Developmental Immunotoxicity, Perinatal Programming, and Non-Communicable Diseases: Focus on Human Studies

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Abstract

Developmental immunotoxicity (DIT) is a term given to encompass the environmentally-induced disruption of normal immune development resulting in adverse outcomes. A myriad of chemical, physical and psychological factors can all contribute to DIT. As a core component of the developmental origins of adult disease, DIT is interlinked with three important concepts surrounding health risks across a lifetime: 1) the Barker Hypothesis, which connects prenatal development to later life diseases, 2) the Hygiene Hypothesis, which connects newborns and infants to risk of later-life diseases and, 3) fetal programming and epigenetic alterations, which may exert effects both in later life and across future generations. This review of DIT considers: 1) the history and context of DIT research, 2) the fundamental features of DIT, 3) the emerging role of DIT in risk of non-communicable diseases (NCDs) and 4) the range of risk factors that have been investigated though human research. The emphasis on the human DIT-related literature is significant since most prior reviews of DIT have largely focused on animal research and considerations of specific categories of risk factors (*e.g.,* heavy metals). Risk factors considered in this review include: air pollution, aluminum, antibiotics, arsenic, bisphenol A, ethanol, lead (Pb), maternal smoking and environmental tobacco smoke, paracetamol (acetaminophen), pesticides, polychlorinated biphenyls, and polyfluorinated compounds.

Introduction

Early life environmental insults affecting the developing immune system can have significant

health ramifications not only for the exposed offspring but also potentially extending to

additional generations. Developmental immunotoxicity (DIT) appears to play a significant role

in the current global epidemic of non-communicable diseases (NCDs) [1,2]. This review of DIT

begins with the history of DIT placed in the context of the area of immunology known as

immunotoxicology and charts the emergence of recent concepts concerning early developmental

programming as it impacts later-life health. It also describes the current state of the science for

DIT and the likely applications of DIT assessment as it may impact both human health and

environmental protection. In particular, the article discusses: 1) the history of DIT research, 2)

the role of critical windows of vulnerability for the developing immune system, 3) frequent

outcomes of DIT, 4) consideration of the microbiome in DIT, 5) the role of prenatal epigenetic

alterations in immunotoxicity, and 6) the connection between DIT, elevated risk of comorbid

chronic diseases, and current epidemic of NCDs.

History of DIT Research

Emergence Within Immuntoxicology

Immunotoxicology, the study of the adverse impact of environmental conditions (*e.g.,* exposure

to food, drugs, chemicals, microbial agents, physical and psychosocial factors) on the immune

system, began to gain recognition during the 1970s and early 1980s [3] with the initial focus on

use of surrogates for host resistance in animal models [4,5] and concern about environmentally-

induced immunosuppression [6]. The search for assays and parameters that were predictive of

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chemical- or drug-induced immunotoxicity centered on measures that could replace then cumbersome and costly host challenges with infectious agents or transplantable cancer cells. Not surprisingly loss of immune protection (*i.e.,* immunosuppression) and increased susceptibility to infections and/or cancer was a driving concern. This also coincided with the era in which the HIV-induced immunosuppression associated with AIDS was an increasing human health challenge [7,8]. A coordinated effort to identify the best predictor of immunotoxicity resulted in the development of the tier system of assays providing a strategy of immunotoxicity testing [9] and the concept that a limited combination of immune measures could be effective for identifying immunotoxic chemicals [10,11]. In addition to the identification of chemicals that could produce immunosuppression in humans [12], the detection of chemicals with sensitizing potential was an early systematic concern within immunotoxicology [13,14].

The examination of adverse insults to the developing immune system, a subsection of immunotoxicology known as developmental immunotoxicity (DIT), was among the first research initiatives within immunotoxicology. As early as the 1970s, animal studies revealed the persistent nature of immune problems resulting from early-life insult. Studies involving drugs [15,16], heavy metals [17,18], pesticides [19], mold toxins [20-22], and polycyclic aromatic hydrocarbons [23] suggested that the developmental periods during which the immune system formed, disseminated to different tissues and acquired the capacity to work with host cells and defended against threats to host integrity were periods deserving of both research and public safety priorities. Yet, developmental studies represented only one of several aspects of immunotoxicogical research and the term "developmental immunotoxicity" was not prevalent in the literature until the mid-1990s [24,25].

DIT did not achieve a priority position for research and safety evaluation within immunotoxicology until approximately the late 1990s-early 2000s. Among the important events were a workshop on childhood health risks coordinated by the March of Dimes and EPA [26-28], the publication of a seminar text on compiling DIT research [29], and the increasing recognition of fetal programming of later-life health and disease [30-32]. Table 1 shows the basic features of DIT that have emerged during decades of research on the developing immune system.

DIT and the Barker Hypothesis

The impetus for a greater focus on DIT was aided by the findings of Barker and colleagues that maternal undernutrition during prenatal development could increase the risk of cardiovascular disease (CVD) in the offspring [33-35]. This led to what has been termed the "Barker Hypothesis" [36]. Originally, the linkage between fetal environment-adult disease was focused solely on maternal nutrition and CVD (including both coronary heart disease and hypertension) as an example linking early developmental conditions and fetal programming to later-life adult disease. But it became clear that the same relationship could exist for many other adult chronic diseases and conditions (*e.g.,* renal disease and type 2 diabetes, in adult offspring) that were also affected by the fetal nutritional environment) [37,38].

DIT and Developmental Origins of Adult Health and Disease (DOHaD)

As the net was cast beyond just maternal-fetal nutritional status to include a wide array of environmental conditions and factors, the concept of developmental origins of health and disease (DOHaD) emerged [39,40] to connect critical windows of development with specific childhood and adult health risks. Immune damage, dysfunction and/or imbalances are now known to persist long after either toxicant levels of chemical exposures return to normal or physical-

psychosocial stressors have been removed [41,42]. In fact, part of the challenge in deciphering

pathways resulting in DIT and fetal programming of adverse immune status is that evidence of

prior problematic exposure conditions may remain largely hidden. For this reason, DIT testing

usually requires careful consideration about exposure windows and immunological assessment

tools [43-45]. The opportunity to examine the different functional responses of the immune

system in response to various challenges has emerged as a key component of safety assessment

[43,46,47].

