

REVIEW

Neonatal immune function and inflammatory illnesses in later life: lessons to be learnt from the developing world?

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Clinical & Experimental Allergy

Summary

With the emergence of allergic and autoimmune diseases in populations that have started to transit to a western lifestyle, there has been an increasing interest in the role of environmental factors modulating early immune function. Yet, most of the information concerning neonatal immune function has been derived from studies in westernized countries. We postulate that comparative studies of early immune development in children born under conditions that are typical for a westernized vs. that of a still more traditional setting will provide a crucial insight into the environmental-driven immunological mechanisms that are responsible for the world-wide rise in inflammatory disorders. In this review, we summarize the current understanding of early-life immune function in humans in general and the literature on some major lifestyle factors that may influence neonatal immune function and potentially the risk for disease in later life. An understanding of the mechanisms of 'prenatal/early-life programming' in populations living in traditional compared with modern societies is crucial to develop strategies to prevent a further rise in 'western diseases' such as allergic disorders. Indications exist that prenatal conditioning of the innate immune system by low-grade inflammatory responses is key to inducing more tightly regulated postnatal adaptive immune responses.

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Introduction

Over the past decades, inflammatory diseases such as allergic and autoimmune disorders have become an important and ever increasing health problem in industrialized countries. A similar trend is beginning to emerge in populations that have more recently started to transit to a more western lifestyle [1–3]. These observations suggest that modern lifestyle factors contribute to the increase in inflammatory diseases, in contrast to a protective role of more traditional lifestyle factors.

There is increasing evidence that the risk for inflammatory diseases in later life is to a certain extent determined by environmental risk factors influencing prenatal and early postnatal immune development [4–7]. The emerging paradigm is that under the influence of traditional lifestyle conditions, the developing immune system is 'educated' to tightly regulate pro-inflammatory immune responses and by doing so protects against inflammatory conditions that are characteristic for the developed world.

Under the assumption that this hypothesis proves to be true, there is much to be learnt from the process of immune development in infants born under still more

traditional lifestyle conditions. Yet, our knowledge of neonatal immune function and postnatal immune development in young children is predominantly derived from populations living in western societies, while we have limited understanding of how this compares with those born in traditional societies. In this review, we will first outline the current understanding of neonatal immune function, as studied predominantly in western populations, and then discuss the available evidence that several traditional lifestyle factors influence *in utero* immune development away from chronic inflammatory disorders.

Neonatal immune function

Innate immune responses in the newborn

The innate immune system provides a first line defence against invading pathogens and other danger signals and plays a pivotal role in the activation of adaptive immune responses. Recognition and immune activation occur through pattern recognition receptors (PRR) expressed on innate immune cells, of which Toll-like receptors (TLR) are

the most studied. Neonatal innate immune function crucially varies from that of an adult due to differences in both TLR signalling and antigen-presenting cells (APCs).

Toll-like receptor signalling pathways. Placental factors such as progesterone, prostaglandin E₂ and high plasma adenosine levels [8] may be responsible for the typical neonatal TLR-mediated cytokine response that, compared with adults, is characterized by a reduced production of pro-inflammatory cytokines such as TNF- α [9, 10], IL-12p70, IFN- γ and type I IFNs [8, 11–16]. In addition, neonatal innate immune cells show a reduced expression of certain TLRs [17, 18] and downstream components of the TLR signalling cascades such as CD14, MyD88 [19, 20], TNF-associated receptor factor, mitogen-activated protein kinase (MAP3K6), nuclear factor κ B (NF- κ B) and IFN regulatory factors-2, -5 and -7 [21], which may further contribute to this neonatal response profile. However, there are indications that activation of TLR-7/8 by the ligand resiquimod (R-848) provides a possible exception and in contrast to other TLR pathways can induce pro-inflammatory cytokine responses in neonatal innate immune cells [13, 22].

To date, there are little or no data available on human neonatal responses to other types of PRRs, such as nucleotide oligomerization domain-like receptors. Understanding the function of these in the neonate is likely to become an important new area of future research, in particular with respect to vaccine development.

