthma in later childhood.³⁹ In the study the diagnostic label of "asthma" was applied by community physicians, and further follow-up of the cohort beyond age 5 years will be necessary before we can determine how many of these children will have asthma that persists into later childhood. Seventy percent of the children with physician-diagnosed asthma were being treated with asthma medications. Exhaled nitric oxide levels were not measured in the study but are unlikely to be helpful in clarifying the diagnosis of asthma in children because they appear to be more indicative of atopy than asthma.⁴⁰ Attempts were made to perform spirometry at age 5 years, but it was difficult to obtain reproducible data in many of the children. These issues do not detract from the links between DC subsets and the more robust clinical outcomes, such as LRIs and wheezing.

Based on these findings, we propose that a reduced frequency of blood pDCs during early life predisposes young children to more frequent and more severe respiratory tract infections, especially viral infections. As a consequence of repeated lower airway inflammation, these children are more likely to wheeze and to be labeled as having asthma. A lower frequency of pDCs thus appears to antedate the development of respiratory tract infections, asthma, and related clinical phenotypes.

We acknowledge the tremendous support and enthusiasm of the study families and their children throughout the study and the dedication of the nursing staff involved in recruitment and follow-up of the families.

Clinical implications: Relative deficiency of circulating pDCs during infancy appears to be a risk factor for LRIs and wheezing in children up to age 5 years.

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METHODS

Monitoring of respiratory tract infections

Symptom diaries were mailed to the families and collected on a monthly basis. The child's mother recorded the presence of runny nose, blocked nose, cough, wheeze, and fever ($>38^{\circ}\text{C}$) on a daily basis for 5 years. The parents were instructed to contact the study center within 24 hours if the child had any symptoms of an ARI, and a home visit was arranged. Detailed clinical information regarding each episode of ARI was obtained at the home visit and then by means of follow-up telephone calls every 2 weeks until resolution of the child's symptoms. Information obtained from telephone contacts and diary cards was used to classify the episodes of ARI, as described in Table I.

Allergic sensitization

At 5 years of age, the children underwent SPTs to a panel of 7 allergens (cow's milk, egg white, rye grass, *Alternaria* species, *Aspergillus* species, house dust mite, and cat dander). Histamine was used as the positive control, and normal saline was used as the negative control. The wheal size was read after 15 minutes, with a wheal size of 3 mm or greater considered a positive reaction. Antihistamines, if taken, were withheld for at least 96 hours before the SPT. Children were considered atopic if they had a positive SPT reaction to at least 1 allergen.

Blood sampling and enumeration of DCs

PBMCs were isolated and cryopreserved for later batch analysis^{E1} by using methods first developed for cryopreservation of sperm and ova for *in vitro* fertilization. We and others have shown that careful cryopreservation of a variety of blood leukocytes does not distort their cellular function or surface phenotype.^{E1-E6} All samples were processed and analyzed without knowledge of the children's clinical status.

Flow cytometry

PBMCs were available for flow cytometry from the 6-month, 12-month, 2-year, and 5-year blood samples. Flow cytometry was not performed on blood samples collected at 3 and 4 years of age. For enumeration of DC subsets, cells were stained with a commercial 4-color antibody kit (BD Biosciences, San Jose, Calif) containing lineage FITC (a cocktail of mAbs specific for B cells, T cells, monocytes, and natural killer cells conjugated to fluorescein isothiocyanate), CD123 phycoerythrin, HLA-DR peridinin chlorophyll protein, and/or CD11c allophycocyanin. Circulating DCs were defined as lineage-negative cells expressing HLA-DR and further divided into plasmacytoid and myeloid subsets based on differential expression of CD123 (pDCs) and CD11c (mDCs). Basophils also express CD123 but lack HLA-DR and can thus be readily distinguished from pDCs by using this strategy. At least 150,000 mononuclear

cells were acquired on the flow cytometer to obtain sufficient numbers of DC subsets for accurate enumeration, and the results were expressed as a percentage of mononuclear cells. In preliminary experiments ($n = 7$) we determined that cryopreservation had no significant effect on the levels of pDCs and mDCs in blood samples from young children (data not shown).

