MiniReview

Prenatal Exposures to Persistent and Non-Persistent Organic Compounds and Effects on Immune System Development

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Abstract: Immune system development, particularly in the prenatal period, has far-reaching consequences for health during early childhood, as well as throughout life. Environmental disturbance of the complex balances of Th1 and Th2 response mechanisms can alter that normal development. Dysregulation of this process or an aberrant trajectory or timing of events can result in atopy, asthma, a compromised ability to ward off infection, or other autoimmune disease. A wide range of chemical, physical and biological agents appear to be capable of disrupting immune development. This MiniReview briefly reviews developmental milestones of the immune system in the prenatal period and early life, and then presents examples of environmentally induced alterations in immune markers. The first example involves a birth cohort study linked to an extensive programme of air pollution monitoring; the analysis shows prenatal ambient polycyclic aromatic hydrocarbons (PAH) and fine particle (PM2.5) exposures to be associated with altered lymphocyte immunophenotypic distributions in cord blood and possible changes in cord serum immunoglobulin E levels. The second example is a study of prenatal-polychlorinated biphenyl (PCB) exposures and the foetal development of the thymus, the organ responsible for lymphocyte maturation. Mothers with higher serum concentrations of PCBs gave birth to neonates having smaller indices of thymus size. Finally, this report underscores the tight connection between development of the immune system and that of the central nervous system, and the plausibility that disruption of critical events in immune development may play a role in neuro-behavioural disorders.

The premise of this paper is that immune system development, particularly in the prenatal period, has far-reaching consequences for health during early childhood, as well as throughout life, and that environmental disturbance of the complex interactions and balances of Th1 and Th2 response mechanisms can alter that normal development. Dysregulation of this process or an aberrant trajectory or timing of events not only may result in atopy, asthma, other autoimmune disease, a compromised ability to ward off infection, or leukaemias, but also has the potential to induce neurobehavioural deficits and/or psychiatric disorders. Interestingly, both increased hypersensitivity or autoimmune responses and decreased immune competence have been observed in association with, in some instances, the same environmental exposures [1,2].

Dietert et al. summarized a workshop that identified five stages of immune development, three of which occur before birth in human beings: initiation of haematopoiesis, migration of stem cells and expansion of progenitor cells, and colonization of bone marrow and thymus [3]. Specific examples of developmental immunotoxicity from early life exposures were also discussed, including the pesticide chlordane, the air pollutant benzo(a)pyrene, the pharmaceutical diethylstilboestrol, as well as two pervasive pollutants, lead and 2,3,7,8-TCDD (tetrachlorodibenzo-dioxin). More recently, Luebke provided an updated review of data on five environmental immunotoxins: diethylstilboestrol, diazepam (valium), lead, tributyltin and 2,3,7,8-TCDD [1]. In each case, sensitivity was greater when exposure occurred during early development as compared to adulthood; in some cases, the effects were far more persistent. For example, T-cell maturation was impaired by 2,3,7,8-TCDD exposure, with 100-fold greater sensitivity in T-cell suppression when exposure took place during the third trimester. West [4] further elaborated on the delineation of potential windows of susceptibility in human beings and Landreth [5] published a similar review for rodents.

This presentation begins with a short description the salient developmental events that occur in utero to elucidate issues of critical windows for susceptibility to immunotoxic insults. The remainder of the paper is devoted to recent work on environmental factors affecting immune markers or outcomes. The first case study is an investigation of the air
pollutants polycyclic aromatic hydrocarbons (PAH) and fine particles, and their associations with several immune biomarkers at birth and with risk of respiratory illness in the preschool period. The second example is that of persistent halogenated compounds, namely PCBs, in relation to thymus size, an outcome that features prominently in the animal toxicology literature for these exposures. We conclude with brief discussions of the state of knowledge on asthma and the immunological correlates of autism in relation to early life exposures.

More work is needed in this area. Aberrant immune system development in prenatal and early postnatal life is likely a common denominator for a number of serious childhood disorders, and a focus on the immune system will shed light on mechanisms of action, helping us to focus attention on the environmental contaminants that disrupt those systems and that place the health of future generations at risk.