Differences in neonatal TLR-mediated cytokine responses have been reported in relation to maternal allergy [23], ethnicity and season of birth [24]. Additionally, children that developed allergy within the first 12 months of life were found to produce higher inflammatory cytokine responses at birth than those that did not [23].

Differences in neonatal innate immune function have been also been demonstrated between children born in Papua New Guinea (PNG) and Australia, with cord blood mononuclear cells (CBMC) of PNG newborns producing significantly higher *Bacillus Calmette-Guérin* (BCG)-induced IL-10 and IFN- γ responses and expressing significantly higher TLR2 and TLR9 mRNA levels but significantly lower TLR4 mRNA levels as compared with cells of Australian newborns [25].

Antigen-presenting cells. Compared with adult cells, cord blood dendritic cells (DC) maintain a relatively immature phenotype with an attenuated expression of activation markers such as CD40, human leucocyte antigen (HLA)-DR, CD86 and CD80, even after stimulation with lipopolysaccharide (LPS) [9, 26–28]. In addition, cord DC express relatively low chemokine levels such as CXCL9, CXCL13, CCL18 and CCL24; have a reduced endocytic activity and suboptimal antigen presentation/co-stimulation capacity [27, 29–32]; and – as mentioned earlier – produce low

levels of the pro-inflammatory cytokine IL-12. Consequently, the immunogenic potential of cord DC and in particular their ability to induce T helper (Th) 1 cells, is poorer as compared with adult DC [33, 34]. In fact, it has been suggested that due to the relative functional immaturity of neonatal APC, early postnatal immune challenges are likely to result in immunological tolerance. For example, exposure of neonatal mice to killed bacteria or LPS was found to induce IL-10-producing tolerogenic DC, while reducing the risk for allergic and autoimmune disease development [35, 36]. Moreover, suboptimal co-stimulation and antigen presentation by immature neonatal DCs have been suggested to favour the development of regulatory T cells (*T_{reg}*) during the perinatal period [37, 38]. Nevertheless, an immuno-regulatory role for human neonatal APCs has not yet been conclusively identified; in fact, we have recently demonstrated that vaccination with a pneumococcal conjugate vaccine at birth does not result in immunological tolerance but in normal memory Th1 and Th2 responses [39] (A. H. J. van den Biggelaar, manuscript in preparation).

Variations in APC function in early life have been related to the development of allergic diseases in later life. For example, the capacity to produce IL-12 at birth has been inversely associated with allergen-specific immune responses in later infancy [40]. In addition, the maturation status of cord blood monocytes [41] and the number of circulating plasmacytoid DCs (pDCs) in infancy have been inversely associated with the development of atopic symptoms in later life [42].

A study comparing the composition of cord blood cell populations in Gabonese (Central African) and Austrian newborns found the first to have significantly higher percentages of monocytes and myeloid DC (mDC), but a significantly lower proportion of pDC [43]. In contrast, we found that cord blood of PNG compared with Australian newborn consisted of a significantly lower proportion of pDC as well as B cells and monocytes, but no difference in mDC proportions (J. G. Lisciandro, unpublished data). Finally, APC priming with IFN- γ has been reported to enhance BCG-induced pro-inflammatory responses including TNF- α , IL-12p70 and in particular IFN- γ production to a significantly higher extent in Australian compared with PNG newborns [25].

Adaptive and regulatory immune responses

At birth, the capacity of the adaptive immune system to induce memory responses is limited and is believed to gradually develop under the influence of postnatal microbial exposure.

T cell responses. There are only a limited number of human studies comparing T cell responses in neonates and adults, with little agreement between them on the

functional capacity of this immune compartment during the neonatal period. Some studies have reported evidence of *in utero* CD4⁺ Th cell priming, including proliferation and cytokine secretion in response to environmental allergens [44, 45]. In addition, functional CD8⁺ T cells displaying characteristics of adult CD8⁺ T cells were found to develop in response to human cytomegalovirus infection during fetal life [46]. Others have argued that neonatal T cell responses are very different from that of an adult. Holt and colleagues found that neonatal T cells display the characteristics of naïve recent thymic emigrants that undergo high levels of apoptosis and lack conventional T cell memory development. In addition, proliferation of cord T cells in response to allergens was found to be limited, short-lived and did not appear to involve conventional memory T cells, but was rather driven by functionally immature T cells following low-affinity T cell receptor–major histocompatibility complex (MHC)–peptide interactions [37].

