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The risk of neurodevelopmental disorders at age 10 years associated with blood concentrations of Interleukins 4 and 10 during the first postnatal month of children born extremely preterm.

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Abstract

Background—Interleukin (IL)-4 and IL-10 are viewed mainly as anti-inflammatory cytokines. Yet, high concentrations have also been associated with inflammation-related diseases in newborns.

Methods—We measured the concentrations of IL-4 and IL-10, as well as IL-8 and ICAM-1 in blood specimens collected on postnatal day 21 (N = 555), day 28 (N = 521), and both days 21 and 28 (N = 449) from children born extremely preterm (EP) (< 28 weeks gestation) who at age 10 years had a DAS-II IQ Z-score > −2 (which approximates a score of > 70) and the following assessments, CCC-2, and CSI-4, DAS-II, NEPSY-II, OWLS-II, SCQ, and WIAT-III. Selected children also were assessed with the ADI-R and the ADOS-2. We modeled the risk of low scores or dysfunctions associated with top quartile concentrations of IL-4 and IL-10 on each day and on both days.

Results—The risks of low scores on the Animal Sorting and Arrows components of the NEPSY-II, both components of the OWLS-II, and the PseudoWord and Spelling components of the WIAT-III were heightened among children who had top quartile concentrations of IL-4 on postnatal days 21 and 28. Children who had high concentrations of IL-10 on days 21 and 28, individually and collectively, were at increased risk of low scores on the WIAT-III Spelling component. High concentrations of IL-4 on day 28 were associated with autism spectrum disorder (ASD). High

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concentrations of IL-10 on day 28 were also associated with a doubling of ASD risk, but this did not achieve statistical significance. Top quartile concentrations of IL-4 and IL10 on both days were not associated with increased risk of social, language, or behavioral dysfunctions

Conclusion—Among children born EP, those who had top quartile concentrations of IL-4 and/or IL-10 on postnatal days 21 and/or 28 were more likely than their peers to have low scores on components of the NEPSY-II, OWLS-II, and WIAT-III assessments, as well as identification as having an ASD.

Keywords

Developmental outcome; neurodevelopment; reading; spelling; autism spectrum disorder; inflammation; very premature infant; interleukin-4; interleukin-10

Introduction

Sustained systemic inflammation appears to contribute to brain damage in extremely low gestational age newborns (ELGANs).[1] We do not yet know why the inflammation once initiated continues.[2] One possibility is the limited ability of ELGANs to resolve inflammation.[3]

Interleukin-4 (IL-4) and interleukin-10 (IL-10) are viewed as anti-inflammatory,[4] and capable of resolving inflammation.[5,6] Indeed, one group of authors wrote, “Interleukin-10 (IL-10) is arguably the most potent anti-inflammatory cytokine.”[7] Others express hope that increasing IL-10 availability might be therapeutic for a number of neuroimmune disorders.[8] The possibility that this hope might apply to the developing brain comes from a report that the neuroprotective effects of early administration of umbilical cord blood cells to fetal sheep are attributable, in part, to the subsequent elevation of IL-10 blood concentrations.[9]

We found only one study that assessed the risk of brain damage among preterm newborns associated with IL-4 and IL10 concentrations.[10] In a sample of 74 infants whose mean gestational age was 27 weeks, area-under-the-curve assessments of IL-4 and IL-10 concentrations were not associated with sonographically-defined brain damage (hyperechoic or hypoechoic lesions for ≥ 7 days).

We are not aware of any assessment of the risk of indicators of brain dysfunction at older ages associated with IL-4 and IL-10 concentrations during the first postnatal month. The ELGAN Study[11] provided us the opportunity to evaluate to what extent elevated concentrations of these proteins were associated with reduced risk of neurocognitive, language, social, and academic dysfunctions at school age among children born extremely preterm (i.e., < 28 weeks gestation).[12]

Methods

Participants

The ELGAN study is a multi-center prospective, observational study of the risk of structural and functional neurologic disorders in extremely preterm infants.[11] A total of 1506 infants

born before the 28th week of gestation were enrolled during the years 2002–2004 and 1200 survived to 2 years. For the assessment at age 10 years, we enrolled 889 (92%) of the 966 children for we had information about the concentrations of proteins measured in blood samples obtained during the first postnatal month. Of these children, 870 had an IQ assessment and of these, 735 had a DAS-II IQ Z-score > −2 (approximating an IQ of > 70) (Table 1). We limited the sample for most analyses to children with at least this IQ level to avoid attributing neurocognitive and other limitations to impaired cognition. Enrollment and consent procedures for this follow up study were approved by the institutional review boards of all participating institutions.