**Development of the immune system in utero**

West [4] provides a detailed description of critical windows in development of the human immune system. Figure 1 shows salient events in the *in utero* development of immune structures and function. Early production of B cells begins in the first few weeks after implantation. Production of immunoglobulin E (IgE) begins in the liver first, followed by the spleen and lung, appearing towards the end of the first trimester [6]. The thymus is seeded with pro-T cells in the late first trimester of pregnancy, while macrophage differentiation and seeding takes place in the first and early second trimester. IgM is produced for circulation near the end of the first trimester, around the time of appearance of the thymus, which functions to promote maturation of lymphocytes, especially in the first few years of life. [6]. T-cell receptors begin to be expressed towards the end of the first trimester, and an intense expansion of T-cell populations occurs in the second trimester; these cells are influenced by exposures during this period [4]. Diethylstilboestrol-induced alterations in T-lymphocyte function may therefore be linked to the mid-trimester of human gestation, although diethylstilboestrol may also target precursor cells before that time.

Macrophage maturation also occurs in the second trimester, while allergen and microbial-specific antibodies have been documented to appear beginning in the late second trimester. Throughout this time, there is placental transfer of maternally produced IgG of various isotypes, with preferential transfer of IgG(1) > IgG(3) > IgG(4) > IgG(2) [7]. Small amounts cross early in gestation, with a rapid increase in transfer after 20 weeks. Developmental lead exposure results in altered IgG levels [1], and could be acting either in late gestation, or might plausibly target B cells, produced relatively early in gestation, but precise mechanisms are likely to be complex.

During foetal life, there is a skewing of the immune response towards the Th2, or pro-inflammatory end of the spectrum [8]. For the foetus, it can be presumed that this confers a survival advantage; one suggested mechanism is that of protection of the foetoplacental unit against toxic cytokines such as interferon-γ (IFN-γ) [9]. In the first few months of life, a majority of infants show reactivity to cow’s milk, and atopic dermatitis is common. This skewing can be measured using IFN-γ [6]. Most infants outgrow these responses over the first 6 months of life. In fact, beginning at birth, the infant begins the process of re-calibrating towards a Th1 response; it appears that those who do not, because of a genetic predisposition that favours expansion of the Th2 response and hence suppresses this development, are the neonates most likely to develop atopy or asthma.

Furthermore, notable during gestation is the ability of the foetus to mount antigen-specific adaptive responses. These have been recorded for rubella virus and helminth parasites in the second trimester [10]. Other evidence of priming of the immune system *in utero* is the presence of high IgE levels in a sizable fraction of newborns [11,12]. In addition, detectable cord blood IFN-γ responses to respiratory syncytial virus have been observed in association with lower risk of asthma [13].
Acquired immunity develops as lymphocytes learn to recognize specific antigens and some clonal expansion occurs that permits a stronger response upon repeated presentation of stimulus. The acquisition of capacity to respond to a multitude of specific foreign invading micro-organisms continues throughout life. Toll-like receptors (TLR), genetically programmed to enable cells to recognize molecular patterns common to major classes of microbes, are present on dendritic cells and macrophages, and on a variety of dermal and other cells, where they orchestrate cutaneous host responses to bacterial, viral and fungal pathogens [14]. TLRs are also involved in adaptive immune responses, such as activation of dendritic cells to produce CD40, CD80, CD86 and interleukin-12 (IL-12) and activation of macrophages to increase production of tumour necrosis factor-α and IL-6 [14]. Thus, antigen-specific immunoglobulin production is regulated in part by cytokines produced in response to the stimulus present. This response can be demonstrated to occur even during prenatal life.