Fundamental Features of DIT

Heightened Sensitivity of the Developing Immune System

One of the hallmarks of the developing immune system is that it exhibits an increased sensitivity

for most environmentally-induced toxicity compared with the fully-matured immune system of

the adult. Additionally, DIT often occurs at exposure doses that are below those producing other

developmental effects [48-51]. Luebke et al. [41] reviewed the evidence of comparative age-

based sensitivity for five of the most extensively studied drugs and environmental chemicals

[diethylstilbestrol (DES), diazepam (DZP), lead (Pb), 2,3,7,8-tetrachlorodibenzo-p-dioxin

(TCDD), and tributyltin oxide (TBO)].   They concluded that early development appears to be a

time of increased sensitivity to xenobiotics and risk of adverse immune outcomes that are likely

to persist into later life.

This increased risk of developmental immune insult compared with that of the adult has been

seen across broad categories of drugs and chemicals as well as among different dietary and

physical/psychological factors [1,29,52]. This differential, age-based sensitivity can take

different forms, which are reviewed in detail in Dietert and Piepenbrink [53].   In many cases,

the lowest dose required to produce immune disruption is several fold to several magnitudes

lower in early-life than in the adult [41]. Additionally, a broader array of immune parameters are likely to be affected following exposure of the non-adult vs. the adult [50,54]. Prenatal and early postnatal exposures are more likely to produce persistent adverse immune outcomes [55-58].

Critical Developmental Windows

The identification and consideration of both systemic and tissue-oriented developmental vulnerabilities for the immune system have undergone progressive evolution since the original series of immune "critical windows" emerged from a national workshop [27,28]. As was illustrated in Dietert [1], for most key developmental steps of immune maturation, multiple environmental disruptors have been identified. The effect of inhibition or delay of the critical developmental step can increase the risk of multiple later life diseases. For example, key processes of T cell selection in the thymus can be affected by maternal exposure to certain heavy metals, plasticizers, dioxins, polycyclic chlorinated biphenyls, tobacco smoke, and certain drugs. Not surprisingly, the adverse health outcomes that have been associated with environmental targeting of thymus-directed processes are largely restricted to prenatal development and cover virtually every category of disease including cancer as well as autoimmune and allergic diseases and childhood vaccine failures [1].

Each immune developmental window has its own unique vulnerabilities that are best detected via age-relevant safety screening [53]. For example, Bunn et al. [59] demonstrated that while Pb was immunotoxic across all windows of prenatal developmental, later gestational maternal exposures were more likely to result in profound T helper 2 (Th2)-favored functional skewing in the juvenile rat.

Application of the critical windows concept for enhanced immune-associated disease prevention has been explored by Jenmalm and Duchen [60]. These authors stressed that dietary interventions capable of aiding prevention of allergy are most likely to be effective if directed toward specific prenatal, perinatal and early postnatal developmental windows [60].

Frequent Outcomes of DIT and Risk of Non-Communicable Diseases (NCDs)

One of the outcomes of the recent human studies on DIT and fetal programming of immune-based disease is an increasing realization that these processes are major contributors to the ongoing epidemic of non-communicable diseases (NCDs) (most of which are chronic diseases). NCDs are the major cause of death globally and include cardiovascular disease as well as cancer [61]. What has become clear is that the vast majority of NCDs cannot be maintained in the absence of misregulated (usually unresolving) inflammation [62-64]. This means that improper immune homeostasis in tissues is likely to be required for NCD onset and/or maintenance. Human studies supported by animal model research have established the importance of the prenatal and early postnatal environment for maturation of innate immune cells in concert with formation of the microbiome in mucosal tissues and other sites (*e.g.,* skin).

One of the impediments to recognizing this DIT-NCD linkage is that historic examination of immunotoxicity often focused on changes in primary and secondary immune organs. However, the majority of immune cells actually reside outside of these organs in mucosal and other tissues such as the gut, brain, skin, liver, endocrine, reproductive, urogenital, and cardiovascular systems. It is these tissues that are most often involved with NCDs, and the status of these resident immune cells are often at issue relative to tissue pathology. A shift in focus in immune evaluation to consider the impact of DIT and later life status of cells such as skin dendritic cells,

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microglia, Kupffer cells, and immune cells of the BALT and GALT should provide a clearer picture of the cause-effect relationship between DIT and certain NCDs [65]. Table 2 provides examples of environmental factors and conditions that are thought to contribute to later-life human disease via DIT and immune dysfunction. NCDs represent the majority of examples shown in Table 2.

The significance of the prevention of DIT as a strategy to reduce the prevalence of NCDs has been strengthened with the awareness that NCDs exist as tightly intertwined patterns of comorbid risks. This paradigm of tightly interlinked chronic diseases and conditions was described in a series of papers illustrating the health risk trajectories that exist when children are diagnosed with a number of different immune-/inflammatory-driven conditions: asthma, recurrent infections, schizophrenia, autoimmune thyroiditis, celiac disease, inflammatory disease, psoriasis [2,42,66,67]. Cancer is one of the common outcomes in the tissue receiving the primary inflammatory insult [68]. Even end-stage conditions such a chronic kidney disease and frailty form part of these interlinked patterns of chronic diseases and conditions [63,69].

As an example, one interconnected pattern of comorbidity exists among a triad of autoimmune conditions, type 1 diabetes, celiac disease, and autoimmune thyroiditis. Children diagnosed with type 1 diabetes have predictably greater risk for developing celiac disease and/or autoimmune thyroiditis [70,71]. While the mechanism remains to be elucidated, childhood asthma, obesity and sleep disorders are similarly interlinked in a triad [72,73]. Tanaka et al. [74] and Anders et al. [75] have pointed out that clinical depression is a largely immune driven, inflammatory-based condition that is another comorbid outcome intrinsically connected to many different NCDs/chronic diseases.

The ramification of these comorbid disease interconnections is that there is increased value in avoiding fetal programming that results in childhood-onset, immune dysfunction-based NCDs. These implications led four immunotoxicologists to call for required DIT testing of chemicals and drugs as a step to better protect children from the risk of NCDs [2].

Human Studies Involving DIT: Alphabetical List of Risk Factors

Most prior reviews of DIT have focused largely on animal research. This section examines the wide range of risk factors for DIT that has been evaluated among human populations. Evidence supporting the occurrence of DIT among human populations has been obtained from both exposed populations as well as via epidemiological studies. The risk factors are presented alphabetically rather than being grouped into different categories (*e.g.,* chemicals, drugs, physical and psychological factors).

