Maternal pregnancy and postnatal factors affecting immune development and risk of childhood acute lymphoblastic leukemia

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Abstract
Despite the challenges of studying the epidemiology of a rare disease, the last couple decades have uncovered tremendous knowledge about the probable causes of childhood leukemia, which currently suggests an important role for exposures that influence a child’s immune development and maturation in early life. Advances in our knowledge of fetal immune development and how it is affected by maternal exposures and in utero conditions provide reason to renew our interests in the pregnancy period for pursuing immunological hypotheses in childhood leukemia etiology. This review provides a summary of the epidemiological evidence on immune-related exposures in childhood leukemia risk and places them in the context of known mechanisms for fetal immune development and postnatal immune modulation.

Key words: childhood leukemia, immune development, epidemiology, infection, pregnancy

Introduction
Leukemia is the most common cancer in children with an annual incidence in Japan of about 32 diagnoses per million children under age 20 years. This equates to about 700 newly affected children per year in Japan. Despite vast improvements in treatment represented by about a combined 80–90% survival rate across the different leukemia subtypes, progress in understanding its causes has been slow, thus delaying efforts for prevention. Within the major subtypes of childhood leukemia defined by cell lineage, acute lymphoblastic leukemia (ALL) is most common comprising nearly 80% of diagnoses in developed countries, followed by acute myeloid leukemia (16%) and other rare subtypes. Childhood leukemia incidence varies markedly by age in developed countries primarily due to a sharp peak in ALL incidence among children aged 2 to 5 years. This incidence peak characteristic of ALL is not observed in childhood AML patients, where incidence appears relatively stable across age groups.

Current evidence supports a two-hit disease model for the natural history of childhood leukemia, particularly pertaining to the predominate subtype, precursor B cell ALL. This model explains that a minimum of two genetic events or “hits” are necessary for leukemia onset, the first of which probably occurs in utero producing a pre-leukemic clone, followed by a second genetic “hit” during the postnatal period. Through the pioneering work by Greaves, Wiemels, and colleagues conducted among concordant twin childhood leukemia patients, and with genetic “backtracking” analyses using archived newborn血 specimens, substantial evidence is now available that show a prenatal origin for several chromosomal abnormalities seen in childhood leukemia, including t(12;21) ETV6-RUNX1, 11q23 MLL-AF4, t(8;21) AML1-ETO, t(15;17) PML-RARA, inv(16) CPFB-MYH11, and hyperdiploidy. This conclusion is consistent with recent studies showing that translocations such as ETV6-RUNX1 may be up to 100-fold more common in the population than that observed in childhood leukemia. Only a very small proportion of children carrying the translocation will advance to overt leukemia, which is assumed to be determined by the occurrence of a sufficient postnatal event.

Established risk factors for childhood ALL includes sex, age, race, prenatal exposure to ionizing radiation in utero, postnatal high-dose radiation, chemotherapeutic agents, and several genetic syndromes, but these together account for only a small estimated proportion of childhood ALL cases diagnosed. Exemplified by several recent publications of pooled analyses by the Childhood Leukemia International Consortium (CLIC), a broad range of etiological hypotheses have been previously explored including the role of parental occupational exposures, parental smoking, air pollution, household solvents, pesticides, non-ionizing radiation, maternal and child dietary factors, allergies,
infectious agents\textsuperscript{21,22}, maternal reproductive factors\textsuperscript{23}, and genetic susceptibility\textsuperscript{24}.

Among the various evaluated risk factors, considerable evidence of a probable role for exposures that influence immune development through contact with infectious agents in infancy is beginning to emerge. Interestingly, evidence has also shown that children who developed leukemia had lower levels of IL10 cytokines in newborn samples compared to control newborn samples\textsuperscript{25}, suggesting that immune dysregulation may be initiated prenatally and indicating a potential role for the mothers’ exposures in influencing the immunologic status of the newborn and subsequent leukemia risk.

This review provides an overview of the current evidence supporting a role for early postnatal infection-driven immune modulation and risk of childhood ALL. In addition, this review will describe a rationale for the importance of fetal immune development and maternal exposures during pregnancy that influence it when considering causal risk factors for childhood ALL.

**Postnatal Immune Modulation**

The neonatal immune system is significantly down-regulated and proceeds along a series of postnatal developmental stages, much of which is mediated by environmental exposures during the first year of life. Among the most important and frequently studied characteristics of the neonatal immune system are the presence of high amounts of regulatory T cells and the down-regulated CD4+ Th1 and Th2 activities that display a strong skewing toward Th2 responses\textsuperscript{26}.