Demographic and pregnancy variables

After delivery, a trained research nurse interviewed each mother in her native language using a structured data collection form and following procedures defined in a manual. Shortly after the mother's discharge, the research nurse reviewed the maternal chart using a second structured data collection form. The medical record was relied on for events following admission. The clinical circumstances that led to preterm delivery were operationally defined using both data from the maternal interview and data abstracted from the medical record.[13] Each mother/infant pair was assigned to the category that described the primary reason for the preterm delivery.[13]

Newborn variables

The gestational age estimates were based on a hierarchy of the quality of available information. Most desirable were estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed sequentially on a fetal ultrasound at 14 or more weeks (29%), LMP without fetal ultrasound (7%), and gestational age recorded in the log of the neonatal intensive care unit (1%).

The birth weight Z-score is the number of standard deviations the infant's birth weight is above or below the median weight of infants at the same gestational age in referent samples not delivered for preeclampsia or fetal indications.[14,15]

Blood protein measurements

Drops of blood were collected on filter paper on the 21st postnatal day (range: 19–23 days), and the 28th postnatal day (range: 26–29). All blood was from the remainder of specimens obtained for clinical indications. Dried blood spots were stored at −70 ^{O}C in sealed bags with a desiccant until processed.

The Genital Tract Biology Laboratory at the Brigham and Women's Hospital in Boston Massachusetts eluted all blood spots as previously described,[16] and measured the total protein concentration in each eluted sample by BCA assay (Thermo Scientific, Rockford, IL) using a multi-label Victor 2 counter (Perkin Elmer, Boston, MA). The laboratory used the Meso Scale Discovery multiplex electrochemiluminescence platform to measure interleukin-4 (IL-4), IL-8 (CXCL8), IL-10, and Intercellular Adhesion Molecule −1 (ICAM-1; CD54). These measurements were normalized to mg total protein.

Because the concentrations of inflammation-related proteins in the ELGAN Study varied with gestational age, and with the postnatal day of collection,[17,18] we divided our sample into 6 groups defined by gestational age category (23–24, 25–26, 27 weeks), and postnatal day of blood collection (21 and 28). Approximately half of all children did not have measurable concentrations of IL-4 or IL-10, while the concentrations in the next quartile (51st centile to 75th centile) tended to be just above zero. This provided additional support for our routine procedure of dichotomizing children into the highest quartile and the lower three quartiles of each protein.

Procedures for the assessments at age 10 years

All families who participated in the previous follow up were contacted by mail and then by phone to invite them to participate in the 10-year follow up. Lost to follow-up families were searched for on state vaccination registries, and other openly-available websites. Social media websites were also used where approved by the local institution's IRB.

Families willing to participate were scheduled for one visit during which all of the measures reported here were administered in 3 to 4 hours, including breaks. The assessments were selected to provide the most comprehensive information about neurocognitive and academic function in one testing session.

Neurocognitive measures

General cognitive ability.—General cognitive ability (or IQ) was assessed with the School-Age Differential Ability Scales–II (DAS-II) Verbal and Nonverbal Reasoning scales.[19]

Language ability.—Expressive and receptive language skills were evaluated with the Oral and Written Language Scales (OWLS), which assess semantic, morphological, syntactic, and pragmatic production and comprehension of elaborated sentences.[20]

Attention and executive function were assessed with both the DAS-II[19] and the NEPSY-II (A Developmental NEuroPSYchological Assessment-II).[21] The DAS-II Recall of Digits Backward and Recall of Sequential Order measured verbal working memory, while the NEPSY-II Auditory Attention and Response Set measured auditory attention, set switching and inhibition, the NEPSY-II Inhibition-Inhibition Inhibition-Switching measured simple inhibition and inhibition in the context of set shifting, respectively, and the NEPSY-II Animal Sorting measured visual concept formation and set shifting.

The NEPSY-II Inhibition-Naming component, provides a baseline measure of processing speed and has no inhibitory component. Visual perception was assessed with NEPSY-II Arrows and Geometric Puzzles, while visual motor function was measured with NEPSY-II Visuomotor Precision and Fingertip Tapping.

