Research Article

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Profound Deficit of IL10 at Birth in Children Who Develop Childhood Acute Lymphoblastic Leukemia

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Abstract

Background: Childhood acute lymphoblastic leukemia (ALL) may originate via abnormal immune responses to infectious agents. It is unknown whether prenatal immune development may differ in children who develop the disease. The current study examines the association between neonatal cytokine profiles, a proxy measure for a child's prenatal immune development, and childhood ALL.

Methods: Neonatal blood spots of 116 childhood ALL cases and 116 controls living in California were ascertained. Eleven cytokines associated with Th1, Th2, and Th17 lymphocytes were measured using a multiplex bead–based assay. Unconditional logistic regression was done to estimate the odds ratio (OR) by measuring the association between neonatal cytokines and ALL adjusted for age, sex, race/ethnicity, and household income.

Results: Of the 11 cytokines measured, 5 [interleukin (IL)4, IL6, IL10, IL12, and IL13] were detectable. Except for IL12, the other 4 cytokines were all significantly lower among cases than controls. In a multivariable model including the 5 cytokines, only IL10 remained independently associated with childhood ALL with an OR = 0.04, 95% CI: 0.01 to 0.18, comparing the highest tertile to the lowest tertile.

Conclusions: A child's neonatal level of IL10, a key regulator for modulating the intensity and duration of immune responses, is associated with his/her subsequent risk of developing ALL.

Impact: The current analysis shows that children with ALL may have a dysregulated immune function present at birth. *Cancer Epidemiol Biomarkers Prev; 20(8); 1736–40. ©2011 AACR.*

Introduction

The major hypotheses [Greaves' delayed infection (1) and Kinlen's population mixing (2)] about the causes of childhood leukemia state that childhood leukemia may originate from abnormal immune responses to infectious agents following a lack of immune modulation during early childhood. Though early exposure to infections may be important for a child's immune development, it is clear that a child's immune development begins *in utero* (3, 4), suggesting that a child's baseline immune function at birth may affect his/her response to subsequent infectious exposures and leukemia risk. The current study examines the association between neonatal cytokine profiles, a proxy measure for the prenatal immune development, and childhood acute lymphoblastic leukemia (ALL) among 116 childhood ALL cases and 116 controls living in California.

Materials and Methods

This study was approved by the Institutional Review Boards of the University of California, Berkeley, CA, and all collaborating institutions, and a written informed consent was obtained from the parents or guardians of all participating subjects.

Detailed recruitment process has been described previously (5). Briefly, subjects of this analysis were recruited by the Northern California Childhood Leukemia Study (NCCLS). The NCCLS is an ongoing case-control study that started in 1995 and recruits subjects from 35 counties in Northern and Central California. Case subjects newly diagnosed with leukemia are recruited from 9 hospitals usually within 72 hours of diagnosis. Birth certificate information obtained from California Office of Vital Records is used to select 1 or 2 controls for each case, matching on age, sex, Hispanic ethnicity, and maternal race. The eligibility criteria for all subjects

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are: (i) being a resident of the study area; (ii) being younger than 15 years old at case diagnosis (reference date for the matched controls); (iii) having at least 1 English- or Spanish-speaking parent or guardian; and (iv) having not been previously diagnosed with cancer. We published previously on the association between maternal immunoglobulin (Ig) E and childhood leukemia using 139 ALL cases and 193 controls recruited by the NCCLS between August 2000 and December 2007 (5). We selected the study subjects from these subjects to investigate the correlation between neonatal cytokine and maternal IgE in addition to evaluating the association between neonatal cytokine profiles and childhood ALL. A total of 116 of the 139 ALL cases had an archived neonatal blood spot available to be included in the current study. A total of 116 of the 193 controls were selected to match the 116 ALL cases on age (birthdate), sex, and race/ethnicity.