In addition to impaired APC function, intrinsic deficiencies in the T cell compartment contribute to the limited ability to develop Th1 memory responses in early childhood. For example, the capacity of neonatal T cells to produce IFN- γ is further restricted due to hypermethylation of CpG motifs in the IFN- γ gene promoter [47, 48]. Interestingly, a recent study has disputed the universality of a type-2 skewed nature of Th responses in human newborns. In this large birth cohort study the relative balance of neonatal Th1/Th2 cytokine responses was found to be highly variable, to correlate with parental cytokine production and to be strongly influenced by familial factors including a shared environment [49]. This implies that alike for innate immunity, T cell function is influenced by the origin of the sample population, where both environment and genetics may modulate the response.

Deficiencies in the number of conventional T cells in neonates may further explain their high susceptibility to infectious diseases. Zhao and colleagues demonstrated that a lack of T cells in neonatal mice, which is 1–2 logs fewer than in adults, caused an unrestrained inflammatory response to LPS. Interestingly, adoptive transfer of neonatal or adult conventional T cells into neonatal mice suppressed this response [50]. This regulatory mechanism by neonatal T cells is yet to be confirmed in humans.

Differences in neonatal T cell function have been described in relation to allergy risk, although findings are inconsistent. Neonatal responses have been reported to be relatively Th2 skewed in children with a family history of atopy and/or that became atopic by 3 years of age [51–58]; although a reduced production of Th2 cytokines and higher production of IFN- γ has also been reported [51, 59]. Additionally, whereas some studies have reported that the number of immunocompetent CD4⁺ T cells is reduced in infants with a family history of atopy [60], others found no differences in the phenotype or function of neonatal T cells

in this risk group [61]. Despite these discrepancies, it is generally believed that the ability to re-direct the developing adaptive immune system towards a Th1 phenotype postnatally is delayed or impaired in atopic individuals [45, 62, 63].

Considering the influence of different environments, Kohler reported a significantly higher percentage of CD4⁺ T cells in Austrian compared with Gabonese CBMC, with Gabonese cord CD4⁺ T cells representing a more antigen experienced phenotype than Austrian cord cells [43]. In our preliminary studies, we found the percentage of CD4^{high} T cells to be significantly higher in cord blood samples of PNG compared with Australian newborns, however, cord blood of Australian newborns contained a significantly higher proportion of CD4^{low}, CD11c and CD14-expressing APC (J. G. Liscandro, unpublished data). No differences in maturation status were observed in our experiments.

B cell responses. B cell antibody responses are compromised during the neonatal period for different reasons. Firstly, the architecture of the neonatal spleen is not fully developed due to a lack of pro-inflammatory cytokines (such as TNF- α), which together with a lack of mature follicular DC limits germinal centre formation up until around 4 months of age [64–66]. Secondly, B cell receptor activation results in a failure to up-regulate the expression of MHC class II and co-stimulatory molecules [66]. This hampers cross-talk between B and T cells, which is essential for efficient production of antigen-specific (isotype-switched and affinity matured) antibodies, particularly in response to T cell-dependent antigens, as well as the development of B cell memory [65–67]. Generally, neonatal antibody responses appear to be delayed, short-lived and of lower antigen affinity. Additionally, lower levels of antibody are produced, particularly of the IgG2 isotype as compared with adult antibody levels [66, 68].

CBMC from Gabonese newborns have been demonstrated to contain a higher proportion of mature CD5⁺ B cells compared with that of Austrian newborns, suggestively due to higher *in utero* antigen exposure in the Gabon setting [43]. No other studies have addressed functional differences of B cells between geographically diverse neonatal populations.

