The Urban Environment and Childhood Asthma Study

James E. Gern, MD
Professor of Pediatrics and Medicine, University of Wisconsin-Madison

Abstract

Childhood asthma is not distributed evenly throughout the population, and children who grow up in crowded urban neighborhoods have higher rates of asthma and experience greater morbidity due to asthma. There are several environmental and lifestyle factors associated with urban living that are suspected to promote the development of asthma, particularly in the first few years of life. Collectively, this information suggests the hypothesis that exposure in early life to adverse environmental and lifestyle factors associated with disadvantaged urban environments modifies immune development to increase the risk for allergic diseases and asthma. The Urban Environment and Childhood Asthma birth cohort study was initiated in 2004 to test this hypothesis. The study population was recruited prenatally, and consisted of 560 families from four urban areas who were at high risk for allergies and/or asthma on the basis of parental histories, along with an additional 49 families without atopic parents. Immune development, respiratory illnesses, and exposure to stress, indoor pollutants, microbial products, and allergens were measured prospectively, and the major study outcomes are recurrent wheeze at three years of age and asthma at age seven. This review summarizes the study design, methods, and early findings of the URECA study.

Introduction

Most asthma begins in childhood, and a number of environmental and lifestyle factors are thought to contribute to the onset of asthma. These factors include outdoor pollutants, including ozone and diesel exhaust, and indoor pollutants such as tobacco smoke and NO_2. A number of recent studies have focused on the hygiene hypothesis, and the latest iteration of this theory proposes that exposure to farm animals, pets, and nonpasteurized milk or fermented beverages may promote healthy development of the immune system to reduce rates of allergic diseases and asthma.[1] On the other hand, lack of exposure could lead to a Th2-bias in immune responses, allergic sensitization, and asthma. Lifestyle and nutrition may also be important for lung and immune development, and several studies suggest that nutrients (e.g. omega-3 fatty acids, vitamin D) and consumption of fruits and vegetables protect against asthma, while obesity and lack of exercise could have the opposite effect.[2-4] Virus-induced wheezing episodes in the first 2-3 years of life are a strong risk factor for asthma, particularly in children with other atopic features such as allergic sensitization or atopic dermatitis.[5] Whether these episodes contribute to asthma causation or instead reveal an underlying predisposition to asthma has not yet been established. Finally, other factors

that have been linked to the development of wheezing and asthma include genetics, ethnicity, month of birth, and exposure to high levels of stress.[6-8]

It is expected that diseases with a strong environmental component would not be evenly distributed throughout the population, and this is the case for asthma. The ISAAC study has documented over 30-fold differences in the local prevalence of childhood wheezing and asthma on a global scale,[9] and although there is less information about the distribution of asthma within the US population, the same appears to be true.[10] Asthma in the US appears to more frequent in ethnic minorities and in children growing up in poor urban neighborhoods, and is least common in rural areas in combination with farm animal exposure.[11] Notably, asthma morbidity follows a similar pattern.

These findings raise questions about the reasons for increased asthma in densely populated urban areas. What are the specific factors or combinations of factors that lead to asthma in American inner cities? Considering the list of asthma risk factors, the urban environment has a number of features that could have adverse effects on children's respiratory health, especially during the first few years of life when the lung and immune system are rapidly developing. It is possible that asthma risk is high because of exposure to adverse conditions, such as pollutants, cockroach or mouse allergens, stress, or the development of obesity. Conversely, it is also possible that children who grow up in an urban environment lack exposures or experiences that are necessary for healthy lung and immune development. Potential examples include reduced exercise, less availability of nutrients such as vitamin D, or low exposure to beneficial microbes that promote normal immune development. Although the urban environment is not usually considered hygienic, it may in fact be deficient in dirt (soil)!

To begin to identify environmental and lifestyle factors related to asthma causation in economically disadvantaged neighborhoods in large US cities, the Inner City Asthma Consortium initiated the Urban Environment and Childhood Asthma (URECA) study in 2004.[12] The study hypothesis, design, and early findings from this observational birth cohort study are described in the following sections.