A critical element of immune maturation during the first years of life is to increase the functional capacity of Th1, which is necessary for effective cell-mediated immunity, anti-tumor defense and progression towards a normal immune balance. Various endogenous conditions and environmental exposures ranging from dietary factors to chemical and microbial agents have been shown to influence this Th1/Th2 balance both positively and negatively during immune maturation. However, microbial exposures have been shown to be among the most potent and effective stimuli in promoting Th1 capacity\textsuperscript{27}. Capacity for T regulatory cell activity is also stimulated by early life microbial exposures and has the ability to enhance the regulatory networks that are responsible for down-regulating immune responses after infection is controlled\textsuperscript{28}. A reduced T regulatory cell function can contribute to unfavorable hyperactivity of the Th1, Th2, and Th17 segments of the immune system upon exposure to an immune challenge. Taken together, extensive research seems to indicate that proper immune modulation, largely through exposures to infections early in life, may have a significant impact on later life responses to immunologic stimuli and risk for disease.

**Delayed Infection Hypothesis and Childhood ALL**

Descriptive evidence has provided clues about the causes of childhood leukemia\textsuperscript{29}. Firstly, childhood ALL has a peak incidence at age 2 to 5 years, a time when children begin formal schooling and increase their exposure to infectious antigens. Secondly, this peculiar age distribution for childhood ALL is only observed in the more economically developed regions of the world often associated with improved hygienic environments. These observations were instrumental in developing the “delayed infection” hypothesis in 1989 which describes that the lack of sufficient immune priming of the child in early life, followed by a normal pattern of subsequent infectious exposures starting from pre-school age (2–5 years), may lead to an adverse immunologic response that contributes to ALL risk (Figure 1)\textsuperscript{4}.

Establishing a role for infection in childhood leukemia etiology has been challenging mainly due to the unsuccessful attempts in identifying the causal agent(s)\textsuperscript{30} and difficulties in directly quan-
tifying a child’s exposure and/or response to infections. Evidence to date in support of the delayed infection hypothesis originates from a substantial body of literature based on surrogate measures of exposure to infections such as birth order, child’s history of infections, and child’s day care and play group attendance. Exposure to infections in early childhood can originate from a number of sources. A surrogate measure used to test the delayed infection hypothesis should accurately represent the child’s primary source of infectious exposures during early childhood, especially during the first year of life. The occurrence of common infectious diseases in early childhood is strongly associated with the frequency of the child’s social contacts with other children. For this reason, higher birth order through contact at birth, or shortly after, with potentially infected older siblings is considered a useful surrogate measure of early life exposures to infections. Similarly, social contacts through day care attendance or activities in other similar types of settings are widely accepted as strong predictors of a child’s early exposure to infections in developed countries. The transmission of infectious agents is believed to be promoted through this type of social setting due to the immaturity of children’s immune systems in combination with the lack of appropriate hygienic behavior. Previous studies have consistently shown day care attendance to be associated with an increased risk of infectious diseases in children, particular those of the respiratory and gastrointestinal tracts.

While some inconsistencies in results are observed across studies, the cumulative evidence appears to be supportive of the delayed infection hypothesis where reduced risk of childhood ALL is associated with surrogate measures of early life immunomodulatory exposures including infections through day care attendance and higher birth order, breastfeeding, and vaccination. A recent pooled analysis performed through CLIC comprising over 6,000 ALL cases and 10,000 controls across 10 case-control studies showed a significantly reduced risk of childhood ALL associated with day care attendance during infancy. Interestingly, a trend for a greater reduction in risk with younger age at start of daycare was observed, consistent with the delayed infection hypothesis. Having an older sibling and breastfeeding for greater than six months were also associated with a reduction in risk.

The Developing Fetal and Neonatal Immune System

Chronic inflammatory and immune disorders have been linked epidemiologically to perinatal exposures and indicators of aberrant early life immune development. Mechanisms explaining this relationship are still actively being explored, but these observations indicate that factors affecting the developmental process of the immune system, which is initiated as early as the first trimester of pregnancy, are likely important.