For each subject, one eighth of an archived newborn blood spot was excised, placed in an Eppendorf tube with 160 μL of extraction buffer [PBS, pH 7.4, 0.5% Tween-20, 1% BSA, and 1× complete protease inhibitor cocktail (Roche)], shaken at 600 rpm under room temperature for 1 to 1.5 hours, then incubated at 4°C overnight. Extracts were assayed in duplicate and block randomized on 96-well plates, with each plate containing equal number of cases and controls and the same proportion of racial/ethnic groups along with a 7-point standard curve per plate. Eleven cytokines (IL2, IL4, IL5, IL6, IL10, IL12, IL13, IL17, GM-CSF, IFN-γ, and TNF-α) were measured using a Luminex bead–based assay (Bio-Rad). Protein content of each extract was determined with the Bradford Assay (Bio-Rad). Cytokine measurements for each duplicate were averaged and normalized to protein concentrations.

Demographic characteristics were compared between cases and controls using chi-squared tests or *t* tests. Univariate analyses were first performed to compare the neonatal cytokine levels between cases and controls using Wilcoxon rank-sum tests and *t* tests. In addition, cytokine levels were divided into tertiles on the basis of levels among the controls, and the proportions of cases and controls in different tertiles were compared using chi-squared tests. Multivariable analyses were performed using unconditional logistic regression with case/control status as outcome and each cytokine as the independent variable adjusted for child's age, sex, race/ethnicity, and annual household income. Annual household income, a proxy measure of socioeconomic status, was included as a covariate because its distribution was different between cases and controls. Finally, all cytokines were included in the same statistical model to assess for the independent effect of each cytokine on the risk of childhood ALL.

Table 1. Univariate analysis comparing neonatal cytokine profiles between 116 childhood ALL cases and 116 controls, the NCCLS, 2000–2007

	Control ($n = 116$)	ALL ($n = 116$)	*P* value
IL4			
Median, pg/mL[a]	0.18	0.13	0.0004
Mean Log_{10} IL4 (SE)[b]	−0.82 (0.02)	−0.95 (0.02)	<0.0001
Tertile, pg/mL[c]			
<0.11	38 (33.0%)	51 (44.4%)	0.006
0.11–0.179	38 (33.0%)	46 (40.0%)	
≥0.18	39 (34.0%)	18 (15.6%)	
IL6			
Median, pg/mL[a]	0.69	0.58	0.002
Mean Log_{10} IL6 (SE)[b]	−0.08 (0.03)	−0.19 (0.03)	0.003
Tertile, pg/mL[c]			
<0.57	36 (31.0%)	56 (48.3%)	0.03
0.57–0.889	40 (34.5%)	29 (25.0%)	
≥0.89	40 (34.5%)	31 (26.7%)	
IL10			
Median, pg/mL[a]	1.37	0.84	<0.0001
Mean Log_{10} IL10 (SE)[b]	0.14 (0.03)	−0.08 (0.02)	<0.0001
Tertile, pg/mL[c]			
<1.08	38 (32.8%)	83 (72.1%)	<0.0001
1.08–1.69	38 (32.8%)	21 (18.3%)	
≥1.69	40 (34.4%)	11 (9.6%)	
IL12			
Median, pg/mL[a]	2.49	2.29	0.79
Mean Log_{10} IL12 (SE)[b]	0.38 (0.03)	0.40 (0.03)	0.73
Tertile, pg/mL[c]			
<1.5	38 (32.8%)	34 (29.3%)	0.70
1.5–3.69	38 (32.8%)	44 (37.9%)	
≥3.7	40 (34.4%)	38 (32.8%)	
IL13			
Median, pg/mL[a]	0.98	0.85	0.0006
Mean Log_{10} IL13 (SE)[b]	0.04 (0.02)	−0.06 (0.02)	0.0003
Tertile, pg/mL[c]			
<0.85	37 (31.9%)	60 (51.7%)	0.005
0.85–1.139	39 (33.6%)	33 (28.5%)	
≥1.14	40 (34.5%)	23 (19.8%)	

[a]*P* value comparing the median cytokine level was calculated using Wilcoxon rank-sum test.
[b]*P* value comparing the mean cytokine level was calculated using *t* test.
[c]*P* value comparing the distribution of cytokine level in tertiles was calculated using chi-squared test.

Results

Cases and controls were identical in the distributions of sex (61% men) and race/ethnicity (48% Hispanics, 33% non-Hispanic whites, and 19% others). Cases and controls were similar in age (mean age in years: 3.8 for cases and 3.9 for controls, $P = 0.8$), but controls had a higher proportion

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of subjects who had annual household income greater than $75,000 compared with cases (50% vs. 38%, $P = 0.11$).