Study hypothesis and outcomes

The study hypothesis was based on the concept that postnatal lung and immune system development occur most rapidly in utero and during the first few years of life, and during this period of time, may be especially susceptible to environmental influences.[13] Furthermore, specific patterns of immune development in early life, such as enhanced Th2-like cytokines and reduced interferon responses, affect the risk of developing atopic diseases, wheezing illnesses, and asthma.[14,15] The URECA study has a two-stage hypothesis (Figure 1). First, the unique environmental exposures in the inner city interact with genetic factors during the prenatal and postnatal periods to adversely influence the development of innate and adaptive immunity, which in turn increases the risk for allergic sensitization and atopic diseases. Second, immune dysregulation in infancy increases the risk of developing lower respiratory infections caused by viruses and perhaps atypical bacteria. Those infections cause airway inflammation and structural changes during a particularly vulnerable period of lung development, leading to an increased risk of asthma by age 7 years.

The primary objective of URECA is to identify in inner-city children the immunologic risk factors for the development of recurrent wheeze by age 3 years and asthma by age 7 years. As an indicator of immune development, blood mononuclear cells from each infant are stimulated ex vivo yearly beginning with cells from cord blood to measure cytokine responses. Patterns of cytokine responses over time will be compared between children with

and without recurrent wheeze at age 3 years and with and without asthma at age 7 years. The two main secondary objectives are to identify environmental exposures that modify the developmental pattern of cytokine responses, and to identify the immunologic correlates of the development of atopic features such as total IgE, allergic sensitization, and atopic dermatitis.

Study population

To obtain sufficient power to test the main hypothesis, 500 subjects were needed, and 560 were recruited, from four study sites: Baltimore, Boston, New York, and St. Louis. The entry criteria specified that the families had to live in neighborhoods where at least 20% of the population had income below the poverty line, and one of the parents had allergic diseases or asthma. The participants were recruited prenatally, and to be study eligible had to be born at 34 weeks gestation or later and without significant respiratory problems in the neonatal nursery. The goal was to recruit a high risk population with as few restrictions as possible, while excluding children who had other respiratory conditions (e.g. hyaline membrane disease, bronchopulmonary dysplasia) that would confound the diagnosis of asthma.

Some studies have shown that babies of atopic vs. nonatopic families have distinct patterns of blood mononuclear cell cytokine responses at the time of birth.[16] With this in mind, a second group of babies born to non-atopic parents were enrolled during the latter stages of the study. The goal was to enroll 50-60 families and 49 were enrolled; the sample size was based on power calculations to enable detection of a 50% reduction in interferon responses in the atopic families, as had been suggested by previous studies.

Both study groups are predominantly ethnic minority. Of the mothers in the allergic cohort, 71% are black and 19% are Hispanic (predominantly of Dominican and Puerto Rican heritage), and the nonatopic cohort has similar demographics. The ethnicity of this population, together with high rates of exposure to stress, tobacco smoke, cockroaches, and poor housing conditions and low socioeconomic status represent many of the potential risk factors for allergies and asthma in the US.

Study procedures

Overview

The URECA protocol was approved by human subjects committees at each of the study centers. Following informed consent, a series of questionnaires were administered to the mother at the prenatal visit (Table I). After birth, the child's mother responds to quarterly telephone questionnaires to assess the child's respiratory and allergy symptoms, medications, tobacco smoke exposure, and diet. Stress-related questionnaires were administered to the mother at the prenatal visit and are re-administered at selected annual clinic visits. Each year, the child visits the study site for a physical examination, eczema assessment, and a blood sample. A sample of the mother's blood was collected at the child's 12-month visit. The child undergoes allergy skin testing at selected visits, and annual pulmonary function testing begins at age 3 years. Each year, URECA staff visit the child's home to collect settled dust samples for analysis of allergens and selected microbial products. Airborne nicotine and nitrogen dioxide (NO_2) are measured in the homes at month 3, and at age 4 and 6 years. A home environment questionnaire is administered at month 3, and annually thereafter. The goal of the periodic environmental sampling is to provide data for estimating longitudinal exposures for the individual study participants.