The relationship between immune responses and neurodevelopment is an emerging area of research that suggests considerable cross-talk of these two systems and has implications for how environmental health research questions can be formulated. The immune system interacts extensively with the nervous system, including during the prenatal and early postnatal developmental periods. Cytokines act on receptors on glial cells, and receptors on immune cells respond to neuropeptides. For example, thymic epithelial cells express oxytocin, a neuropeptide, and platelet serotonin levels are elevated in certain neurodevelopmental disorders such as autism [15–17]. Cytokines modulate neuronal survival [18] and differentiation [19]. The IL-6 plays a key role in neuroprotection and neuroregeneration and is released by astrocytes [20]. Hippocampal neurogenesis is enhanced by the presence of T cells and impaired in immune-deficient mice; the T cells appear to interact with microglia [21]. Major histocompatibility complex I molecules not only play a key signalling role in immune function, by displaying proteins that T cells recognize, but also play a role in neural development, through activity-dependent changes in synaptic strength [22,23]. These interactions of immune system components with the central nervous system and the induction of neuropahtology by inflammatory processes constitute a further rationale for focusing attention on the immune system in addressing the effects of environment on health and development.

Rodent experiments, for example, have shown that early immune challenges can alter the neural circuitry and associated behaviours. An interesting experimental study links time-dependent exposures to both behavioural and cytokine responses and to gene expression in mouse pups. Meyer et al. exposed pregnant dams to poly I:C (polynosinic:polycytidylic acid) at different gestational days (GD9 and GD17) and showed time-dependent aberrant behaviours [24]. The earlier exposures resulted in reduced open field exploratory behaviour, reduced neurogenesis in the dentate gyrus, reduced foetal brain concentrations of IL-10, massive elevations in IL-6, higher IL-1 6 hr after treatment, and differences in IL-1β mRNA [24]. A different set of behavioural, neuropathologic, immune signals and immune-regulating gene expression markers resulted from later (GD17) exposure. Thus, these experimental models provide strong evidence for systemic changes in neurobehavioural development resulting from stimulation of immune responses, which in turn are highly sensitive to timing of exposures and appear to be mediated through gene expression pathways.

In human beings, prenatal infections have been linked to a number of neurodevelopmental or neuropsychiatric disorders, including schizophrenia [25,26], autism [27,28] and mental retardation [29], effects currently believed to be linked to inflammatory responses rather than to any specific micro-organism. In rodents, exposures that induce cytokine response in the absence of infection give rise to neuropathology [30]. Thus, disruption of the well-modulated development of the body’s immune system can have unexpected consequences other than immune deficiency, atopy or autoimmune disorders. Nevertheless, further understanding of mechanisms is needed and will likely require multidisciplinary approaches and use of new technologies.

Dietert and Dietert have reviewed a range of developmentally immunotoxic exposures, which include, as examples, metals, alcohol, cigarette smoke, 2,3,7,8-TCDD, diethylstilboestrol, xenoestrogens, genistein, trichloroethylene, C-sections, maternal infections, tributyltins, bisphenol A, diazepam, pre-eclampsia and antibiotics [31]. Because development of the immune system is influenced not only by biological challenges, but also by early life exposures to chemical and/or physical agents, it follows that research focused on understanding immune health and its relationship to neurodevelopmental disorders must account for the broad array of human interactions with the natural and man-made environment.

Prenatal air pollution exposures and early immune development

A few in vitro studies support a role of air pollution in impairment of immunological competence. Lundborg et al. found that ultrafine carbon particles impair the effectiveness of alveolar macrophage killing of bacteria [32]. Coarse particles were shown to induce high inflammatory activity in mouse macrophages, but fine particles from areas with high photochemical activity were associated with the highest inflammatory activity. Ultrafine particles from wintertime in Prague, on the other hand, were highly cytotoxic [33]. Pertinent to the discussion of air pollutant developmental immunotoxicity is the experimental research conducted on lead, commonly present in ambient air. Critical windows for effects of prenatal lead exposure on T-cell maturation and macrophage function were identified in rodent studies [34]. Late gestational exposures gave rise to gender-specific effects of lead on thymic weight, delayed type hypersensitivity, numbers of monocytes, and IL-10 and IL-12 production. Also relevant is the finding of altered cytotoxic
T-cell activity in mice prenatally exposed to mainstream cigarette smoke [35].