Of the 11 cytokines measured, 5 (IL4, IL6, IL10, IL12, and IL13) were detectable. In the univariate analysis (Table 1), the levels of IL4, IL6, IL10, and IL13 were significantly lower among childhood ALL cases than controls. We adjusted for the potential confounders (child's age, sex, race/ethnicity, and annual household income) and found that increasing levels of IL4, IL6, IL10, and IL13 were all associated with a significantly ($P < 0.05$) decreased risk of childhood ALL (Table 2). IL12 did not show any significant relationship with childhood ALL. In a second multivariable analysis, we included all of the 5 cytokines in the same statistical model, and only IL10 remained statistically significant (Table 2). By using the lowest tertile of IL10 level as the referent group, the second tertile had an odds ratio (OR) of 0.16 (95% CI: 0.07–0.39), and the highest tertile had an OR of 0.04

Table 2. Multivariable analysis examining the association between each cytokine and risk of childhood ALL, the NCCLS, 2000–2007

	Multivariable analysis 1[a]		Multivariable analysis 2[b]	
	OR (95% CI)	*P* value	OR (95% CI)[b]	*P* value
IL4				
Tertile, pg/mL				
<0.11	Referent		Referent	
0.11–0.179	0.92 (0.49–1.72)	0.79	2.00 (0.88–4.52)	0.10
≥0.18	0.35 (0.17–0.71)	0.004	2.01 (0.57–7.12)	0.28
Trend	0.61 (0.43–0.87)	0.006	1.57 (0.88–2.81)	0.13
Continuous Log_{10} IL4	0.11 (0.03–0.35)	0.0002	4.47 (0.48–41.58)	0.19
IL6				
Tertile, pg/mL				
<0.57	Referent		Referent	
0.57–0.889	0.50 (0.26–0.95)	0.03	0.61 (0.27–1.36)	0.22
≥0.89	0.47 (0.24–0.89)	0.02	0.78 (0.31–1.93)	0.58
Trend	0.67 (0.49–0.93)	0.02	0.89 (0.57–1.38)	0.59
Continuous Log_{10} IL6	0.20 (0.07–0.57)	0.002	0.44 (0.12–1.67)	0.23
IL10				
Tertile, pg/mL				
<1.08	Referent		Referent	
1.08–1.69	0.25 (0.13–0.49)	<0.0001	0.16 (0.07–0.39)	<0.0001
≥1.69	0.12 (0.06–0.27)	<0.0001	0.04 (0.01–0.18)	<0.0001
Trend	0.33 (0.22–0.48)	<0.0001	0.21 (0.11–0.39)	<0.0001
Continuous Log_{10} IL10	0.04 (0.01–0.13)	<0.0001	0.01 (<0.001–0.07)	<0.0001
IL12				
Tertile, pg/mL				
<1.5	Referent		Referent	
1.5–3.69	1.29 (0.68–2.47)	0.44	1.84 (0.86–3.94)	0.12
≥3.7	1.08 (0.55–2.12)	0.83	1.78 (0.80–3.96)	0.16
Trend	1.04 (0.74–1.45)	0.83	1.29 (0.87–1.92)	0.20
Continuous Log_{10} IL12	1.17 (0.53–2.58)	0.70	2.09 (0.81–5.42)	0.13
IL13				
Tertile, pg/mL				
<0.85	Referent		Referent	
0.85–1.139	0.54 (0.29–1.01)	0.05	0.98 (0.43–2.22)	0.96
≥1.14	0.36 (0.18–0.70)	0.003	2.10 (0.63–7.03)	0.23
Trend	0.59 (0.42–0.83)	0.002	1.27 (0.73–2.21)	0.41
Continuous Log_{10} IL13	0.07 (0.02–0.33)	0.0006	4.00 (0.25–65.06)	0.33

[a]Each cytokine was analyzed separately (5 analyses are shown) and adjusted for child's age, sex, race/ethnicity, and annual household income using unconditional logistic regression.

[b]All 5 cytokines were analyzed together in 1 unconditional logistic regression model, adjusted for child's age, sex, race/ethnicity, and annual household income.