From the fourth week of gestation, the first innate immunity cells, including monocytes/macrophage, can be found in low numbers, followed by the production of granulocytes and natural killers (NK) cells initiated a few weeks later. Precursors for B and T cells of the adaptive immune system are also detected from about eight weeks gestation with gradual maturation into differentiated states over time. Overall, although present from early fetal development, effector function of the immune cells are poorly developed in order to encourage maternal-fetal tolerance.

While it is known that postnatal stimuli can determine a range of trajectories for immune maturation, there is emerging evidence to suggest that discrete immune cell/maturation pathways from the fetal stage as well can be influenced by specific maternal in utero conditions and exposures. Thus, from an etiological perspective for diseases believed to involve immune maturation pathways, such as childhood leukemia, consideration of maternal pregnancy conditions and exposures is likely to be important.

Role of Maternal Pregnancy Factors in Fetal Immune Development and Disease Risk

Biological plausibility is supported largely by the fact that there is constant fetal-maternal crosstalk via the placenta throughout pregnancy. A range of maternal factors and exposures have a variety of hypothesized routes for affecting disease risk in the offspring. Based on current understanding derived from the scientific literature, those that may be related to childhood ALL through mechanisms of inhibited fetal immune development include maternal malnutrition, obesity, stress, smoking, and microbial exposures.

Malnutrition during pregnancy can cause fetal growth restriction, preterm birth, as well as obstruct fetal immune maturation processes. Vitamins A and D are strong immune-modulators that support formation of secondary lymphoid organs and enhance the suppressive capacity of T regulatory cells, respectively. Maternal deficiency in zinc has been associated with impair T and B cell activity and reduced IgG levels in the fetus. Maternal over-nutrition and obesity may also negatively impact the fetal immune system where studies have shown associations of increased risk of inflammatory and metabolic diseases in the offspring. Mechanistic insight originating from studies in mice have shown decreased lymphocyte counts and reduced antigen-specific antibody production in pups born to obese mothers. In a recent large records-based California statewide study of childhood cancers, children born to mothers who were...
overweight prior to pregnancy had an increased risk of childhood leukemia\(^\text{x20}\). However, studies evaluating this associations are still scarce.

Epidemiological associations have been reported that show maternal stress and anxiety during pregnancy and increased risk of asthma, allergy\(^\text{x25}\), and compromised adaptive immune responses in the offspring\(^\text{x26}\). These observational findings are supported by animal models which demonstrated immunosuppressive effects of maternal stress on the offspring by modifying lymphocyte effector function and reducing NK cell cytotoxicity\(^\text{x27}\). A plausible mechanism for these associations may be through transfer of maternal stress hormones, which are known to have immune-modulatory functions, via the placenta to the fetus. There is a need for studies to begin exploring this hypothesis in childhood leukemia studies; however, as retrospective studies are the most feasible study design for rare diseases like childhood leukemia, ensuring valid assessment of stress levels during pregnancy is a challenge.

In addition to being linked to lung development in the fetus, maternal smoking during pregnancy has been associated with chronic inflammatory diseases in the offspring. This relationship is thought to be facilitated through a reduction in fetal T regulatory cell production and inhibited innate immunity by altering responsiveness to Toll-like receptor ligands\(^\text{x28,29}\). Smoking during pregnancy and childhood leukemia risk has been the topic of investigation of several studies, but findings have been inconsistent. Several studies have reported increased risks associated with light smoking, but an unexpected reduction in risk was found to be associated with heavy smoking\(^\text{x30}\). It is suspected that this non-linear association may be due to competing risks, such as miscarriage or fetal birth defects, at higher levels of smoking\(^\text{x31}\).

Maternal infection during pregnancy has the potential to affect the fetus and birth outcomes in various ways depending on the type of infection and whether it is localized or has entered the maternal bloodstream. Transmission to the fetus resulting in the stimulation of fetal/placental inflammatory pathways may lead to fetal growth restriction, spontaneous abortion, and/or induction of labor\(^\text{x32}\). Another line of evidence suggests that exposures to the diverse microbial repertoire through living on a farm during pregnancy may confer protection against immunological conditions such as asthma and allergies in the offspring\(^\text{x33}\). A previous study observed higher T regulatory cell numbers and decreased Th2 immune responses in cord bloods of neonates born to farming mothers. The magnitude of immunological changes seen in the cord blood corresponded to the amount of farm animals to which mothers were exposed during pregnancy\(^\text{x34,35}\). Previous studies have also shown evidence of a role for maternal infections in the risk of childhood leukemia. In a nested case-control study that measured maternal first trimester serum antibodies for Chlamydia, Helicobacter pylori, and Mycoplasma pneumoniae, statistically significant increased risk of leukemia in the offspring were observed for M. pneumoniae IgM and H. pylori IgG levels\(^\text{x36}\). Another study using questionnaire to assess maternal illnesses during pregnancy observed an increased risk of childhood ALL in the offspring associated with history of influenza/pneumonia\(^\text{x37}\).