Academic Function—The Wechsler Individual Achievement Test-III (WIAT-III [C]) provides standard scores in word recognition and decoding, spelling, and numeric operations.[22]

Child Symptom Inventory-4—While the child was tested, the parent or caregiver completed questionnaires regarding the child's medical and neurological status and behavior, including the Child Symptom Inventory-4 Parent Checklist (CSI-4).[23] The child's current teacher was also asked to complete the Child Symptom Inventory-4 Teacher Checklist. Although the parent checklist has 20 more items than the teacher version (97 vs 77), both include the same 18 items specific for ADHD symptoms (9 for the inattentive domain and 9 for the hyperactive/impulsive domain) that are each rated on a scale from 0 (never) to 3 (very often). Teachers and parents did not make any DSM-IV diagnosis. Rather, the CSI-4 program identified children as screening positive for these diagnoses based on the parents' or teachers' acknowledging selected characteristics of the child.

Social Responsiveness Scale

The SRS identifies social impairment associated with autism spectrum disorder (ASD) and quantifies its severity.[24] This 65-item instrument provides a total score reflecting severity of social deficits in the autism spectrum, as well as five subscale scores: social awareness, social cognition, social communication, social motivation, and restricted interests and repetitive behavior.

Children's Communication Checklist-2 (CCC-2)

The Children's Communication Checklist-2 (CCC-2) has 70 items that assess speech, vocabulary, sentence structure, and social language skills.[25] The 10 subscales are discourse, syntax, semantics, coherence, inadequate initiation, stereotyped language, use of context, non-verbal communication, social relations, and interests. We calculated Z-scores using normative data.[26]

Autism assessment

All children were screened by parent report for risk of ASD with the Social Communication Questionnaire (SCQ).[27] Children determined at risk on the SCQ, were assessed with the Autism Diagnostic Interview – Revised (ADI-R), and in-depth parent interview.[28] Children meeting ADI-R criteria for ASD[29] were administered Autism Diagnostic Observation Schedule-2 (ADOS-2).[30] All children meeting standardized research criteria for ASD on both the ADI-R and ADOS-2 were classified as having ASD.[31] In addition, 11 children were included who met ADOS-2 criteria, but did not have an ADI-R assessment. Nine additional children who had a prior clinical diagnosis of ASD, or who the site psychologist thought likely to meet diagnostic criteria for ASD, were assessed with the ADOS-2.

Data Analyses

We evaluated the null hypothesis that top-quartile concentrations of IL-4 and IL-10 are not associated with any cognitive or executive dysfunction, academic achievement or social/behavioral limitation. We began by assessing the maternal, pregnancy, and newborn characteristics associated with top quartile concentrations of IL-4 and IL-10, first in regard to maternal, pregnancy and delivery variables (Supplement Table 1), then newborn variables (Supplement Table 2), and IL-8 and ICAM-1 concentrations (Table 2). Information from these three sets of analyses provided information about potential confounders. Based on this

information and what we know about this sample,[32–34] we decided to adjust for gestational age category (i.e., 23–24 and 25–26 weeks), birth weight Z-score < −1, and same-day top quartile concentration of IL-8.

We then created multiple logistic regression models of the risk of a score one or more standard deviations below the normative mean of each neurocognitive assessment at age 10 years (Table 3), and of selected social and behavioral dysfunctions, including an autism spectrum disorder (Table 4). These parsimonious models allowed us to calculate odds ratios (and 95% confidence intervals) of each 10-year characteristic associated with top quartile concentrations of IL-4 and IL-10.

RESULTS

Sample (Supplement Tables 1 and 2)

Of the 966 children eligible for recruitment at age 10 years, 889 returned for follow-up at 10 years, and 870 had their IQ measured. Of the 735 who had an IQ Z-score > −2 (approximately equivalent to > 70), 555 had concentrations of IL-4 and IL-10 measured in specimens from day 21, and 521 had measurements made in day 28 specimens.

In a normally-distributed sample, only 2.2% would have a Z-score of ≤ −2, while 16% would have a Z-score less than −1. In our sample, however, at least 20% of children had a Z-score of less than −2 on three NEPSY-II components (Inhibition Inhibition, Inhibition Switching, Inhibition Naming), while only 4 to 6% had a Z-score ≤ −2 on each of the WIAT-III components. More than half the sample had a Z-score ≤ −1 on three NEPSY-II components (Inhibition Switching, Animal Sorting, and Visuomotor Precision). In contrast, only 17% of children had a Z-score ≤ −1 on the Spelling component of the WIAT-III.