T regulatory cell responses. In line with the naïve phenotype of neonatal CD4⁺ T cells, cord blood T_{reg} cells express the gut homing receptor $\alpha 4\beta 7$, CD27 and CD45RA, which is in contrast with the CD45RO⁺ and CCR4⁺ memory phenotype of adult peripheral blood T_{reg} cells [69–71]. Interestingly, it has been reported that maternal allo-antigens can cross the placenta to induce CD4⁺CD25^{hi}-Foxp3⁺ T_{reg} in the fetus that are capable of suppressing fetal anti-maternal immune responses and can be detected until late childhood [72].

Relatively little is known about T_{reg} function in newborns and findings are sometimes contradictory; while

some studies have reported that cord blood T_{reg} cells express normal levels of Foxp3 and are efficient immunosuppressors [73, 74], others have found the expression of Foxp3, the production of TGF- β and the suppressive activity of cord blood $CD4^{+}CD25^{\text{hi}}$ cells to be reduced as compared with adult cells [75–77].

Interestingly, children born to atopic mothers have been reported to have reduced T_{reg} cell numbers and reduced T_{reg} -mediated suppressive activity at birth in comparison with children born to non-atopic mothers [78, 79].

With respect to geographically diverse populations, cord blood samples from Gabonese newborns have been shown to contain significantly lower numbers of $CD4^{+}CD25^{\text{hi}}$ cells relative to Austrian newborns [43], and in accordance we have found a lower proportion of $CD4^{+}CD25^{\text{hi}}CD127^{-}$ cells in PNG compared with Australian cord blood samples (J. G. Lisciandro, unpublished data). No differences in T_{reg} function between newborns of different environments have been reported, although the expression of Foxp3 and CTLA-4 was found to be reduced on Gabonese compared with Austrian neonatal T_{reg} [43].

The potential impact of traditional lifestyle-associated factors

There is increasing evidence that several factors that are common but not restricted to more traditional lifestyles may modulate early immune function and development. Of these, we discuss here the prenatal influence of microbial and parasite exposure, nutrition and biomass fuel/tobacco smoke exposure. Preterm birth will be reviewed as a separate entity although most of the factors discussed here are also risk factors of preterm delivery. The effects of these prenatal exposures on neonatal immune function and the associated outcomes are summarized in Table 1.

Microbial exposure

The 'hygiene hypothesis' postulates that microbial stimulation in early life is required to drive immune maturation and the induction of regulatory mechanisms that are protective against inflammatory conditions in later life. This is supported by epidemiological studies demonstrating inverse relationships between allergic or autoimmune outcomes and exposure to surrogate measures of microbial burden such as living in a farming environment, larger family sizes, daycare attendance and having pets [145–150].

Microbe-driven immune modulation already begins before birth. As demonstrated in experimental studies, postnatal immune responses were modified and characterized by reduced allergic responses in animals exposed to microbial products *in utero* [4, 5, 82]. Accordingly, in humans prenatal exposure to a farming environment has been associated with Th1-skewed immune responses and

enhanced T_{reg} numbers in cord blood [7, 83] as well as altered innate immune function and reduced risk of allergic disease at school-age [6, 81]. Furthermore, maternal intake of probiotic bacteria by consumption of unpasteurized farm-produced yoghurt has been associated with reduced cord blood pro-inflammatory cytokine production [83]. Thus, *in utero* microbial exposure may 'educate' the immune system before birth to appropriately respond to allergens and auto-antigens and hence prevent disease in later life. The mechanism by which prenatal immune function is microbially modulated is unknown; however, a recent animal study showed that the asthma-protective effects of prenatal exposure to cowshed bacteria were dependent on maternal TLR signaling and potentially implicate a role for neonatal DC as mediators of these effects [63, 80].

Parasite infections

In contrast to microbes, parasites can survive in the human host for an extended period of time by evading or suppressing the host's immune response. Parasite-driven immuno-modulation is not confined to parasite-specific responses per se, but as demonstrated for chronic and/or high-intensity infections with *Schistosoma haematobium* [93] as well as *Ascaris*, *Trichurias*, hookworms and filarial worm infections [151], can spill-over and suppress bystander responses mediating atopy.