In many of these studies antibody titers against either a common virus or childhood vaccinations have been used as a biomarker of DIT. While limited as an overall immune measure, there are significant benefits to this approach: 1) serum antibody levels are easily determined, 2) a majority of children will have been vaccinated according to a predictable and standard schedule, 3) the microbial infection or vaccine challenge of the child's immune system will enable a detection of potential dysfunction in an actively responding immune system and, based on animal data, these are among the most sensitive parameters for measuring DIT. Other studies reach beyond vaccination data to examine associations between exposure/environmental conditions and immune-based chronic diseases during childhood. Among the most commonly used are asthma, allergic rhinitis, atopic dermatitis, type 1 diabetes, celiac disease, and inflammatory bowel disease. Only a portion of these disease-association studies have overt

human immune function associated with them. For the remainder, there has been a tendency to rely more on linking DIT immune function animal data with information on human immune disease-associations.

Air Pollution

Ambient air pollution including specific components (e.g., polycyclic aromatic hydrocarbons, particulate matter) has been implicated in respiratory and cardiovascular diseases via improperly-controlled inflammation. Nadeau et al. [76] examined groups of asthmatic and non-asthmatic children in Fresno, CA for pollutant exposure, T regulatory (Treg) cell activity (that would help to control Th2 mediated asthma symptoms) and DNA methylation. The researchers found that increased exposure to ambient air pollutants was associated with increased methylation of CpG islands at the Foxp3 locus as well as reduced Foxp3 expression [76]. They also reported reduced numbers of Fox3p+ Treg cells and reduced Treg activity particularly among the asthmatic children. The authors concluded that increased air pollution exposure in children is associated with increased asthma morbidity via epigenetic alterations and a possible immune mechanism [76].

Kerkhof et al. [77] found evidence in children that traffic-related air pollution [e.g., (particulate matter (PM) 2.5, 10, soot, and nitrogen dioxide] increased the prevalence of doctor-diagnosed asthma by year 8 particularly among children with specific variant alleles for the toll-like receptor (TLR) genes 2 and 4. The investigators suggested their results are consistent with the suspected involvement of innate immune response in the linkage between exposure to traffic pollution and risk of childhood asthma [77].

Calderón-Garcidueñas et al. [78] compared immune markers in asymptomatic children from two different city areas (Southwest Mexico City vs. Polotitlán, Mexico as a control city) with vastly different burdens of urban air pollution. They found that children exposed to the severe air pollution had immune dysregulation with reduced levels of IFN-γ and natural killer cells with evidence of elevated systemic inflammation (elevated C-reactive protein and prostaglandin E metabolites).

Indoor air pollution, beyond that of environmental tobacco smoke, which is discussed in a later section, has also associated with human DIT. Herberth et al. [79] studied the effects of home renovation (e.g., painting, flooring, new furniture) on inflammatory biomarker profiles of six year old children. Significant increases in serum IL-8 and macrophage chemotactic protein 1 (MCP-1) were associated with home renovation activities. Installation of new wall-to-wall carpet gave the strongest single activity association with these markers.

Aluminum

Aluminum exposure during prenatal and childhood development can occur via a variety of routes including via food, certain drugs (aluminum-containing antacids), drinking water and air [80] including some parenteral nutrition products [81]. The immune system appears to be a sensitive target for aluminum [82]. However, a prevalent opportunity for repeated exposure is alum (aluminum oxyhydroxide)-containing vaccines. Alum is an adjuvant designed to promote a protective immune response, which may include a component of local inflammation (via specific cytokine release). One of the concerns with aluminum is the potential to sometimes induce inappropriate inflammation involving innate immune cells such as macrophages. In some individuals, such as those carrying HLA-DRB1*01, there appears to be an elevated risk of

persistent macrophagic myofasciitis [83,84], and this link has been proposed as one route to autoimmune/inflammatory syndrome induced by adjuvants (ASIA) [85].

There is evidence to suggest that febrile responses in children following alum-containing vaccination may represent an inflammation-driven hyperresponse that occurs in a subset of children, possibly those possessing certain cytokine gene alleles [86]. A proposed mechanistic basis for alum-induction of DIT in a subpopulation of children was discussed by Terhune and Deth [87]. These authors suggested that the Th2 biasing and inflammasome activating effects of aluminum may present a problem for children carrying genetic variants of certain cytokine genes (e.g., IL-4, IL-13, IL-33, and IL-18). In some subpopulations of children aluminum adjuvants might enhance the production of non-target directed IgE thereby elevating the risk of allergy and atopy [87]. Other investigators have suggested that alum may play a role in the induction of Crohn's disease in genetically susceptible individuals [88].

Antibiotics

Antibiotic use in early life has been associated with an elevated risk of immune-based diseases such as childhood asthma. Raciborski et al. [89] found that antibiotic use during the first three years of life was associated with a significantly elevated risk of asthma by 6-8 years of age among 1461 children in Warszawa, Poland. The highest association was found between infants who completed three courses of antibiotic within the first year of life and later childhood asthma (OR = 5.59, 95% CI: 2.6-12.01) [89]. Not all authors agree on this association. Heintze and Petersen [90] argue that various forms of bias weaken the literature on perinatal antibiotic use and risk of childhood asthma. However, the impact of repeated antibiotic use on the microbiome

during immune development provides a potential mechanistic basis for DIT, Th2 skewing and misregulated inflammation [91].

Extensive antibiotic use is of particular concern when viewed in the context of the hygiene hypothesis or the recently-described "Completed Self" model (*i.e.,* where unimpeded co-maturation of the development immune system and infant microbiome is critical) [91] (see figure 1). Under the "Completed Self" paradigm, successful development of a balanced, well-regulated immune system needs co-maturation with a complete microbiome in the infant. The developing immune system receives important signals from the commensal microbes and eventually matures to perceive self as a combination of the mammalian cells and commensal microbes. The successful merger of the infant's mammalian and microbial components into the fully-formed Human-Microbial Superorganism may well represent the single most important step distinguishing later-life health from disease. As a result, any prenatal or postnatal environmental exposure that interferes with timely and effective self-completion is a significant health risk [91]. This new immunological view of what constitutes a fully-completed infant could impact risk-benefit considerations for antibiotic administration in early life.