Distribution of concentrations

More than three quarters of all children had IL-4 and IL-10 concentrations of less than 0.1 picograms/mg protein on both days 21 and 28. In contrast, the maximum IL-4 concentration on both days was greater than 3.0 picograms/mg protein, while the maximum IL-10 concentration on day 21 was 2.9 and on day 28 was 6.4 picograms/mg protein.

Associations with inflammation (Supplement Table 3)

Children who had top quartile concentrations of IL-8 on days 21 and 28, and separately, those who had top quartile concentrations of ICAM-1 on days 21 and 28, were more likely than others to have top quartile concentrations of IL-4 and IL-10 on those days.

Maternal, pregnancy and delivery variables (Supplement Table 4)

Of all the maternal, pregnancy and delivery variables assessed, only maternal and fetal indications for extremely preterm delivery were associated with top quartile concentrations of IL-4 and IL-10 (Supplement Table 1).

Newborn variables (Supplement Table 5)

Of all the newborn characteristics assessed, six characteristics were associated with high concentrations of IL-4 and IL-10. Growth restricted newborns were more likely than others to have high concentrations of both of these proteins, as were singletons and children who had high illness severity scores (SNAP ≥ 30), low PaO2 and high PCO2 measurements on two of the first three days, and those who had bacteremia.

Neurocognitive, language, and academic associations (Table 1)

Children who had top quartile concentrations of IL-4 on postnatal day 28, and those who had similarly high concentrations of IL-10 on day 21 and on day 28 were at increased risk of an IQ Z-score ≤ −2. These low IQ children are excluded from the rest of this table.

Top quartile concentrations of IL-4 on day 21, and on both days 21 and 28 were associated with heightened risk of a Z-score ≤ −1 on the Animal Sorting and Arrows components of the NEPSY-II, both the Listening Comprehension and Oral Expression components of the OWLS, and both the PseudoWord and Spelling components of the WIAT-III. Top quartile concentrations of IL-4 on day 21 were also associated with heightened risk of a low score on the WIAT-III Word Read component. The same point estimate of the odds ratio (*i.e.*, 1.6) was also seen among children who had IL-4 elevated concentrations on days 21 and 28, but did not achieve statistical significance because of the smaller sample (449 vs 555).

Children who had top quartile concentrations of IL-10 on day 21 were at increased risk of a Z-score ≤ −1 on the Animal Sorting, Inhibition-Naming, and Arrows components of the NEPSY-II, both the Listening Comprehension and Oral Expression components of the OWLs, and the Word Reading component of the WIAT-III. Children who had top quartile concentrations of IL-10 on day 21, and separately on day 28, and both days 21 and 28 were at increased risk of a low score on the Spelling component of the WIAT-III.

Social, language, and behavioral associations (Table 2)

Top quartile concentrations of IL-4 on day 28 were associated with being classified as having an autism spectrum disorder (ASD). The risk of ASD was also more than doubled among children who had a day-28 concentration of IL-10 in the top quartile, but this did not reach statistical significance. No other concentration elevations were associated with any other social, language, or behavioral difficulty identified with the assessments listed in this table.

DISCUSSION

What we found

Because sustained (or less likely, intermittent) inflammation has been most clearly associated with increased risk of brain damage in the ELGAN Study cohort,[1,32,35] we place most credence on elevated concentrations on multiple days. We evaluated 16 neurocognitive correlates (DAS-II working memory, 9 components of the NEPSY-II, both components of the OWLS, and four components of the WIAT-III). By chance, we would expect one of these to be associated with high concentrations on both days 21 and 28. Nevertheless, we found

that children in the ELGAN Study cohort who had top quartile concentrations of IL-4 on both days were at increased risk of low scores on six of these neurocognitive correlates (NEPSY-II Animal Sorting and Arrows components, both the Listening Comprehension and Oral Expression components of the OWLS, and both the PseudoWord and Spelling components of the WIAT-III). On the other hand, children who had top quartile concentrations of IL-10 on both days were at increased risk of low scores on the WIAT-III spelling assessment only. Children who had high concentrations of IL-4 or IL-10 on both days were not at increased risk of any social, language, or behavioral dysfunction.