First evidence that maternal parasite infections can have a prenatal immunomodulatory effect came from malaria studies showing that infants born to *Plasmodium falciparum*-infected mothers were at greater risk to become malaria-infected during childhood than children whose mothers were non-infected [87, 88]. The same phenomenon has been observed for children born to mothers infected with the filarial parasite *Wuchereria bancrofti* [89].

Several studies have investigated the immunological effects of *in utero* parasite exposures. Malhotra and colleagues demonstrated that children born to mothers infected with *S. haematobium*, *Wuchereria bancrofti* and/or other intestinal helminths underwent *in utero* sensitization and were capable of producing parasite-specific cytokine responses that were similar to those observed in adults [84, 85, 89]. This is in accordance with studies demonstrating prenatal priming of adult-like $CD8^{+}$ T lymphocyte responses to *Trypanosoma cruzi* infection [86]. At the same time, *in utero* parasite exposure may lead to the development of immune tolerance, potentially via the generation of T_{reg} cells [87, 90–92]. Interestingly, the regulatory response was found to dominate the effector response in infants born to mothers with chronic placental malaria infection whereas in children born to mothers with resolved infections at the time of delivery both regulatory and Th1 pro-inflammatory responses had been induced [152]. In addition, malaria-in-pregnancy

Table 1. Prenatal exposure to various environmental and lifestyle factors and the impact on neonatal immune function and associated outcomes

Prenatal exposure	Impact on neonatal immune function	Reference	Outcome(s)	Reference
High microbial burden*	↑ Proportion of mature CD5 ⁺ B cells in CBMC	[43]	↓ Risk of allergic disease at school-age	[6, 81]
	↑ Cord blood T_{reg} numbers and functions, suppression of Th2 responses	[7]	↓ Allergic responses in animal models	[4, 5, 82]
	Th1-skewed CBMC responses and also ↓ cord blood pro-inflammatory cytokine production	[83]		
	↑ Gene expression of TLR2, TLR4, CD14	[6]		
Parasite infections*	Adult-like parasite-specific CBMC cytokine responses	[84–86]	↑ Risk of parasite infection postnatally	[87–89]
	Generation of T_{reg} cells mediating immune tolerance	[90–92]	Non-specific effects: ↓ Atopy at school-age Altered vaccine responses	[93] [94, 95]
	Altered neonatal innate immune responses: Partially matured DCs (↑ HLADR expression but not CD86) and ↑ cytokine response to TLR9 stimulation	[96]		
	↑ Numbers of mDC	[97]		
	Altered pro-inflammatory cytokine production in responses to LPS and PolyI : C (↑ IFN- γ but ↓ TNF- α)	[98]		
Under-nutrition/low birth weight*	Potential atrophy of thymus and ↓ production of thymic hormones, which affect thymic T cell development and function in the periphery	[99–101]	↑ Risk of asthma, but protective against atopic eczema	[102, 103]
			↑ Risk of infection-related mortality in adulthood	[104, 105]
Preterm birth*	Lack of passively derived maternal antibodies	[165]	↑ Risk of wheezing and asthma	[114, 115]
	Impaired innate immune responses: Low mannose-binding lectin levels	[165]	No association with eczema or food allergy	[106, 116, 168]
	↓ capacity of neonatal monocytes to produce IL-12 in response to IFN- γ priming and LPS	[166]		
	↑ expression of immune biomarkers: IL-2, -4, -5, -8, -10, monocyte chemoattractant protein 1, macrophage inflammatory protein and TNF- α	[167]	↑ Risk of infections and sepsis, particularly those caused by Gram-positive bacteria	[132–134]
High fat (Western) diet	↓ Numbers of splenic lymphocytes, thinning of the thymic cortex, impaired antigen-specific immune responses and ↑ TNF- α and IgE production (shown in mouse model only)	[117]	↑ Risk of atopic disease	[118–120]
			↑ Wheeze and eczema	[121, 122]
ETS	↑ IgE cord blood levels	[139], [181]	↑ Risk of childhood asthma, allergies and respiratory tract infections	[131, 138, 163]
	Attenuated cytokine responses to innate TLR stimuli	[185]		
	↑ Neonatal Th2 responses (particularly IL-13) to allergens and ↓ IFN- γ production	[140, 185]	Impaired lung function	[182, 183]
	Enhanced CBMC-proliferative responses to allergens	[130]	↑ Bronchial hyper-reactivity	[184]
Industrial/traffic-related air pollution	↓ Total T cells (particularly CD4 ⁺) but ↑ CD19 ⁺ B and natural killer cells in cord blood	[123, 124, 128, 160]	↑ Risk of developing airway hyperresponsiveness and allergic sensitization	[125, 126]
			↑ Risk of childhood asthma	[127]
Biomass fuel use*	Unknown	–	Low birth weight, intrauterine growth restriction and preterm parturition	[144, 160–162]
Vitamin D intake	Induces cord blood APCs with tolerogenic properties	[109]	Controversial: Protective effect against wheezing, asthma and allergic rhinitis in childhood	[107, 110]
			But ↑ atopy also reported	[108]