In addition to these scheduled activities, mothers are instructed to contact URECA staff whenever their children experience respiratory symptoms, such as rhinorrhea, cough, or wheezing. Nasal lavage specimens are collected for moderate colds or worse, as assessed by a respiratory symptom score card based on the questionnaire used in the Childhood Origins of Asthma (COAST) study.[17]

Cytokine secretion assays

In designing this multicenter study, one of the first tasks was to design cytokine secretion assays using peripheral blood mononuclear cells as an indicator of immune development in children. A panel of stimulants was selected to elicit innate, adaptive, and antiviral responses, along with mitogenic stimuli to induce polyclonal T cell responses (Table II). Two cytokine panels were also designed to correspond with the innate and adaptive stimuli.

Previous multicenter studies had used different protocols to isolate mononuclear cells from blood specimens; sometimes fresh cells had been used, and in other studies cells were cryopreserved so that the cells could be sent to a central laboratory for processing. Since technical details of mononuclear cell stimulation protocols and outcome measures vary widely, the URECA group conducted preliminary experiments to determine the optimal conditions for our four-center study. In these experiments, cryopreservation vs. use of fresh cells changed patterns of cytokine secretion, and did not improve reproducibility.[18] As a result, processing of fresh mononuclear cells was selected for the URECA study. These preliminary studies underline the need to test the performance characteristics of immunologic assays before their adoption into a clinical protocol.

Viral diagnostics

Recurrent bouts of virus-induced wheezing in infancy signal an increased risk of developing asthma, and the viral etiology of these wheezing illnesses appears to modify the degree of risk. Results from two birth cohort studies suggest that children who wheeze with rhinoviruses (HRV) may be at especially high risk for asthma, especially if there is concurrent atopy.[19,20] Viruses in urban environments could differ significantly from those reported in other populations, and this could affect the subsequent risk of asthma. Given the large sample size and repeated sampling that was expected in this study, URECA investigators worked with a biotechnology company (EraGen Biosciences, Madison, WI) to develop a high throughput multiplex PCR-based system that detects all common respiratory viruses.[21] This system has been validated by comparison with standard diagnostic techniques, and like other PCR-based systems, greatly improves detection of respiratory viruses that are difficult to grow in tissue culture (e.g. HRV, bocaviruses, metapneumoviruses). Furthermore, this technique, when paired with partial sequencing of viral genomes, has been used successfully to identify additional members of the newly discovered HRV-C species, which so far have only been detected with molecular techniques.[22]

Preliminary Results

Maternal and prenatal influences on cytokine responses at birth

Prenatal exposures and maternal characteristics can affect immune development, and these effects are measurable in cord blood mononuclear cells.[16,23,24] To test for these relationships in the URECA population, several selected maternal, perinatal, and newborn characteristics were examined as predictors of cytokine responses in the URECA newborns. [25] The predictors with the most associations with innate immune responses included season of birth, ethnicity, birth weight/gestational age (which are highly correlated), and maternal asthma/use of inhaled corticosteroids. Although cytokine responses to protein antigens were

low, it was nevertheless possible to evaluate associations between maternal and child characteristics and antigen-induced responses in cord blood cells.

Over half of the cytokine responses, including both innate and adaptive responses, varied by season of birth. There were up to 3-fold fluctuations in specific IFN-α and IFN-γ responses, and antigen-induced IFN-γ responses were enhanced during the winter months (Figure 2). Ethnicity, which in this study was largely a comparison between babies of black vs. Hispanic backgrounds, affected a variety of innate, but not antigen-induced, cytokine responses. Birth weight was inversely associated with IFN-γ responses to RSV ($R = -0.16$), but positively associated with IL-8 responses to a variety of innate stimuli ($R = 0.08$-0.12). Interestingly, children of mothers with asthma had generally lower RSV–induced cytokine responses. This finding suggests that babies born to mothers with asthma could have impaired antiviral responses, perhaps increasing the risk of virus-induced wheeze. Finally, cytokine responses of the 49 babies from non-atopic families were compared to an equal number of babies from allergic families, matched for season of birth and study center. Cytokine responses were generally lower in babies born to parents with allergy/asthma, and this was most pronounced for IL-12p40 and IL-8 responses.