What others found

a. newborn—Although by and large, preterm newborns have distributions of blood concentrations of IL-4 and IL-10 that are similar to those of term-born newborns, among very preterm infants, the younger the gestational age, the higher the concentrations of IL-4 and IL-10.[36] Others have also reported that infants born before 36 weeks tend to have lower IL-10 blood concentrations than infants born during or after the 36th week of gestation.[37] In addition, umbilical cord blood cells from (human) infants born preterm (< 37 weeks) secrete lower levels of IL-10 than cord blood cells from infants born at term.[38] Concentrations of IL-4 and/or IL-10 in the blood of preterm infants are elevated among preterm newborns who have sepsis[39–43] or necrotizing enterocolitis.[44] The production of anti-inflammatory cytokines, such as IL-4 and IL-10, however, appears to follow, rather than accompany, the early production of pro-inflammatory cytokines.[45]

b. School age—Among 25 children who had sickle cell disease and normal MRI studies of the brain, the higher the contemporaneous concentrations of IL-4, the lower the scores on the Verbal Fluency component of the Delis-Kaplan Executive Function System.[46] We do not know of any assessment of the relationship between very high concentrations of IL-4 and IL-10 during the first postnatal month with dysfunctions at school age.

Inferences about our findings

IL-4 and IL-10 are offered as surrogates for all the other cytokines that are part of the inflammatory process.[47] Thus, what we attribute to them might well be attributable to the other proteins that they represent.

Our finding that high concentrations of IL-4 and IL-10 are associated with undesirable scores on assessments of neurocognition, language, communication, and social function is compatible with several possible explanations. We offer three.

a. elevated concentrations of IL-4 and IL-10 indicate the severity of inflammation—Given what we know about systemic inflammation and brain damage in very and extremely preterm newborns,[1] one of the most obvious inferences is that high concentrations of IL-4 and IL-10 provide (supplemental) information about systemic inflammation severity. Because IL-10 blood concentrations and the ratio of IL-10 to TNF-alpha concentrations were higher in human adults who did not survive sepsis, the authors concluded, “the sustained overproduction of the anti-inflammatory cytokine IL-10 is the main predictor of severity and fatal outcome.”[48]

In children, “IL-10 is produced in massive amounts in the initial phase of fulminant meningococcal septic shock.”[49] The IL-10 blood concentrations did correlate (albeit weakly) with severity of disease as measured by the Glasgow meningococcal septicemia prognostic score. Also, among very low birth weight infants, elevated blood concentrations of IL-10 not only distinguish between those who do and do not have early onset bacteremia, but also identify those preterm newborns most likely to develop disseminated intravascular coagulation.[50]

Yes, the “overproduction” of IL-10 might be an indicator of severity of inflammation, with the presumed “inflammatory proteins” (e.g., IL-8, ICAM-1, etc) contributing to the brain damage. In this situation, high concentrations of IL-4 and IL-10 are epiphenomena.

b. IL-4 and IL-10 can contribute to brain damage—In support of the possibility that elevated blood concentrations of IL-4 and IL-10 contribute to the risk of adverse effects (including contributing to brain damage) is the observation that an anti-IL-10 monoclonal antibody increased the probability of survival of mice that developed polymicrobial sepsis induced by cecal ligation and puncture.[51] Thus, reducing IL-10 concentrations appears to reduce the risk of adversity, in this case death.

Additional support for this concept that high concentrations of IL-4 and IL-10 contribute to adversity comes from other sources. Because of its anti-inflammatory properties, one strategy to ameliorate inflammation-exacerbated neurologic disorders is to increase the availability of IL-10.[5] This, however, has sometimes made things worse in models of multiple sclerosis,[52] and Alzheimer’s disease.[53] Limiting the availability of IL-10 in a model of Alzheimer’s disease preserved synaptic integrity and mitigated cognitive disturbance,[54] adding support for the view that IL-10 can contribute to brain damage. Similarly, increased IL-4 expression in an Alzheimer’s model increased (not decreased) amyloid deposition.[55] Perhaps IL-4 contributes to brain damage via its ability to potentiate the effects of oxidative stress on neurons.[56]

c. High concentrations of IL-4 and IL-10 are surrogates for maturity/vulnerability—The Score for Neonatal Acute Physiology (SNAP-II) is a measure of illness severity during the first 12 postnatal hours.[57] Among children in the ELGAN Study cohort, disproportionately more of those who had a high score (indicative of physiologic instability, which we equate with immaturity) had top-quartile concentrations of IL-4 and IL-10 than their peers (Supplement Table 2). In addition, children who had a high SNAP-II during the first 12 postnatal hours were at increased risk of cognitive, educational, executive, language, and social dysfunctions at age 10 years.[58]

Limitations.