   Arsenic

Arsenic is found in both inorganic and organic forms. Most of the environmental health concerns have focused on the inorganic forms of arsenic (e.g., arsenite or arsenate) with exposure occurring primarily via ingestion of contaminated food and water and secondarily via inhalation. Some forms of arsenic (*e.g.,* arsenic trioxide) have been used in the treatment of leukemias. The topic of arsenic-induced immunotoxicity was recently reviewed by Dangleben et al. [92]. These authors stressed the increased vulnerability of infants and children to arsenic-

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induced immune dysfunction and the potential for early-life exposures to produce later-life health problems.

Studies of exposed human populations also suggest that arsenic is a major concern for DIT, and several studies have examined children in Mexico and Bangledesh among highly exposed populations. Soto-Peña et al., [93] found that children in Mexico (6-10 years old) with arsenic exposure primarily from drinking water (evaluated based on urinary levels) had peripheral blood mononuclear cells (PBMs) that were shifted in ex vivo stimulated function (proliferation and cytokine secretion). Rocha-Amador et al. [94] found that children in Mexico living in an area with high exposure to arsenic via drinking water had increased apoptosis among PBMs compared with those from an area with lower exposure levels. In a study of Bangladeshi children where a significant exposure to arsenic can occur via drinking water, Ahmed et al. [95] found that prenatal exposure to arsenic interfered with thymic function affecting T cell development. The proposed route of insult was via oxidative damage and possible misregulation of apoptosis. The same investigators demonstrated that prenatal exposure to arsenic was associated with increased placental inflammation increasing oxidative stress and altering both T cell and cytokine profiles in cord blood [96].

This suggests that, at physiologically-relevant exposures, arsenic-induced DIT can manifest almost immediately during fetal development. This is consistent with the findings of arsenic-exposed children in Mexico where increased arsenic levels were associated with increased basal nitric oxide production by monocytes and increased superoxide anion produced by activated monocytes [97]. Taken together these studies suggest that a proinflammatory state is part of the profile of arsenic-induced human DIT. Lower resistance to certain infectious diseases has been associated with early life exposure to arsenic. In a prospective population-based cohort study,

1,552 Bangledeshi infants were examined for both lower respiratory tract and diarrhea-associated infections and compared vs. maternal arsenic levels during the pregnancy (measured at two time points via urine) [98]. Rahman et al. [98] found that the highest quadril of maternal arsenic exposure vs. the lowest had a significantly elevated risk of both forms of mucosal tissue infections. Lower respiratory tract infections had a 69% increased relative risk for infants of high arsenic exposure mothers [adjusted relative risk (RR) = 1.69; 95% confidence interval (CI), 1.36-2.09)], whereas there was a 20% increased risk of diarrheal-associated infections (RR = 1.20; 95% CI, 1.01-1.43) among the same groups.

Bisphenol A

Bisphenol A (BPA) is used in a variety of food and beverage containers. Most human chemical exposure occurs via food and beverages although exposure via air, dust, and water is also possible. Sources of BPA include food storage containers, water bottles, baby bottles and polycarbonate tableware. BPA has received significant immune system evaluation in recent years although the majority of studies, to date, have been performed in rodents.

Rogers et al. [99] recently reviewed the immunotoxicologic profile of BPA suggesting that it: 1) increases Th2 polarization of dendritic cells, 2) alters macrophage inflammatory cytokine production and metabolism but with different dose-dependent effects, 3) decreases T regulatory cells, 4) alters the relative proportions of immunoglobulin (Ig) producing cells and 5) polarizes CD4+ T helper (Th) cells although the direction of polarization (e.g., Th1 vs. Th2) has differed among studies.

Human studies for BPA and DIT are comparatively limited to date. In a National Health and Nutrition Examination Survey (NHANES) study, children and teens less than 18 years of age

exhibited an inverse correlation of BPA exposure (urinary levels of BPA) with antibody levels against cytomegalovirus [100]. Kim et al. [101] examined the genomic alteration patterns of Egyptian pre-pubescent girls (ages 10-13) relative to both genome–wide methylation and methylation of genes previously identified as sensitive to BPA exposure. Among those genes prominently modified were those involved with immune response and autoimmune thyroid disease. Taken together, the animal and human studies suggest that early-life exposure to problematic doses of BPA produces altered gene expression related to immune function and inflammatory responses. However, more research is needed to define the boundaries of these alterations and the impact on various immune-related diseases in later life.

Cesarean Section

Cesarean section (CS) can be a medical necessity in some circumstances. However, the increased prevalence of elective CS [vs. vaginal delivery (VD)] has created a public health concern [102]. CS has been reported to alter the course of immune development by producing Th skewing, innate immune dysfunction and an increased likelihood of exacerbated inflammatory responses [reviewed in 103-105]. There are a minimum of four possible factors with Cesarean delivery that may contribute to subsequent DIT: 1) failure to properly seed the newborn's mucosal tissues with microbiota from the maternal vaginal tract, 2) the prophylactic use of antibiotics, 3) other drug administration connected with the Cesarean operation and 4) the contrasting placental immune-stress-hormonal environment between the two delivery modes.

In the first category, birth delivery mode can significantly affect the microbiota and the subsequent immune-microbiome interactions. In a Canadian study, Azad et al. [106] found that infants delivered by elective Caesarean were much lower in the bacterial diversity and richness

of their microbiome. In the fourth category from above, the immune physiology of vaginal delivery (vs. CS) appears to create a strikingly different environment for the full-term fetus. A cross-sectional study of 375 women in the Netherlands compared spontaneous term VDs vs. elective CSs for signs of intrauterine inflammation. Houben et al. [107] found that measures of placental inflammation and amniotic fluid proinflammatory cytokines (IL-6, TNF-α, IL-8) were significantly elevated with VD vs. CS. The investigators suggested that increased sterile inflammation during labor and VD delivery may play a critical role in normal parturition and facilitate subsequent maturational processes (*e.g.,* immune and airway maturation) in the newborn [107].

CS has been associated with altered levels of immune cell population, cytokines, and chemokines in neonates leading Cho and Norman [105] to suggest that it should not be recommended except where there is a clear medical indication or a benefit over risk estimate including long-term consideration for the infant-child. For example, CS has been found to skew the infant immune profiles toward a Th2 biased capacity [108]. Innate immune maturational markers are also affected. Elective CS without labor was found to be associated with reduced surface expression of two different toll-like receptors (TLRs): TLR2 and TLR4. In contrast, labor and vaginal delivery appears to upregulate these TLRs to adult levels [109]. Because these TLRs are important in innate immunity, the authors suggest that labor is an important component of ongoing immune maturation [109]. The concentrations of the chemokine, RANTES (CCL5), a chemokine important in recruiting immune cells to inflammatory sites, was found to be lower in neonates from CS than VD [110]. In a prospective study of full term deliveries, Malamitsi-Puchner et al. [111] found that VD neonates had elevated levels of both soluble IL-2 receptor and TNF-a compared with CS delivered babies. Taken together, these studies suggest that

neonatal immune profiles, including early inflammatory interactions, are locked into a less mature, more-fetal-like state following CS vs. VD deliveries. Not surprisingly, this appears to have consequences relative to risk of childhood chronic diseases.