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(95% CI: 0.01–0.18), representing a 25-fold difference. Because the 5 cytokines levels are correlated with one another (Pearson's correlation coefficients ranged from 0.14 to 0.78), there is a potential for multicollinearity when they are included in the same logistic regression model. The variance inflation factor (VIF) was calculated for each cytokine (on the $\log_{10}$ continuous scale) to evaluate for the presence of multicollinearity. It has been recommended that a VIF > 10 is an indication for the existence of multicollinearity (6). The VIFs for the 5 cytokines ranged from 1.1 to 3.7, suggesting that multicollinearity is not a serious problem for our logistic regression model that included all 5 cytokines.

The association of IL10 level and childhood ALL did not differ significantly by sex, birth order, or age at diagnosis (data not shown). Little correlation was found between maternal IgE levels, assessed previously (5), and the levels of the 5 neonatal cytokines (Spearman correlation coefficients ranged from −0.09 to 0.10).

Discussion

Results of the current study suggest that children who develop ALL may already have abnormal immune function at birth as indicated by their lower level of IL10 at birth. IL10 is a key regulator for modulating the intensity and duration of immune responses to infections (7). The United Kingdom Childhood Cancer Study (UKCCS) reported, using general practitioner records (rather than parental interview), that children diagnosed with ALL had significantly more clinically diagnosed infectious episodes in the first year of life than controls (8). The number of infectious episodes in children with ALL increased with increasing indices of infectious exposure (birth order, regular social activity outside the home, and social deprivation at birth), a phenomenon not observed among healthy control children (9). It has been reported that an increased risk for repeated respiratory infections during infancy and childhood, associated with an elevated production of IL5 by T cells at birth, could be attenuated by IL10 production (10). It is possible that children with a dysregulated immune function at birth are at higher risk for developing leukemia due to constitutively lower expression of IL10, a cytokine that is critical to prevent an overactive inflammatory response to pathogenic infections. Biological stress from postnatal infection in combination with a dysregulated immune response may confer a growth advantage for a preleukemic clone leading to its rapid expansion and an increased opportunity for the occurrence of a second mutation required for the development of childhood leukemia (1). Though some may argue that the presence of a leukemia clone prenatally may affect the level of IL10 at birth by suppressing the immune system, preleukemia cells at birth are rare, usually around 1 cell for every 1,000 normal cells, and therefore unlikely to affect IL10 levels (11, 12). Second, though age at diagnosis and levels of preleukemic cells at birth were roughly correlated among some leukemia subtypes (13), there was no relationship between age at diagnosis and neonatal IL10 levels among the cases in our analysis (data not shown).

Both the Greaves and Kinlen hypotheses emphasize a role for early infections on a child's risk of leukemia. Clearly, risk of childhood leukemia is inversely associated with early daycare attendance (14) and parental report of a child's infection during early childhood (15–17) and higher birth order (18, 19). There is only a single study that we are aware of that assessed general practitioner records for evidence of clinically diagnosed early childhood infection. This study, the UKCCS, showed that early life (less than 1 year old) infections are a *risk* factor (8), whereas exposures to childhood contacts are a *protective* factor for leukemia (20). Our current study implicates an altered congenital responder status to infection in newborns; newborns with lower IL-10 are likely to respond more vigorously to infections resulting in a visit to the clinician, which is compatible with the UKCCS result (8).

A dysregulated immune function at birth for children diagnosed with ALL implies that maternal factors may influence the leukemia risk of the child by modulating the child's *in utero* immune development. Schaub and colleagues reported an increased induction of IL10 and Foxp3 in cord blood mononuclear cells among children with nonatopic mothers, suggesting that children with atopic mothers may have a decreased capacity to respond to microbial challenge (3). We previously reported that an elevated level of maternal IgE was associated with an increased risk of childhood leukemia (5), although we did not find any correlation between maternal IgE and neonatal IL10 level in the current analysis. We measured IL10 levels at baseline rather than after infectious challenge which may account for the discordance.

In summary, our data suggest that children who develop leukemia may already have a dysregulated immune function at birth. Future studies need to confirm this association and consider maternal and genetic factors in the development of childhood leukemia through their influence on the child's immune development.

Disclosure of Potential Conflicts of Interest

The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIEHS of the NIH.

Contributions

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