We adjusted for IL-8 concentrations in the top quartile as our indicator of severity of systemic inflammation. Nevertheless, we acknowledge the possibility of residual confounding.

We are also limited by the relatively small number of proteins measured. Inflammation is a broad and complex phenomenon,[47] and we assessed a very small part of it.

We relied on blood specimens obtained for clinical indications. As a result, ELGANs with the most stable physiology were less likely than their sicker peers to have blood drawn on days 21, and 28. Consequently, selection bias probably occurred.

Finally, we are unable to distinguish between causation and association as explanations for what we found.

Strengths

With more than 555 children for whom we had day 21 measurements, we were able to identify odds ratios as low as 1.5 as statistically significant. Even though we had protein concentrations from only 449 children on both days 21 and 28, we were able to identify odds ratios as low as 1.7 as statistically significant.

Other strengths are the selection of infants based on gestational age (and not birth weight),[59] prospective collection of all data, examiners who were not aware of the medical histories of the children they examined, thereby minimizing "diagnostic suspicion bias,"[60] modest attrition, and finally, protein data of high quality [19,20], and high content validity.[17,61–63]

Conclusions

Children with an IQ in the normal range (i.e., Z-score > −2) who had elevated blood concentrations of IL-4 during both the third and fourth postnatal weeks were at increased risk of low scores on assessments of concept generation and mental flexibility (NEPSY-II Animal Sorting), perception of line orientation (NEPSY-II Arrows), listening comprehension and oral expression (OWLS), and both reading achievement and spelling (WIAT-III). Similar associations were not seen with elevated concentrations of IL-10 during these two weeks. In addition, top quartile concentrations of neither IL-4 nor IL-10 during both the third and fourth postnatal weeks were not associated with increased risk of social, language, or behavioral dysfunctions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADI-R	Autism Diagnostic Interview-Revised
ADOS-2	Autism Diagnostic Observation Schedule, Second edition
ASD	Autism Spectrum Disorder
CCC-2	Children's Communication Checklist-2 (CCC-2)
CSI-4	Child Symptom Inventory-4
DAS-II	Differential Ability Scales, Second Edition
ELGAN	Extremely Low Gestational Age Newborn
ICAM-1	Intercellular Adhesion Molecule-1 (CD54)
IL-4	Interleukin-4
IL-8	Interleukin-8 (CXCL-8)

IL-10	Interleukin-10
NEPSY-II A	Developmental NEuroPSYchological Assessment, Second Edition
OWLS	Oral and Written Language Scales, Second Edition
SCQ	Social Communication Questionnaire
SRS-2	Social Responsiveness Scale, Second Edition
WIAT-III	Wechsler Individual Achievement Test, Third Edition
WM	Working memory

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What is known

- IL-4 and IL-10 are viewed as predominantly anti-inflammatory proteins.
- Less commonly, high concentrations of IL-4 and IL-10 have been associated with adverse health outcomes.

What is not known

- We do not know to what extent elevated concentrations of IL-4 and IL-10 during the third and fourth postnatal weeks are associated with increased risk of neurocognitive, behavioral, language, and social dysfunctions among children born very preterm

What this study adds

- Children born very preterm who have elevated concentrations of IL-4, but not IL-10, on both postnatal days 21 and/or 28 are at increased risk of low scores on assessments of processing speed, visuospatial skills, listening comprehension, oral expression, and reading and spelling achievement.

Table 1.

Sample description

	Yes	No
Recruited at 10 years	966	
Seen at 10 years	889	77
DAS IQ measured	870	19
and IL-4 & IL-10 measured on day 21	661	209
and IL-4 & IL-10 measured on day 28	616	254
and IL-4 & IL-10 measured on day 21 & 28	539	331
DAS IQ measured & IQ Z-score > −2	735	135
and IL-4 & IL-10 measured on day 21	555	180
and IL-4 & IL-10 measured on day 28	521	214
and IL-4 & IL-10 measured on day 21 & 28	449	286

Table 2.

The proportion of children who did or did not have an IL-8 concentration in the top quartile (top half of table) or an ICAM-1 concentration (bottom half of table) on days 21 and/or 28 who also had a top quartile concentration of IL-4 and/or IL-10 on each of these days. These are column percents.