*Exposures that are more common in developing vs. Western world environments.

APC, antigen-presenting cells; DC, dendritic cell; mDC, myeloid DC; CBMC, cord blood mononuclear cells; LPS, Lipopolysaccharide; TLR, Toll-like receptor.

can affect neonatal innate immune responses in the newborn, as demonstrated by a studies in Africa where children born to *P. falciparum*-infected mothers exhibited partially matured DCs [96], higher numbers of mDCs [97] as well as significantly modulated IFN- γ and TNF- α responses following stimulation with TLR agonists LPS and PolyI:C at birth [98].

Exposure to helminths during pregnancy has been reported to compromise the infant's ability to develop protective Th1-mediated responses to BCG vaccination, indicating a non-specific prenatal effect of helminth infections on T cell development [94]. In contrast, *in utero* exposure to *T. cruzi* has been associated with enhanced Th1 responses to vaccine antigens [95].

Altogether these studies indicate that the increase in allergic and auto-immune diseases in the western world may to some extent be explained by the eradication of chronic parasite infections whose immuno-suppressory specific and non-specific effects are likely to start *in utero* and last until later life.

Nutrition

According to the 'fetal and infant origins of adult disease' or Barker hypothesis, reduced fetal growth increases the risk to develop chronic degenerative diseases in adult life [153]. It is now emerging that this hypothesis extends to the development of chronic immunological disease. For example, a Swedish twin cohort study recently reported that low birth weight is a risk factor for asthma, but protects against atopic eczema [102, 103]. However, the effect of prenatal malnutrition on immune development is not restricted to inflammatory illnesses, but can also impair the development of protective immune responses. In a study performed in rural Gambia, children who were born during the 'hunger' season and had low birth weights were demonstrated to be at a 10-fold higher risk of infection-related mortality in adulthood compared with those born during the 'harvest' season [104, 105]. And in a Filipino study, prenatal malnutrition has been associated with reduced antibody responses to typhoid vaccination in adolescence [99, 100]. These studies are suggestive of an irreversible change in postnatal immune function as a result of starvation during fetal life. This may be explained by the extreme vulnerability of lymphoid tissues, particularly the thymus, to atrophy [101] and the reduced production of thymic hormones that are involved in thymic T cell development and peripheral function in states of malnutrition [99, 100]. In addition, exposure to prenatal famine has been linked to epigenetic changes that persist in adult life and potentially alter disease risk [154].

In contrast to starvation, the increasing epidemic of obesity in the western world have been associated with changes in immune function that enhance the risk for atopic disease [118–120]. Additionally, the increased con-

sumption of a low-fibre diet in the western world has been associated with changes in the composition of gut microbiota that may explain the rising incidence of colitis and other autoimmune conditions [155]. The immuno-modulatory effects of diet/nutrition likely begin prenatally [130, 156, 157]; a recent murine study reported that a high fat diet in the fetal period resulted in reduced numbers of splenic lymphocytes, thinning of the thymic cortex, impaired antigen-specific immune responses and elevated TNF- α and IgE production, which are suggestive of immune regulatory dysfunction and a greater tendency towards inflammation [117]. Other components of a western diet that may be immunomodulatory and/or increase inflammatory responses in early life include a reduced intake of n-3 polyunsaturated fatty acids, dietary contaminants such as by-products from industrial and agricultural practices, as well as feeding newborns and infants on formula instead of breast milk (reviewed in [158]). In addition, a reduced consumption of antioxidant-rich foods such as fruit and vegetables and a higher intake of meat during pregnancy has been linked to a higher risk of wheeze and eczema in infants [121, 122].