Although an abundant epidemiological literature has addressed asthma in relation to air pollution, very little attention has been paid to prenatal influences on immune development. Nevertheless, research on tobacco smoke is relevant to ambient air pollution due to their similar chemical composition, as both are generated by combustion of organic matter. Tobacco smoke induces a wide range of immune responses. Early life vulnerability to cigarette smoke manifests as increased rates of lower respiratory infections, asthma or wheeze, middle ear disease and sudden infant death syndrome [36,37]. IL-13, a Th2 cytokine, appears to be induced by cigarette smoke exposure in a dose–response fashion [38]. A genetic polymorphism for the IL-1 receptor antagonist increased the effect of maternal smoking during pregnancy on the risk for childhood asthma [39] demonstrating how environmental influences interact with genetic predisposition to affect immune development.

An experiment in primates indicated that cigarette smoke exposure in either the prenatal period or the first few months of life, alters maturation of the immune system. Levels of IFN-γ, IL-2, and IL-10 mRNA levels and the ratio of CD4:CD8 lymphocytes differed in environmental tobacco smoke (ETS)-exposed rhesus monkeys as compared to those who received pure filtered air [40]. IL-6 mRNA was increased as a result of postnatal exposure. In contrast, mice exposed prenatally demonstrated a higher incidence of tumours and reduced cytokotoxic T-lymphocyte activity, but no changes in cytokine levels of lymphoid organ histology [35].

A series of studies on a birth cohort from two districts in the Czech Republic represents a major contribution to the epidemiological data available on immune system effects from prenatal and early postnatal exposures to ambient airborne particles and other pollutants. These studies have taken place in Teplice, a district with a long history of coal mining and coal-fired power plants, and Prachatice, characterized by lighter industry.

Beginning in the early 1990s, an air monitoring programme was put in place in these two districts [41], in response to serious concerns about air quality in the larger region of the Czech Republic known as the Black Triangle, which includes northern Bohemia, where Teplice is located. Daily measurements of SO₂, NO, NOₓ, NO₂, NO₃ and CO were initiated. Additionally, measurements were made of fine and coarse particles and numerous PAHs on a daily basis during the five most polluted (winter) months of the year, every third day for another 5 months in spring and fall, and every sixth day during 2 summer months. In May 1994, the Pregnancy Outcome Study was launched, which, over the next 4+ years, enrolled about 7500 mother-infant pairs, or close to 90% of all births in the two districts, and collected maternal information at the time of delivery. Simultaneously, a study of a stratified random sample of about 20% of the births began collection of maternal and cord blood specimens as part of the Immune Biomarker Study. A total of 1492 mother-infant pairs were enrolled in this study.

Specimens were transported to Prague and processed at the Institute of Experimental Medicine, Academy of Sciences, for lymphocyte immunophenotyping using flow cytometry [42].

In multivariate-adjusted linear regression, the distributions of lymphocytes were associated with district of residence and season of birth in a consistent manner. Specifically, children born in Teplice or in winter months (locations and times of the highest air pollution) had reduced T cells and higher proportions of natural killer cells in cord blood. These results suggest that long-term or medium-term higher exposures to particles or other associated air contaminants, such as SO₂, NOₓ or PAHs, alter the relative production of specific immunophenotypes in late gestation [42]. Furthermore, when the daily monitoring data were incorporated into the analysis, neonates with higher short-term (14-day) late gestational exposures to PAHs and fine particles had a relative reduction in all T cells, and in CD4+ and CD8+ cells separately, with a concomitant increase in B lymphocytes (CD19+ cells) [43]. Thus, the Teplice Programme Immune Biomarker Study indicates long-term chronic exposures, seasonal influences and short-term elevations in ambient PAHs and fine particles can perturb either the development or functioning of the neonatal immune system. The target site or mechanism remains to be elucidated.