CS with the outcome of low bacterial diversity in the infant is reported to increase the risk of several immune-based diseases emerging in children including asthma [112,113], atopic dermatitis [114], celiac disease [115], and type 1 diabetes [116,117]. A meta-analysis of 23 studies on CS and asthma estimated that the increased risk associated with this birth delivery mode was estimated at 20% [118]. Of note is the observation that specific subpopulations may be at an increased risk for the disease-promoting aspects of CS. For example, Magnus et al. [119] found that the association between CS and childhood asthma (at 3 years of age) was strongest among children of non-atopic mothers.

Childhood Abuse

In children who experience abuse, the developing immune system appears to become wired for dysfunctional responses. In the Nurses' Health Study II, Bertone-Johnson [120] found that women reporting moderate to severe childhood or adolescent abuse had significantly elevated levels of two inflammatory markers CRP and IL-6 as adults. The authors argued that early life stress may program the immune system for dysregulation and that subsequent immune dysregulation elevates the risk of certain chronic diseases. Slopen et al. [121] make a similar link between childhood adverse experiences, misregulated inflammation and risk of cardiovascular disease.

Diethylstilbestrol

While human immunological studies on diethylstilbestrol (DES) are limited compared with other health-related studies, there are reports suggesting that in utero-exposed offspring are at a higher risk of immune-based disease. Overall DES daughters exposed in utero self-reported an increased risk of all immune-based diseases (infections, allergies, autoimmune conditions). Within specific categories, the women experienced more infectious diseases than non-DES exposed daughters [122]. In a separate study, Strohsnitter et al. [123] examined the incidence of selected autoimmune conditions among DES daughters. They found no overall increase in disease associations for rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), optic neuritis (ON), or idiopathic thrombocytopenic purpura (ITP) However, there was a significant increase in the onset of RA by 45 years of age in the DES-exposed vs. non-exposed groups [123].

Ethanol

There is substantial data from animals suggesting that developmental exposure to alcohol produces DIT [51,124] and can elevate the risk of non-communicable diseases possibly via inflammatory processes [125]. Maternal consumption of alcohol during pregnancy can produce immune-related adverse outcomes in the offspring. In fact, later gestation appears to be particularly sensitive to the effect of ethanol [126]. Among the reported long-term effects was interference with the immune response to influenza virus challenge in mice [127].

Remarkably, human studies are limited for low-level ethanol intake and DIT-related outcomes. Most studies following children exposed in utero to alcohol have focused on growth and behavioral outcomes [128,129]. Carson et al. [130] utilized the COPSAC prospective birth cohort comprising 411 children born to asthmatic mothers. The children were considered full

term and lacked congenital abnormality, systemic illness, or history of mechanical ventilation or lower airway infection. For this study group, the risk of atopic dermatitis was reported to be significantly elevated for any maternal alcohol consumption during pregnancy (HR 1.44, 95% CI 1.05-1.99, p=0.024) even after exclusion of effects of maternal smoking or atopic dermatitis [130].

Two studies reported negative results for maternal alcohol intake and childhood asthma. Yuan et al. [131] examined the incidence of hospitalization for asthma to age 12 among children from 10,440 singleton full-term births in Denmark between the years 1984-87. The authors reported no significant associations between alcohol vs. no alcohol consumption (HR 0.95; 95% CI 0.70-1.29) including different doses of alcohol as well as binge drinking. In a second study, Shaheen et al. [132] examined maternal alcohol consumption during pregnancy relative to risk of childhood atopic disease (asthma and hayfever) measured at seven years of age within the Avon Longitudinal Study of Parents and Children (ALSPAC). They found no elevated risk for late gestational alcohol consumption with asthma or hayfever and no difference among mothers carrying different alleles for the alcohol dehydrogenase gene [132].

A case-controlled study in Ireland with infants born 1994-2001 examined factors that are potentially involved with Sudden Infant Death Syndrome (SIDS) [133]. McDonnell-Naughton et al. [133] reported that mothers of infants with SIDS were more likely to have consumed alcohol during pregnancy than were controls (HR 3.59, 95% CI 1.40-9.20).

Lead (Pb)

A cadre of heavy metals has been examined for DIT and associated health risks in both children and adults. Among the most consistent observations with lead (Pb) are elevated risk of oxidative

damage and a skewing toward Th2-driven responses with elevated levels of IgE. As an indicator

of Pb's ability to produce misregulated inflammation, Pineda-Zavaleta et al. [134] found the

macrophages isolated from Pb-exposed children stimulated in vitro with lipopolysaccharide

overproduced superoxide anion. Karmaus et al. [135] reported that Pb exposure was associated

with elevated IgE in children. Li et al. [136] reported a negative correlation between circulating

CD4+T cells and blood lead levels. Lutz et al. [137] found that combined exposure to Pb and

environmental tobacco smoke was strongly associated with elevated serum IgE levels in

children. The human data are consistent with the animal studies suggesting that Th skewing,

increased oxidative stress and tissue damage, and misregulated inflammation are among the

adverse immune outcomes following developmental exposure to Pb [138].

Maternal Smoking and Environmental Tobacco Smoke

There are several suggested developmental risk factors for asthma. Among these,

maternal smoking during pregnancy and exposure of the infant to environmental tobacco smoke

[ETS] were identified by Selgrade et al. [139] as having the most convincing body of evidence

connecting environmental exposure to DIT and risk of childhood asthma. Additionally, Prescott

[140] identified early life exposure to tobacco smoke producing altered immune function as

being an important contributor to risk of allergic diseases. Among the pathways proposed to be

involved is the capacity of maternal smoking to alter TLR-mediated responses in infant innate

immune cells [140]. Noakes et al. [141] suggest that smoking induced TLR alterations will

affect not only the developing immune system but also the "hygiene hypothesis" effects of

immune-microbiome interactions in the newborn. The capacity of DIT to disrupt integrity of the

immune-microbiome (The Completed Self Model) is depicted in figure 1.