Lastly, vitamin D intake during pregnancy was shown to induce cord blood APCs with tolerogenic properties including higher Ig-like transcript (ILT)-3 and -4 expression [109], and was associated with a protective effect against wheezing, asthma and allergic rhinitis in childhood [107, 110], although the opposite has also been reported [108]. These contradictory findings may be explained by differing study designs, where maternal vitamin D status was ascertained either by food frequency questionnaire, report of supplementation and/or a single serum measurement of maternal vitamin D. Given that maternal vitamin D status may change over the course of pregnancy and is not only solely determined by food intake and supplementation but also sun exposure, the melanin content of the skin and internal regulatory processes that tightly govern the conversion of vitamin D to its active form, calcitriol, future observational studies measuring serum calcitriol multiple times throughout pregnancy with a follow-up of the children may help to resolve these conflicting findings.

Overall, these findings suggest that low birth weight and prenatal starvation are risk factors for reduced immune function in later life, whereas obesity and a western diet are likely associated with an increased inflammation.

Air pollution

The use of biomass fuels such as wood, charcoal and other organic matter, often in the absence of adequate ventilation, is commonplace in many developing countries. The burning of this form of energy results in the release of hundreds of toxic air pollutants that have been associated with an increased risk of developing respiratory illnesses

[141–143, 159]. With respect to prenatal exposure, biomass fuel use has been associated with low birth weight, intrauterine growth restriction and preterm parturition [144, 160–162].

The impact of biomass fuel pollution on prenatal immune development and long-term immune function is unknown. Relatively more is known about prenatal exposure to environmental tobacco smoke (ETS), which is another form of indoor air pollution that has been associated with an increased risk of childhood asthma, allergies and respiratory tract infections [131, 138, 163]. Indeed, *in utero* ETS exposure has been linked with enhanced IgE cord blood levels, increased production of IL-4 and IL-13, reduced serum levels of IFN- γ and attenuation of cytokine responses to innate TLR stimuli [139, 140]. Furthermore, the rise in traffic-related pollutants that results from progressive urbanization has been associated with an increased risk of developing airway hyperresponsiveness, allergic sensitization [125, 126] and childhood asthma [127]. In addition, a study conducted in the Czech republic during the 1990s reported that children born in locations at times of greatest industrial air pollution had reduced percentages of total T cells, particularly CD4⁺ cells, but increased proportions of CD19⁺ B lymphocytes and natural killer cells in their cord blood [123, 124, 128, 160].

The relative exposures to ETS and other types of industrial/traffic-related air pollutants in developing compared with developed world regions is highly variable, with exposure likely being the lowest in the poorest areas and the highest in populations that are transmitting or have transmitted to modern western lifestyles. On the contrary, biomass fuel use is the highest in poorer populations, and studies addressing the impact of biomass fuel use on immune development in developing countries would be of high interest.

Preterm birth

Preterm birth, defined as parturition at <37 weeks of gestation, is the most common cause of perinatal morbidity and mortality in developing countries. In fact, infants born prematurely are 40 times more likely to suffer neonatal mortality compared with term infants [129]. Premature birth may result from a number of causes including intrauterine infection (30–50% risk of preterm birth) and/or inflammation, stress and uterine overdistension [135, 136]. In the case of infection, preterm birth is thought to be mediated by pro-inflammatory cytokines such as TNF- α and IL-1 β [17, 137].