In this same study population, associations between gestational age-specific air pollution and cord serum IgE levels were investigated. These data suggest that an elevated cord IgE concentration is more frequent if high PAH exposures occur during the second trimester [prevalence ratio (PR) = 1.27, 95% confidence interval (CI) = 1.00, 1.61], while high PAH exposures during the first trimester confer some protection (PR = 0.67, 95% CI = 0.50, 0.91) [12]. Adjusting for season attenuated the odds ratios and the confidence intervals became wider (Herr et al. unpublished results). It is unclear whether an adjustment for season is appropriate; it could be a confounder, if associated with a causal factor, but it could also be serving as a proxy for exposure. Nevertheless, the findings are suggestive of a delicate timing of events for in utero development of an appropriately balanced immune response. The opposing direction of the effects for second as opposed to first trimester exposures is striking.

In further follow-up of the children, all paediatricians in the two districts cooperated, solicited participation from the parents and provided medical records for the study. Complete data were collected on a total of 1133 of these children, including extensive information about their early life home environment (e.g. passive tobacco smoke exposure), day care attendance and breastfeeding history. Longitudinal analysis of diagnoses of bronchitis between birth and either 3 or 4.5 years of age showed an effect from PAH and fine particle exposures [43]. After adjustment for temperature, season, calendar year, day of the week and numerous individual level variables, elevated rate ratios were found for various pollutant averaging periods ranging from 3 to 45 days. For example, an increase in the 30-day average PAH exposure of
100 ng/m³ was accompanied by a 1.29-fold elevation in the daily rate of bronchitis for children under 2 years of age, and virtually identical results were found for an increment of 25 μg/m³ of PM2.5. In children aged 2–4.5 years, these rate ratios were 1.56 and 1.23, with the confidence interval of the latter overlapping 1. Thus, throughout the preschool period, bronchitis diagnoses are increased following a month in which PCB exposures are elevated; in the first 2 years of life, an association is also evident for fine particle exposures, but the effect of particles is weaker after that age [43].

Supporting evidence for a role of PAHs in regard to respiratory problems in early life is also seen in a study from Poland. Personal air monitors were used to measure maternal second trimester exposures to PAHs. These values were then related to parent reports of symptoms over the first year of life. Risk ratios were elevated for barking cough, wheezing without cold, sore throat, ear infection and cough irrespective of respiratory infection. An interaction between these prenatal exposures and postnatal ETS was also observed [44].

Prenatal PCB exposures and early immune development
An abundant literature in experimental animals has established PCBs as potent immunotoxins. Atrophy of the thymus has been induced in numerous species, including rats, rabbits, guinea pigs and Rhesus monkeys [45–48]. Other outcomes observed in two or more species include: reduced skin tuberculin reactivity, reduced antibodies to sheep red blood cells and reduced antibodies to tetanus [49].

Wildlife studies have reported coplanar PCBs correlate with altered ratios of heterophils:lymphocytes and with enhanced mitogen-induced lymphocyte proliferation in sea turtles [50,51]. In bottlenose dolphins and beluga whales, non-coplanar PCBs were associated with neutrophil and monocyte phagocytosis [52]. PCB and PAH tissue residues were associated with immune cell toxicity in mussels [53].

Studies of human populations with high, accidental exposures to PCBs indicate lowered IgM and IgA and increases in respiratory infection during pregnancy or in the three preconception months. For instance, the difference for smokers versus non-smokers was 3%, and for infants whose mothers had a respiratory infection during pregnancy or in the three preconception months, 6%. The thymus growth for a 1-week longer gestation was +3%, while female babies had smaller thymic indices by ~10%.