	Top quartile IL-8 concentration			
	Day 21		**Day 28**	
	Yes	**No**	**Yes**	**No**
Top quartile IL-4				
Day 21	**34**	22	27	25
Day 28	**34**	23	30	23
Top quartile IL-10				
Day 21	**34**	22	28	26
Day 28	**33**	24	28	24
	Top quartile **ICAM-1** concentration			
	Day 21		Day 28	
	Yes	No	Yes	No
Top quartile IL-4				
Day 21	**34**	22	**34**	23
Day 28	**33**	23	**36**	21
Top quartile IL-10				
Day 21	**36**	22	**33**	24
Day 28	**33**	24	**38**	21

Table 3.

Odds ratios (95% confidence Intervals) for low IQs and for Z-scores ≤ −1 on the remaining assessments listed on the left associated with top quartile concentrations of IL-4 and IL-10 on the days at the top of each column. All assessments listed beneath the DAS IQ listings are limited to children whose IQ Z-score was above −2. Adjustment has been made for gestational age category (23–24, 25–26, and 27 weeks), birth weight Z-score < −1, and for IL-8 concentrations in the top quartile on the relevant days.

	IL-4			IL-10		
	Day 21	**Day 28**	**Both days****	**Day 21**	**Day 28**	**Both days****
DAS-IQ						
< −2	1.6 (0.98, 2.6)	**1.7 (1.02, 2.8)**	1.5 (0.8, 2.6)	**1.7 (1.1, 2.8)**	**2.0 (1.2, 3.3)**	1.7 (0.9, 3.0)
> −2, ≤ −1	1.4 (0.9, 2.2)	0.8 (0.5, 1.3)	1.0 (0.6, 1.7)	1.3 (0.8, 2.0)	0.8 (0.5, 1.4)	0.9 (0.5, 1.7)
Maximum N	661	616	539	661	616	539
DAS-WM*	**(Z ≤ −1)**					
	1.4 (0.9, 2.2)	1.1 (0.7, 1.7)	1.3 (0.8, 2.2)	1.3 (0.8, 2.0)	1.0 (0.6, 1.6)	1.0 (0.6, 1.9)
NEPSY-II	**(Z ≤ −1)**					
Aud Attntn	0.8 (0.5, 1.3)	0.9 (0.6, 1.4)	0.8 (0.5, 1.4)	0.8 (0.5, 1.2)	1.1 (0.7, 1.7)	0.9 (0.5, 1.6)
Aud Rspns	1.0 (0.7, 1.5)	0.9 (0.6, 1.4)	0.9 (0.6, 1.5)	1.2 (0.8, 1.9)	0.9 (0.6, 1.4)	1.1 (0.6, 1.8)
Inhib Inhibtn	0.9 (0.6, 1.3)	0.9 (0.6, 1.4)	0.9 (0.6, 1.5)	0.9 (0.6, 1.3)	0.9 (0.6, 1.3)	0.9 (0.5, 1.5)
Inhib Switch	1.2 (0.8, 1.8)	1.3 (0.8, 1.9)	1.4 (0.8, 2.3)	1.5 (0.96, 2.2)	1.0 (0.7, 1.6)	1.0 (0.6, 1.7)
Animal Sort	**1.6 (1.03, 2.3)**	1.5 (0.98, 2.3)	**1.7 (1.1, 2.9)**	**1.6 (1.1, 2.5)**	1.3 (0.8, 1.9)	1.5 (0.9, 2.5)
Inhib Naming	1.3 (0.9, 1.9)	1.2 (0.8, 1.8)	1.3 (0.8, 2.2)	**1.5 (1.01, 2.2)**	0.9 (0.6, 1.4)	1.4 (0.8, 2.3)
Arrows	**1.7 (1.1, 2.5)**	1.3 (0.8, 1.9)	**1.8 (1.1, 3.1)**	**1.6 (1.1, 2.5)**	1.0 (0.6, 1.5)	1.2 (0.8, 2.2)
Geo Puzzle	1.3 (0.8, 2.0)	1.2 (0.8, 1.8)	1.2 (0.7, 2.0)	1.1 (0.7, 1.7)	1.0 (0.7, 1.6)	1.1 (0.7, 1.9)
VisMot Precs'n	1.3 (0.9, 1.9)	1.1 (0.7, 1.7)	1.3 (0.8, 2.2)	1.4 (0.9, 2.1)	1.2 (0.8, 1.8)	1.7 (0.97, 2.8)
OWLS	**(Z ≤ −1)**					
Listen Comp	**1.7 (1.1, 2.6)**	1.5 (0.98, 2.3)	**1.8 (1.1, 2.9)**	**1.7 (1.2, 2.6)**	1.4 (0.9, 2.1)	1.6 (0.95, 2.7)
Oral Exprsn	**1.9 (1.2, 2.9)**	1.5 (0.97, 2.3)	**2.1 (1.3, 3.6)**	**1.6 (1.1, 2.5)**	1.1 (0.7, 1.7)	1.6 (0.9, 2.7)
WIAT-III	**(Z ≤ −1)**					
Word Read	**1.6 (1.03, 2.6)**	1.3 (0.8, 2.1)	1.6 (0.9, 2.6)	**1.8 (1.1, 2.8)**	1.2 (0.7, 2.0)	1.3 (0.7, 2.4)
PseudoWord	**1.7 (1.1, 2.7)**	1.4 (0.9, 2.2)	**1.8 (1.1, 3.0)**	1.4 (0.9, 2.1)	1.2 (0.7, 1.9)	1.7 (0.97, 2.9)
Spelling	**2.0 (1.2, 3.2)**	1.4 (0.8, 2.3)	**2.0 (1.1, 3.6)**	**2.3 (1.4, 3.7)**	**1.8 (1.1, 3.0)**	**2.8 (1.6, 5.0)**
Numerical	1.2 (0.8, 1.9)	1.3 (0.9, 2.1)	1.5 (0.9, 2.5)	1.2 (0.8, 1.8)	1.5 (0.96, 2.3)	1.3 (0.8, 2.3)
Maximum N	555	521	449	555	521	449