Preterm children are at high risk for infections, in particular Gram-positive infections. The risk of invasive disease and the severity of infectious outcomes are inversely related to the degree of prematurity, with those born very preterm (<32 weeks of gestation) being most vulnerable [132–134]. The few studies that have compared

immune function in term with preterm newborns indicate that in addition to the lack of passively derived maternal antibodies, impaired innate immune responses likely play a role in the high susceptibility of preterm newborns [164, 165]. Recent studies have demonstrated that neonatal monocytes of very preterm newborns have an even further reduced capacity to produce IL-12 in response to IFN- γ priming and LPS stimulation as compared with term newborns [166], whereas levels of immune biomarkers such as IL-2, -4, -5, -8, -10, monocyte chemoattractant protein 1, macrophage inflammatory protein and TNF- α were shown to be increased [167].

A number of studies have found that prematurity is associated with an increased risk for asthma [114] and wheezing in later life [115] although it is not clear to what extent this is explained by physiological rather than immunological reasons, considering that such associations have not been found for allergic disorders such as eczema and food allergy [106, 116, 168].

Preterm birth may be an independent risk factor for neonatal immune modulation or be indirectly related to other risk factors such as *in utero* exposure to microbial and parasitic infections, indoor air pollution and/or poor maternal diet.

Other lifestyle factors

In addition to the risk factors discussed above, a number of other environmental factors may alter prenatal immune function and the risk for developing inflammatory diseases in later life. One such factor is the intake of certain vitamins and minerals during pregnancy. For example, prenatal vitamin E and zinc intake have been found to protect against asthma and other atopic symptoms in early childhood [111, 112] and as discussed previously, the intake of vitamin D during pregnancy may also influence the risk of these diseases, although at present its role is controversial.

Exercise during pregnancy may also influence a child's risk of developing inflammatory diseases; maternal exercise has been inversely associated with cord IgE levels [113] and the incidence of pre-eclampsia [169], which suggest it may help to prevent an inflammatory environment during pregnancy. To our knowledge, there are currently no studies specifically addressing the effect of maternal exercise and a sedentary lifestyle on prenatal immune development or the risk for atopic and autoimmune diseases in later life.

Delivery by caesarean section, which is more common in the western world than the developing world, is associated with an enhanced risk of developing atopy [170, 171]. Other maternal lifestyle factors that may affect prenatal immune development but have not been discussed here include parity [172], alcohol intake and antibiotic use (reviewed in [173]).

Environmentally driven epigenetic mechanisms

There has recently been much interest in the contribution of environmentally driven epigenetic mechanisms to the *in utero* programming of susceptibility to immunological disease. This stems from findings that environmental changes associated with westernization, such as reduced microbial exposure and altered diet may propagate the risk for allergies and autoimmunity via the activation or silencing of immune genes (reviewed in [174, 175]). These epigenetic changes occur by modifying patterns of DNA and histone methylation, histone acetylation and chromatin structure, without altering the nucleotide sequence of the genome. Indeed, it is now recognized that a number of immune pathways are under epigenetic control, including Th1/Th2 cell differentiation (reviewed in [176]), Foxp3 expression and the induction of T_{reg} cell populations [177–179]. Further support for epigenetic modulation of early immune development comes from a study showing that maternal supplementation with the methyl donor folate enhanced allergic airway disease in their offspring [180].

Currently, little is known about the contribution of epigenetic mechanisms to *in utero* developmental programming in low-income countries. Future research in this area will be of high relevance to further understanding the role of environment in modulating susceptibility to immunological disease under affluent and adverse living conditions.

Conclusions and clinical relevance

There is increasing evidence that under the pressure of traditional lifestyle conditions, prenatal conditioning of the innate immune system results in more tightly regulated postnatal adaptive immune responses that are associated with a reduced risk to develop allergic and autoimmune diseases. This implies that studying the way in which environmental factors modulate early immune development is key to improve our understanding of the driving force behind the epidemic of allergy and other chronic inflammatory disorders in westernized countries and to limit the emerging rise of 'western diseases' in populations transiting to a more western lifestyle. We suggest that human studies addressing differences in neonatal immune function and postnatal immune development under variable environmental pressures of developing world settings will play a crucial role herein.

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