Prenatal influences on atopy/asthma
A considerable body of research has investigated the role of early environmental exposures in asthma and/or atopy. Yeatts et al. [64] recently reviewed the literature on both susceptibility factors and environmental exposures in relation to incidence of asthma. Prenatal influences include birth order, maternal smoking, obstetric complications and elective caesarean section, with equivocal data on maternal use of antibiotics and maternal diet. Much work on early influences in asthma has focused on the 'hygiene hypothesis,' which posits that proper priming of the immune system in early postnatal life depends on microbial exposures.
Endotoxin has been suggested to play a key role. Nevertheless, the literature on hygiene-related factors is rather mixed. Asthma is known to be lower in children who attend day care or have more siblings, but other early life (first year) exposures have been associated with increased prevalence of asthma [65]: smoke or soot exposures, herbicides and cockroaches. Research on air pollution and asthma has tended to focus on prevalence or severity rather than incidence. One exception is the work on residential proximity to major roadways, suggested to be a better proxy of air pollution exposures than central monitors. Proximity within 75 m was strongly related to higher incidence of asthma in school children [66]. Few human studies, however, have specifically addressed intrauterine factors that may be instrumental in the development of asthma, such as diet, hormones, infections and environmental chemicals, and this gap may be partly responsible for the mixed results on hygiene factors [64]. Furthermore, the failure of many studies to distinguish atopic from non-atopic asthma may have hindered the ability to identify clear causal factors.

Although cord serum IgE was suggested to be predictive some years ago, the search for markers of high risk has intensified, with a variety of cytokines and other functional parameters taking center stage. As early markers of risk become identified, environmental research can focus on the interim end-points. Such studies are easier to conduct, because of the shorter induction period and possibly higher prevalence of the marker, and additionally provide information about mechanisms.

Although IFN-γ is lower in neonates than adults, it is reduced even further in those neonates who later develop atopic disease [67–69]. Likewise, children who develop atopic disease by age 3 exhibit depressed allergen-specific IL-6, IL-10 and IL-13 at birth [70]. It has been suggested that the immune development at birth in children who later develop disease is in a more immature state. In their comprehensive review, Jones et al. suggest that a normal process of apoptosis or anergization of antigen-reactive T cells must occur for the vast majority of babies to outgrow reactivity to cow’s milk [6]. They further postulate that suppression or delay in maturation of this apoptotic or anergic activity may be responsible for autoimmune disease.

IL-4 is a pro-inflammatory cytokine associated with in utero smoke exposure and with later risk of wheeze or atopy [71]. IL10 is an anti-inflammatory cytokine believed to regulate thymus-dependent lymphocyte responses and was found to be reduced in neonates who later developed atopy [70,72]. TGF-β is a family of cytokines involved in regulation of growth, differentiation and apoptosis of cells; these compounds have key roles in embryogenesis and levels in breast milk were inversely related to wheeze [70,72,73]. Thus, newborns may already be programmed for a tendency towards development of a normal versus atopic pattern of immune function, and both prenatal and early postnatal environmental factors may be critical.

### Immunological correlates of neurodevelopmental disorders

A growing number of research studies have reported that autism is characterized by substantial deviations in immune parameters. In 2004, Ashwood and Van de Water published a review of autoimmunity in autism and cited no fewer than eight previous studies or case series showing auto-antibodies against central nervous system proteins such as neuron–axon filament proteins and myelin basic protein [74]. Two separate studies have found mothers of children with autism to have different patterns of auto-antibodies to foetal brain tissue as compared with patterns from mothers of control children. The two studies found similar banding patterns using Western blots [75,76]. An array of cytokines, especially pro-inflammatory ones, are present at higher concentrations in children with autism than in typically developing controls [77]. Decreased plasma IL-23 has been observed in 2–5-year-old children with autism as compared to population-based controls [78]. Plasma leptin levels were also reported to be elevated in autism, especially in those with early onset [79]. Several HLA (human leucocyte antigen) genes have been reported to be associated with autism [80]. The association of autism in epidemiological studies with prenatal infections is also notable [28,81]. Other reports have noted an increase in auto-immune disorders in family members of persons with autism [82].

Parallels between autism and asthma have recently been noted. Becker et al. reviewed the evidence of similarities
between asthma and autism, including larger head size at birth, a shift in cytokines towards Th1 responses, a higher proportion of males affected as compared to females, some shared genes, such as phosphatase and tensin homolog, and a number of polymorphic markers discovered through genome-wide scans [83]. As asthma is known to be lower in children with more siblings, day care attendance or pets at home, it is hypothesized to result from an overly ‘hygienic’ lack of microbial exposure [29].


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