Wilson et al. [142] reported that exposure of children to secondhand smoke produced significant changes in cytokine levels particularly reducing the level of IFN-γ. As previously mentioned in the section on Pb, Lutz et al. [137] reported an interaction of environmental risk factors in which Pb-exposed children also exposed to ETS had elevated IgE and IL-4 levels and altered T cell populations. Similar results were obtained by Tebow et al. [143] for exposure covering both prenatal and postnatal periods. These researchers found that parental smoking was associated with a disrupted balance of IFN-γ to IL-4 among children of smokers. While IL-4 levels were unchanged in the comparison of children with parental smokers vs. non-smokers, reduced IFN-γ was associated with parental smoking and a dose response relationship appeared to exist. Therefore, the balance of IFN-γ to IL-4 was shifted toward the latter.

Elevated risk of allergic diseases is not the only immune-based concern with early-life exposure to tobacco smoke. Kum-Nji et al. [144] reviewed the literature regarding ETS and childhood infection and concluded that there is no longer a doubt about this association. Supporting evidence has been seen using childhood vaccination. In an examination of 200 infants with a history of parental allergy, Baynam et al. [145] found that, among children with parents who smoked, infants carrying a variant of the IL-4 receptor gene (the IL-4Ralpha 551 QR/QQ genotype) exhibited significantly altered immune responses. These included: reduced IgG responses, reductions in certain T cell responses (*e.g.,* those associated with IFN-γ production) and altered innate immune (defective TLR-driven) responses upon vaccination with tetanus toxoid. These studies suggest that early-life exposure to smoking causes immune dysbiosis (targeted inappropriately exaggerated responses as well as suppression) that includes both an elevated risk of certain allergic diseases as well as potentially impaired responses to childhood vaccination. In keeping with many other DIT studies involving other risk factors, it

also suggests that some human subpopulations are likely to have enhanced vulnerability for smoking-related DIT.

Disrupted immune maturation is not the only pathway through which maternal smoking and ETS appear to affect later-life immune function. Wilhelm-Benartzi [146] found that childhood ETS exposure produced epigenetic marks in genes associated with both immune function and immune signaling.

Paracetamol

Prenatal and early infant exposure to paracetamol (acetaminophen) has been associated with an increased risk of a variety of wheeze-associated disorders in the child including asthma. In the case of prenatal exposure, a study from Denmark examined 197,060 singletons born in northern Denmark in 1996-2008 [147]. Paracetamol exposure during any trimester of the pregnancy resulted in an adjusted odds ratio of 1.35 (95% confidence interval: 1.17-1.57) for risk of asthma by the end of 2009 [131]. For infant exposure, Gonzalez-Barcala et al. [148] studied 20, 000 children in Galicia, Spain and reported that paracetamol use during the first year of life led to a significant increased risk of asthma in 6-7 year old children [odds ratio (OR) 2.04 (1.79-2.31).] Henderson and Shaheen [149] recently reviewed the epidemiological data regarding prenatal and infant exposure to paracetamol and an increased risk of childhood asthma. They argue that the evidence is sufficiently strong as to be compelling for this association but also point out that mechanistic causation remains a significant data gap.

One of the potential confounding factors is prevalence of infections and the use of antibiotics, which may coincide with administration of paracetamol [150]. Heintze and Petersen [90] argued that failure to distinguish among the confounding effects of these two factors would significantly

weaken the proposed associations. However, Muc et al. [151] performed a cross-sectional study

of 1063 primary school children in Portugal in which they partitioned the factors of paracetamol

in early childhood and antibiotic administration relative to risk of asthma. Paracetamol use and

antibiotic administration were independently found to increase the risk in children of current

asthma (at the time of evaluation) as well as ever having asthma. Because frequency of

paracetamol use was connected to increased allergic symptoms, the researchers suggested that

dose-dependent associations may be present among the data [151]. Not all studies have reported

positive associations for paracetamol and asthma.   However, based on an understanding of the

pathways through which paracetamol is likely to affect offspring immune status and childhood

health, Thiele et al. [152] called for a reconsideration of safety and dosage recommendation

during pregnancy.

For potential infant use, McBride [153] argued that risk data combined with the likelihood of

glutathione depletion by paracetamol in the airways suggested that children at risk for asthma

should avoid the use of paracetamol. Selgrade et al. [139] pointed out that accompanying animal

data have been generally lacking in DIT models of the human paracetamol-asthma linkage.

However, these authors also point to the overall importance of oxidative stress and inflammation

as likely routes for xenobiotic-induced, DIT-related asthma. This would be consistent with

findings of several research groups.

Evidence from several studies suggests that disruption of effective oxygen species regulation is a

likely route to the elevated risk. Kang et al. [154] reported that postnatal pediatric use of

paracetamol was more likely to produce asthma among children carrying specific genetic alleles

associated with control of oxidative inflammation (*NAT2, Nrf2, and GSTP1*). Shaheen et al.

[155] examined the effect of specific maternal alleles for nuclear erythroid 2 p45-related factor 2

(*Nrf2*) and glutathione S-transferase (GST) polymorphisms within data from the Avon Longitudinal Study of Parents and Children. They found that maternal *Nrf2* alleleic differences had an effect on early gestation exposure to paracetamol and childhood asthma, while the presence of the *GSTT1* allele was important in late gestational exposure to paracetamol [155]. Taken together, these studies suggest that subpopulation differences are likely to exist for the relative risks of association between prenatal exposure to paracetamol and childhood-onset asthma.

Pesticides

Pesticides fall into several different chemical categories (e.g., organophosphate, organochlorine, pyrethroids). However, humans are likely to be exposed to pesticide mixtures rather than to a single pesticide, and mixtures may result in unanticipated interactions among the pesticides at the molecular level [156]. Human exposure to certain pesticides at sufficient doses has been known to produce a variety of effects on physiological systems with some outcomes potentially linked to their endocrine disrupting activity [157] and altered oxidative stress [158]. In particular most of the human findings primarily concern early life exposure and childhood neurodevelopmental impairment. In a prospective longitudinal study conducted in the French West Indies, Boucher et al. [159] reported that exposure to the organochlorine pesticide, chlordecone, was associated with impaired neurodevelopment in 18 month old infants. The effects were seen in boys but not girls.