The results of this study provide evidence that the variability of newborn immune responses is not random, and, that development of the fetal immune system during the perinatal and postnatal periods appears to be responsive to maternal characteristics and experiences. Many of the associations between environmental predictors and cytokine responses were of relatively low magnitude; however, from an immunoepidemiologic point of view, such correlations could have biologic significance. Our findings also suggest that newborns of parents with allergy/asthma could differently to postnatal environmental experiences. This maternal influence may be partially mediated by genetics; however, seasonal variations suggest that either environmental or epigenetic mechanisms also affect the developing immune system. This cohort of infants will be analyzed to determine the long-term effect of these maternal-fetal interactions on immune development, recurrent wheezing (age 3 years), and asthma (age 7 years).

Ancillary studies

In addition to the main outcomes of the study, the prospective cohort study design provided an opportunity to address several other scientific questions related to the development of allergies and asthma. A collaboration was established with researchers in the Channing Laboratory at Harvard University to determine whether deficiencies in CD4+ T regulatory cell number or function increased the risk for asthma in the infants enrolled at the Boston URECA site. Early findings in this study show that CD4+ T cells from the newborns vs. mothers had similar expression of Foxp3, but cells from newborns had lower suppressive function.[26] In addition, investigators at Washington University performed prospective analyses of selected antiviral responses to determine whether deficient antiviral signaling or interferon responses predispose to recurrent wheezing and more severe viral infections. Finally, all URECA participants undergo yearly testing for obesity, consisting of bioelectrical impedance analysis to estimate percent body fat, anthropomorphic measurements, and immunologic tests to assess potential evidence of systemic inflammation associated with overweight status.

Conclusions

Birth cohort studies have been a rich source of information about factors that influence the development of asthma, including viral infections, environmental factors, immune development, and wheezing phenotypes in early life. In the USA, asthma incidence and morbidity are greater in urban areas, and yet there is relatively little information about the

relationship between early life exposures and asthma in this high-risk environment. The goal of the URECA birth cohort study is to identify mechanisms by which urban-specific lifestyle and environmental factors modify immune development in early life, and the subsequent risk of asthma. There are a number of potential explanations for the increased incidence of asthma and increased morbidity due to this disease in large urban areas. In theory, it is possible to improve adverse urban environmental or social conditions to prevent asthma, but due to the magnitude of the problems this is unlikely to occur without definitive information to link specific exposures or lifestyle factors to asthma onset. The design and population of the URECA study are well suited to identify relationships between urban exposures or lifestyles and childhood asthma.