All results of blood sampling, cell staining, and flow cytometry were processed and analyzed without knowledge of the children's clinical status.

Statistical analysis

Because the numbers of blood DCs had a skewed distribution, natural log transformation was conducted before further analyses. Group data are presented as geometric means and corresponding 95% CIs. In those children with paired DC measurements at both 6 and 12 months of age, bivariate correlation and paired-sample *t* tests were used to investigate the relationship between the measurements at the 2 time points, and the results showed no significant difference between blood DC numbers at 6 and 12 months. Therefore the variables of infant pDC or infant mDC numbers refer to the number of blood DCs at age 6 or 12 months or their mean when the child had measurements at both times. The independent-sample *t* test was used to analyze variations in blood DCs in relation to age (Fig 1), to asthma and wheezing up to age 5 years (Fig 2 and Table E1), and to atopy up to age 5 years (Table III).

Poisson regression was used with the number of ARIs as the dependent variable and *z* scores of the log value of numbers of blood DCs as the independent variable in the model to analyze the association between blood DCs in infancy and ARIs in the first 5 years of life. Calculation of *z* scores was as follows:

$(\text{Log value of number of DCs} - \text{Mean of log value of number of DCs}) / \text{SD of log value of number of DCs}$.

Incidence rate ratios and their 95% CIs, which are equivalent to odds ratios in logistic regression analysis, were estimated for every 1-unit increase in the *z* score of blood DC numbers (Table II). Incidence rate ratios were estimated by using 2 methods. First, pooled infant blood DC numbers was fitted as an independent variable in the Poisson regression analysis (method 1). Second, the number of blood DCs at age 6 and 12 months was treated as an individual sample for those who had both measurements at age 6 and 12 months (method 2), and in the Poisson regression analysis a "cluster option" was selected to account for the internal correlation in the same subject. Both methods produced similar results for the association between the number of blood DCs in infancy and the number of ARIs in the first 5 years of life (Table II). Finally, the Spearman method was used to evaluate associations between numbers of blood DCs and total IgE or Phadia IgE levels (Table IV). *P* values of less than .05 were considered significant. Except for the Poisson regression (Intercooled Stata 9), all the analyses were conducted with SPSS 13 software (SPSS, Inc, Chicago, Ill) for the Macintosh computer.

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TABLE E1. Circulating DC subsets during infancy and cumulative rate of asthma or wheezing

	Yes				No				P value
	No.	GM	95% CI		No.	GM	95% CI		
			Lower	Upper			Lower	Upper	
Asthma diagnosed by a doctor up to age 5 y									
pDCs at 6 mo	6	0.13	0.04	0.36	23	0.24	0.20	0.29	.027
mDCs at 6 mo	6	0.13	0.06	0.26	23	0.17	0.14	0.21	.26
pDCs at 12 mo	11	0.08	0.05	0.13	30	0.17	0.14	0.21	.002
mDCs at 12 mo	11	0.11	0.08	0.14	30	0.15	0.12	0.19	.088
Infant pDCs	13	0.11	0.07	0.18	39	0.19	0.16	0.22	.006
Infant mDCs	13	0.12	0.09	0.16	39	0.16	0.13	0.19	.098
Asthma diagnosed by a doctor or wheezing up to age 5 y									
pDCs at 6 mo	7	0.14	0.06	0.33	22	0.24	0.19	0.29	.045
mDC at 6 mo	7	0.13	0.07	0.23	22	0.17	0.13	0.21	.27
pDCs at 12 mo	12	0.09	0.06	0.14	29	0.17	0.14	0.21	.003
mDCs at 12 mo	12	0.11	0.09	0.15	29	0.15	0.12	0.18	.19
Infant pDCs	15	0.12	0.08	0.18	37	0.19	0.16	0.23	.013
Infant mDCs	15	0.13	0.10	0.17	37	0.16	0.13	0.19	.19

Data in boldface are significant at the .05 level. *Infant pDCs* and *infant mDCs* refer to the number of cells per 100 PBMCs at age 6 or 12 months or their mean when the child had measurements at both times.

GM, Geometric mean.