Three epidemiological studies are significant in pointing to similar conclusions regarding prenatal pesticide exposure and later childhood neurodeficits. In the Columbia University study, Rauh et al. [160] found an inverse association between Working Memory Index and Full-Scale

IQ in inner-city children at age seven and the level of prenatal exposure to chorpyrifos, an organophosphate pesticide. In a Mount Sinai Children's Environmental Health Study, Engel et al. [161] reported that prenatal exposure to organophosphate pesticides was negatively associated with cognitive function by 12 months of age but also continuing later into childhood.   In a multi-institutional California study among predominately Latino farmworker families, Bouchard et al. [162] reported that prenatal exposure to organophosphate pesticides was associated with reduced intellectual development at age seven.

Among pesticides, the exposure risks not only involve childhood-onset conditions but also later-life-appearing diseases (*e.g.,* neurodegenerative). Zhou et al. [163] found that early-life exposure of mice to paraquat led to a later silencing in the gene (PINK1) responsible for producing a neuroprotective peptide. At the same time these pesticides activated the brain's innate immune cell resident microglia populations to generate excessive oxidative damage among neurons [164]. The reduced neuroprotection coupled with the increased risk of immune-mediated oxidative damage shifts the equilibrium of the aging brain toward neurodegeneration.

There is a suggestion that pesticide exposure may affect the risk of immune-driven NCDs. In the U.S. Agricultural Health Study, Hoppin et al. [165] found that exposure to pesticides elevated the risk for atopic (but not non-atopic) asthma among farm women. In fact the exposure to pesticides nullified the beneficial effect of growing up on a farm relative to risk of asthma. In this study, a total of 7 of 16 insecticides, 2 of 11 herbicides, and 1 of 4 fungicides were associated with an elevated risk of atopic asthma while permethrin use was the only pesticide associated with an increased risk of non-atopic asthma [165]. The study design [165] did not permit a comparison of differential developmental sensitivities and the potential role of pesticide-induced DIT in risk

of asthma. However, the apparent nullification of immune-microbiome protection against asthma (*i.e.,* hygiene hypothesis) raises intriguing questions.

Corsini et al. [166] recently reviewed the literature on pesticides and immunotoxicity. Based on human studies, these investigators concluded that the potential role of pesticides in immunotoxocity is unclear at present. They pointed out the serious limitations of most of the available studies including problems in accessing exposure levels and quite divergent approaches to assessment. The researchers called for better studies that would include pre-and post-exposure information and be designed with appropriately matched controls. Beyond the weaknesses discussed by Corsini et al. [166], other weaknesses include a general lack of data regarding early developmental exposures and information regarding potential hypervulnerability for pesticide-induced DIT among human subpopulations.

Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are in a category of persistent organic pollutants (POPs) that can present human health challenges long after release into the environment. Stølevik et al. [167] examined the effects of exposure to PCBs and dioxin among Norwegian mother-child pairs and potential immune effects. They found that exposure to PCBs and dioxins was associated with increased incidence of respiratory infections and reduced antibody response against one (measles) of several childhood vaccinations. This is consistent with the findings of Heilmann et al. [168,169] who studied perinatal PCB exposure and immune outcomes among children of the Faroe Islands. These researchers reported reduced antibody titers up to age seven to common childhood vaccinations following largely maternal diet-based perinatal exposure to PCBs. For the strongest associations, these investigators found that a doubling of serum PCB concentrations

resulted in an approximately 20% reduction in antibody levels. Approximately 28% of the Faroe Islands children were found to be effectively unprotected against the childhood preventable diseases based on the extent of antibody suppression [168,169].

Significantly for risk of NCDs, Grandjean et al. [170] found that prenatal and lactational exposure of Faroe Island children to marine pollutants including PCBs increased the risk of allergic sensitization. These findings are consistent with the apparent capacity of PCBs to produce disruption of immune homeostasis with effects including not only immunosuppression but also inappropriately enhanced and misdirected immune responses.

Polyfluorinated and Perfluorinated Compounds

The impact of developmental exposure to perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) was examined in a prospective cohort birth study in the Faroe Islands [171]. The researchers found that a two-fold increase in the levels of PFOS and PFOA at age five resulted in a several-fold increased likelihood of being unprotected against preventable childhood illnesses at age seven. In this case, lack of protection was defined as being below a protective level of antibodies against diphtheria and tetanus [171]. These findings have potentially stark implications for the health protection of children. In fact, the investigators determined that if benchmark dose (BMD) was calculated for the various polyfluorinated compounds using antibody levels as the driver and these were converted to safety limits for PFCs, the current limits may be several hundred fold too high [172].

Conclusions

DIT and fetal programming are emerging as significant contributors not only to later life immune dysfunction and misregulated inflammation but also to increased risk of NCDs and particularly

chronic diseases. Given the present epidemic of NCDs, the inter-related comobidities that exist among a myriad of chronic diseases, and the role of NCDs as the greatest cause of death worldwide, better preventative strategies are needed.

Animal model research of DIT dates back decades and helped to establish the fundamental characteristics surrounding early-life immune vulnerability for later life disease.   Recently, human DIT-related data have shown the relevance of the animal model information concerning dose sensitivity, subpopulation vulnerability, and health ramifications. While data gaps still exist for some categories of environmental risk factors (e.g., bisphenol A, certain pesticides), the way forward seems clear.

Better identification of DIT risk and improved protection of age-, sex-, and genotype-based hypervulnerable subpopulations are needed. This may well require a different approach to safety testing. With the potential for epigenetic alterations to be produced in utero and inheritance of altered immune- and inflammation-related gene expression across generations, it is apparent that efforts to reduce the prevalence of NCDs need to being early in life. Reducing the prevalence of DIT is an important first step in comprehensive efforts to reduce the prevalence and global impact of NCDs.