Acknowledgments

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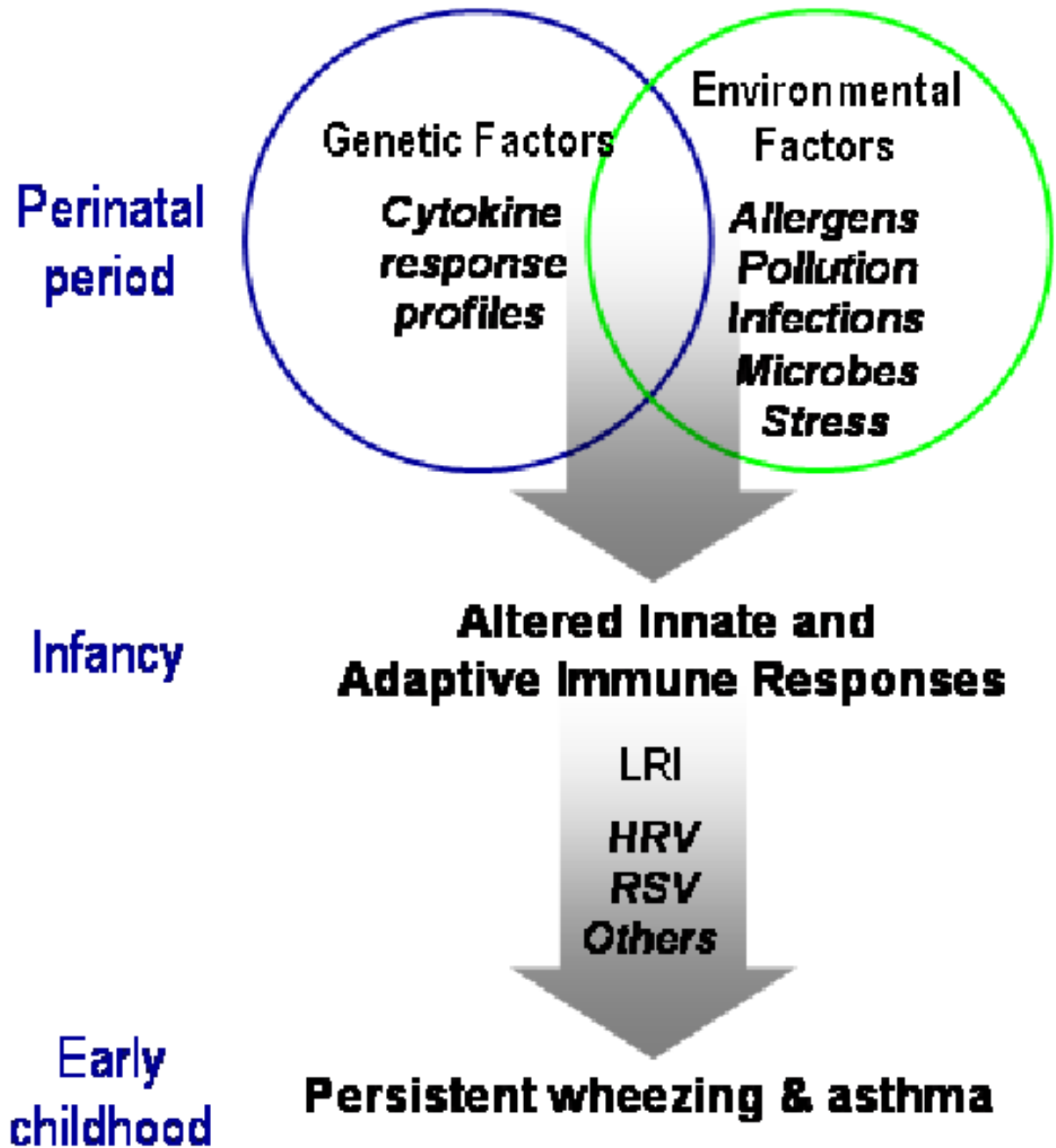
Figure 1.
Factors influencing the onset of asthma in urban settings (from [reference 12]). Abbreviations: LRI, lower respiratory infection; HRV, human rhinoviruses; RSV, respiratory syncytial virus.
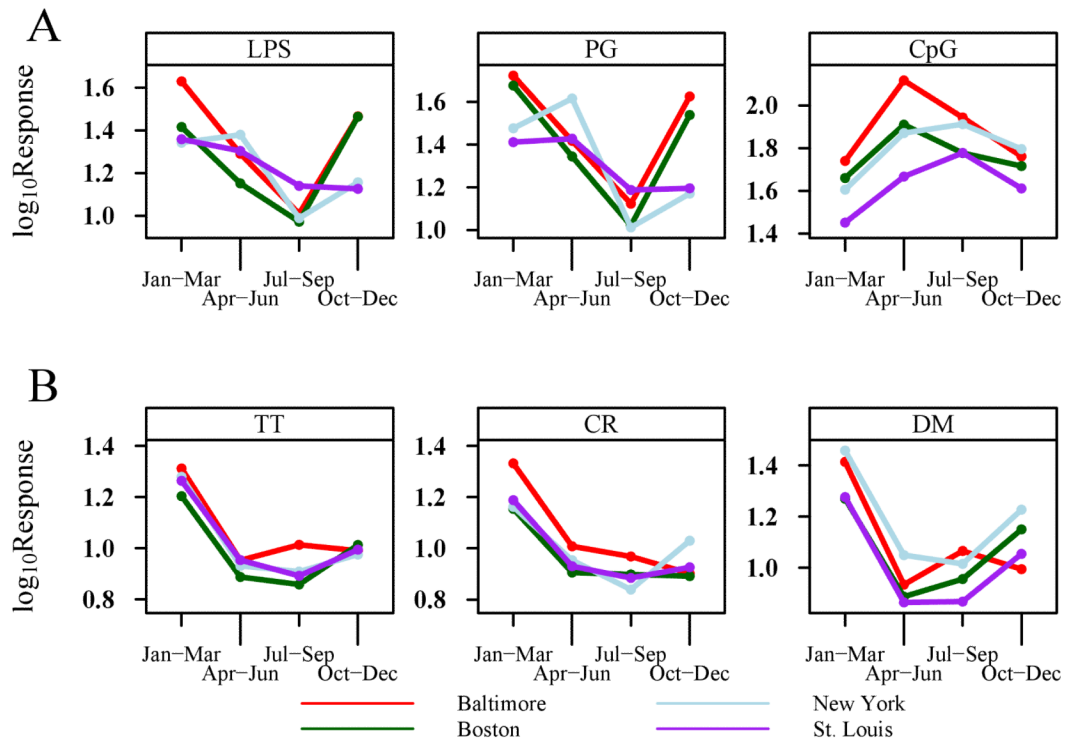
Figure 2.
Seasonal patterns of selected cytokine mean responses. A, IFN-α responses to LPS, peptidoglycan (PG), and CpG according to season (3-month intervals). B, IFN-γ responses to cockroach (CR) and dust mite (DM) extracts and tetanus toxoid (TT). Seasonal patterns from the 4 individual study sites are depicted by the colored lines. The depicted cytokine mean responses are those with the strongest seasonality patterns. All figures demonstrate a statistically significant seasonal effect, with each panel having P < .001 (from [reference 25]).