*Working memory

**Protein in the highest quartile on both days 21 and 28

Table 4.

Odds ratios (95% confidence Intervals) for undesirable scores on the social, language, and behavioral assessments listed on the left among children whose IQ Z-score was above −2. Adjustment has been made for gestational age category (23–24, 25–26, and 27 weeks), birth weight Z-score < −1, and same-day IL-8 concentrations in the top quartile.

Assessment	Definition of abnormal	Top quartile IL-4		
		Day 21	Day 28	Both days*
Social awareness (SRS)	T score ≥ 60	1.2 (0.7, 1.9)	1.4 (0.9, 2.3)	1.2 (0.7, 2.2)
Social cognition (SRS)	T score ≥ 60	1.5 (0.96, 2.3)	1.4 (0.9, 2.3)	1.3 (0.8, 2.3)
Social relations (CCC)	Z-score < −1	1.3 (0.8, 2.1)	1.6 (0.96, 2.7)	1.2 (0.7, 2.3)
Social Communication Questionnaire	Screen positive	1.2 (0.6, 2.2)	1.3 (0.7, 2.4)	1.0 (0.5, 2.1)
Autism spectrum disorder	Positive	1.5 (0.6, 3.6)	**2.4 (1.04, 5.7)**	1.2 (0.4, 3.5)
ADHD (CSI-4)	Parent report	1.4 (0.9, 2.2)	1.0 (0.6, 1.7)	1.1 (0.6, 2.1)
	Teacher report	1.2 (0.7, 2.0)	1.0 (0.6, 1.8)	1.0 (0.5, 2.0)
		Top quartile IL-10		
		Day 21	Day 28	Both days*
Social awareness (SRS)	T score ≥ 60	0.9 (0.5, 1.5)	1.0 (0.6, 1.7)	0.9 (0.5, 1.6)
Social cognition (SRS)	T score ≥ 60	1.4 (0.9, 2.2)	0.9 (0.6, 1.5)	1.0 (0.6, 1.8)
Social relations (CCC)	Z-score < −1	1.1 (0.7, 1.9)	1.5 (0.9, 2.6)	1.2 (0.7, 2.3)
Social Communication Questionnaire	Positive screen	1.1 (0.6, 2.1)	1.0 (0.5, 1.9)	1.1 (0.5, 2.3)
Autism spectrum disorder	Positive	1.2 (0.5, 3.1)	2.1 (0.9, 5.0)	1.8 (0.7, 5.0)
ADHD (CSI-4)	Parent report	1.0 (0.6, 1.7)	1.0 (0.6, 1.8)	1.0 (0.5, 1.9)
	Teacher report	1.0 (0.6, 1.8)	1.0 (0.6, 1.9)	1.1 (0.5, 2.1)
Maximum column N		555	521	449

*Bob's ASD paper describes diagnostic process

**Gross motor function classification system