**Concluding Remarks**

As an early onset disease and evidence that the first genetic events occur before birth, the suspicion that maternal factors and exposures during pregnancy may affect childhood leukemia risk is not unreasonable, nor is it a new concept. Previous studies have observed a role for maternal factors, however, the mechanisms responsible for the associations are not clear and are difficult to elucidate within the possibilities of epidemiological approaches. Emerging concepts and innovative work in understanding the *Developmental Origins of Health and Disease* (DOHaD) indicate that preconception and *in utero* circumstances can influence fetal developmental processes that may, in turn, have long-term effects on the individual even into adulthood\(^\text{x38}\). As an extension to the current immunological hypothesis in childhood ALL (delayed infection hypothesis) focused on the role of postnatal mechanisms, it is possible that developmental influences on the immune system from the fetal period may be important as well. This is particularly supported by previous evidence that showed altered IL10 cytokine profiles at birth in children who developed leukemia\(^\text{x39}\), and risk associations with maternal factors known to modify fetal immune processes. Regarding the natural history of childhood leukemia, whether these *in utero* exposures contribute to the first genetic event effectively producing a pre-leukemia clone or serve as effect modifiers to postnatal exposures that drive the second genetic event, epidemiological findings can be speculative at best.

Another indicator of the *in utero* environment that may be important is maternal genetics. It is known that our genetic composition is responsible for inter-individual variation in the functional capacity of various biological processes, some of which are likely important in determining the environment of the developing fetus. Thus, it is conceivable that maternal genetic factors may affect the risk for childhood leukemia in the offspring independent of, or jointly with, the effect of the child’s genetics. Very few studies have yet explored this possibility. In a rare example from a recent report, a family-based study comprising case-parent trios were genotyped genome-wide for exome single nucleotide
polymorphisms. Using a methodology similar to the log-linear analysis approach, they identified a few loci that showed suggestion of an independent effect of maternal genotypes on childhood ALL risk. More research in this area is warranted.

The interpretation of research findings as true scientific evidence depends on several factors, but among them are criteria for ensuring adequate internal validity and consistency of findings. This is particularly important in epidemiological studies that take observational approaches since there is greater inherent tendency for bias and confounding compared to experimental approaches. While childhood leukemia epidemiology has been pursued for decades, over time, methodology has improved, and the experience of previous studies have informed subsequent studies in terms of the most timely hypotheses and areas for methodologic improvements. What is evident from the examination of the collection of previous work is an obvious lack of comprehensive studies from regions of the world outside of the United States and Europe. With potentially different prevalence of exposures, lifestyle factors, and genetic structure, greater representation from a larger diversity of populations may be highly informative to help bring additional evidence and consistency to the effort of understanding the causes of childhood leukemia.

The rarity of this devastating disease makes it almost methodologically infeasible for any single study to pursue a prospective cohort approach without extensive collaboration as pursued by the International Childhood Cancer Cohort Consortium. Thus, the vast majority of previous investigations originate from case-control studies, most of which are currently a member of the CLIC; notably however, no studies are from an Asian country. In Japan, we have initiated a new study called the Epidemiological Study of Hematological Cancers in Children (Epi-HCC), a case-control study of childhood leukemia comprising DNA sample collection from children and their parents and a comprehensive questionnaire to primarily examine the role of, 1) maternal pregnancy factors and exposures, 2) early postnatal immune-related exposures of the child, and 3) maternal genetics, on the risk of leukemia in the offspring. Patients are recruited through a large network of major clinical centers located in Tokyo and surrounding areas, and controls are individually matched on age and gender randomly selected from the resident registration system. As Japan has a long practice of using a maternal and child health handbook to record detailed information on prenatal care, delivery, and early postnatal conditions of the child, this study has a unique opportunity to contribute high-quality findings to help establish greater consensus, and potentially contribute new evidence for hypotheses that are currently understudied.

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