Table 1

Urban Environment and Childhood Asthma (URECA) Study Overview.

	Ongoing Visits					Clinic Visits (number represents child's age in months)								
	PN	D	3	QC	HV	12	24	33	36	48	60	72	81	84
Questionnaires	x		x	x		x	x	x	x	x	x	x	x	x
Respiratory Illness Score Card					As often as illness is reported									
Study Procedures														
Cord blood sample		x												
Maternal blood sample						x								
Child blood sample			x			x	x		x	x	x	x	x	
Eczema evaluation			x			x	x		x	x	x	x		x
Physical examination						x	x		x	x	x	x		x
Nasal lavage sample*						x			x		x			x
Allergen skin testing								x			x			x
Lung function testing									x	x	x	x		x
Bronchodilator reversibility														x
Methacholine challenge													x	
Lung volume (plethysmography)													x	
Exhaled nitric oxide													x	
Bioelectrical impedance analysis						x	x		x	x	x	x		x
Home Environment Assessment														
Dust sample collection			x		x									
Airborne nicotine and NO$_2$			x		x†									

Notes: PN=prenatal visit; D=delivery; 3= home visit at age 3 months; QC=quarterly calls (occurring at 6, 9, 15, 18, 21, 27, 30, 39, 42, 45, 51, 54, 57, 63, 66, 69, 75, 78, and 81 months);

HV=home visits occur once between each yearly clinic visit, except between 5 and 6 years of age.

*
Also collected during respiratory illnesses

†
Collected at 3 months, and at age 4 and 6 years.

Table II

Stimulants used and cytokines measured in the mononuclear cell assays.[12]

Innate Immune Responses		Adaptive Immune Responses	
Stimulants	**Cytokines**	**Stimulants**	**Cytokines**
Lipopolysaccharide	IFN-α	Phytohemagglutinin	IFN-γ
Polyinosinic-polycytidylic acid	IFN-γ	Cockroach extract	IL-10
Peptidoglycan	IL-10	Dust mite (*D. pteronyssinus*) extract	IL-13
CpG	IL-12p40	Tetanus toxoid	IL-4
Respiratory syncytial virus	TNF-α	CD3 + CD28 Mab[*]	IL-5[†]
Rhinovirus[*]	IL-8	Medium alone	
Medium alone			

[*] Stimulation not conducted on cells from the umbilical cord samples

[†] Not measured in umbilical cord samples