Acknowledgements

Abbreviations

ALSPAC Avon Longitudinal Study of Parents and Children

Alum aluminum oxyhydroxide

BMD benchmark dose

CI confidence interval

COPSAC Copenhagen Prospective Study on Asthma in Childhood

CRP c-reactive protein

CS Cesarean section

CVD cardiovascular disease

DES diethylstilbestrol

DIT developmental immunotoxicity

DOHaD developmental origins of health and disease

DZP diazepam

HLA human leukocyte antigen

Ig immunoglobulin

IgE immunoglobulin E

IFN-γ interferon-gamma

Il-4 interleukin-4

IL-6 interleukin-6

IL-8 interleukin-8

NHANES National Health and Nutrition Examination Survey

NCDs Non-Communicable Diseases

Pb lead

PFOA perfluorooctanoic acid

PFOS perfluorooctane sulfonic acid

POPs persistent organic pollutants

RR relative risk

Th T helper

Th2 T helper 2

TBT tributyltin oxide

TCDD 2,3,7,8-tetrachlorodibenzo-p-dioxin

TLR toll-like receptor

TNF-α tumor necrosis factor-alpha

VD vaginal delivery

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Developmental Immunotoxicity (DIT) in the Context of
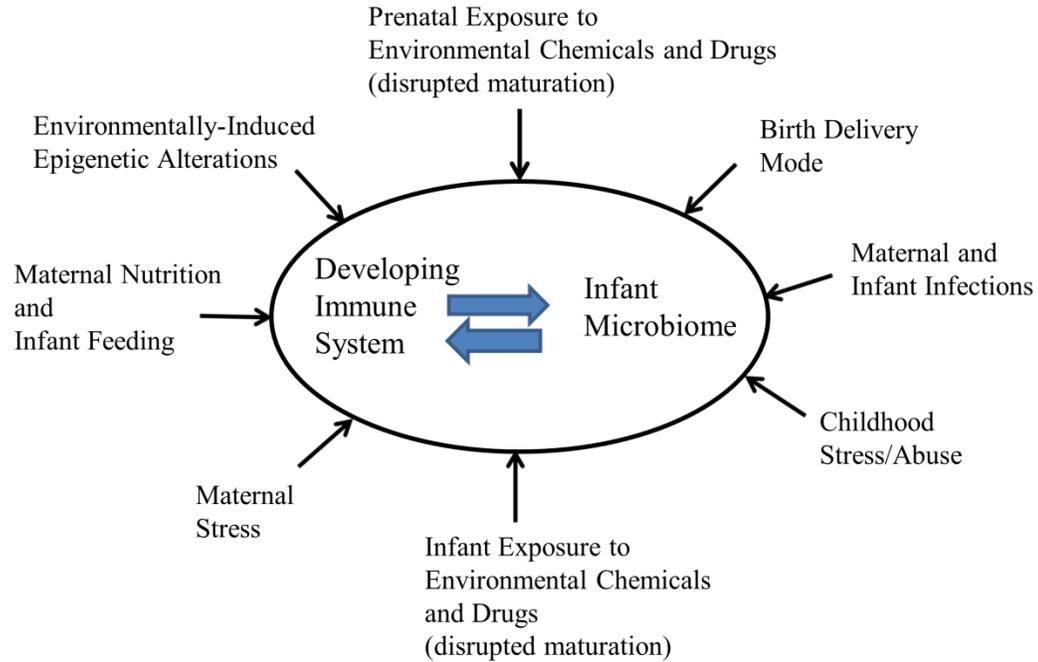## The Completed Self Model for the Human-Microbial Superorganism

Figure1. This figure depicts a model following the "Completed Self" paradigm [91] in which the immune system and infant microbiome need to co-mature without interference or disruption to reduce later-life health risks. The categories of environmental risk factors reported to cause prenatal and/or postnatal disruption are illustrated.

Table 1. Ten Basic Features of Developmental Immunotoxicity (DIT)*

DIT

- is directly linked with immune dysfunction and increased risk of NCDs
- stems from critical developmental windows of immune vulnerability restricted to the young
- can happen at lower exposure levels than usually produce adult-exposure immunotoxicity
- often involves a broader spectrum of adverse immune outcome vs. adult-exposure immunotoxicity
- usually produces more persistent effects that those following adult exposure
- can lead to latent dysfunction that may be masked until it is triggered by an later-life event
- often manifests as immune dysfunctional imbalances (suppression of some immune responses along with the inappropriate enhancement of others)
- may produce different sex-based outcomes
- is not routinely assessed in most required safety testing protocols to date
- can occur via several different biological pathways (*e.g.,* impaired immune maturation, epigenetic alteration, immune-microbiome disruption)

*The DIT literature is sufficiently extensive to permit fundamental characterizations. This information is derived from the following references [1,27,41,42-44,52,53,63,68].

Table 2. DIT and Increased Risk of Human Disease*

Disease, Disorder or Susceptibility State	Suggested Early-Life Immune-Modulating Risk Factor	Reference(s)
Acute myeloid leukemia	Benzene	[173]
Allergic sensitization	Polychlorinated biphenyls	[170]
Asthma	Maternal paracetamol use	[155]
Atherosclerosis	Maternal hypercholesterolemia	[174]
Atopic dermatitis	Maternal smoking	[175]
Allergic rhinitis	Antibiotics in infancy	[176]
Autism spectrum disorders	Maternal immune activation	[177]
Bipolar disorder	Gestational influenza	[178]
Cardiovascular disease	Childhood abuse	[179]
Celiac disease	Elective cesarean delivery	[180]
Crohn's disease	Maternal smoking	[181]
Chronic obstructive pulmonary disease	Smoke from biomass fuels	[182]
Depression	Childhood trauma	[183]
Endometriosis	Environmental tobacco smoke	[184]
Hypertension	Pesticides (DDT)	[185]
Insulin resistance	Maternal diet	[186]
Lack of protection against diphtheria and tetanus following childhood vaccination	Perfluorinated pollutants	[171]
Multiple sclerosis	Vitamin D insufficiency	[187]
Myalgic encephalomyelitis (Chronic fatigue syndrome)	Childhood trauma	[188,189]
Narcolepsy (specific subpopulation)	H1N1 flu vaccination	[190,191]

Table 2. DIT and Increased Risk of Human Disease*

Obesity/overweight risk	Cesarean delivery	[192]
Otitis media	Maternal smoking/ETS	[193-195]
Parkinson's disease	Pesticides	[164,196]
Pre-eclampsia	Traffic-related air pollution	[197]
Psoriasis	Environmental tobacco smoke	[198]
Respiratory infections	Polychlorinated biphenyls	[199,200]
Rheumatoid arthritis	Maternal smoking	[201]
Schizophrenia	Prenatal immune activation	[202,203]
Sudden infant death syndrome	Maternal smoking and alcohol consumption	[133]
Type 1 diabetes	Lack of or short-duration breastfeeding	[204]
Ulcerative colitis	Urban living	[205]

*This table includes both non-communicable and communicable diseases and conditions. Environmental risk factors are provided to illustrate an example and are not intended to be an exhaustive listing. The focus is on human studies and data.