

Transient environmental exposures on the developing immune system: implications for allergy and asthma

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Purpose of review

Early environmental exposures have been extensively studied as potential causes of the observed increase in allergic disease over time. Transient fetal or neonatal exposures in particular are of interest in that they may occur during critical windows of immune system development. Due to the tremendous complexity of variables in early life, as well as the difficulty in randomizing many interventions, it is very difficult to properly study these exposures. Some progress, however, has been made and some more candidates for study may be emerging. Of particular interest are micronutrients, whose ever-changing use and immunomodulatory capabilities make them prime targets for study.

Recent findings

New risk factors for atopic disease have emerged from the pool of early life interventions, such as caesarian section, prolonged labor and infant multivitamin supplementation. Data are emerging regarding micronutrient status and supplementation and their effects on the developing immune system and risk for allergic disease. Clinical trials have yet to demonstrate much causality but, in some cases, it is too early to make any judgments.

Summary

The gold standard of randomized clinical trials has not borne out a number of proposed early-life allergic risk factors, while other trials are too incomplete to draw any conclusions so far. Properly designed studies for other risk factor interventions may still be achievable, provided that there is a proper understanding of the interventions, populations and outcomes.

Keywords

immunomodulation, neonate, vitamins

Abbreviations

Ig	immunoglobulin
PUFA	polyunsaturated fatty acid
RSV	Respiratory Syncytial Virus
Th	T-helper

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Introduction

Fetal or early infant exposure can influence the development of the immune system and possibly explain some of the recent increase in atopy. The changes in the environment and practices of modern culture and medicine provide many variables for study: prenatal diet and supplements, perinatal delivery, resuscitation and feeding practices, as well as vaccination, general health maintenance and hygiene, are all more good examples of practices which have changed markedly over the years.

This review will discuss recent animal and human studies which serve as the basis for understanding how early exposures could have long-term immunomodifying effects. Special attention will be paid to recent basic science and clinical studies which attempt to link a prototypical early exposure – diet and micronutrients – to atopic outcomes. Finally, we will outline tools available to study early exposures and comment on the difficulties of using them to separate association from causality.

The immunologically impressionable infant

Our developing understanding of the neonatal immune system suggests that dynamic processes are occurring and that they differ from adult immunologic processes, beyond mere growth and expansion. Such differences could therefore impart unique susceptibilities to immunomodulation. Conversely, these differences may be evolutionarily adapted to cause the immune system to be relatively resistant to modulation as compared to the adult's, because the growth and development period, by definition, is characterized by so much cellular division and differentiation. Neonatal immune responses have been re-evaluated in the past decade in order to understand why certain neonatal responses are vigorous despite the assumption that the neonate is inherently immunosuppressed and why certain antigenic exposures can actually increase the risk for subsequent immunopathology, despite the

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long-held belief that the antigenic exposures in early life were tolerizing [1].

Animal data show that initial neonatal antigenic exposure increases the risk for subsequent T-helper (Th) 2 responses as compared to initial antigenic responses later on. For example, a recent animal study using Respiratory Syncytial Virus (RSV) infection showed markedly worse, Th2-dominated responses, during re-infection in mice sensitized to RSV very early in life as opposed to those sensitized at several weeks of age [2]. Human data show that initiation of cows'-milk feeding neonatally versus at 9 months of age has marked differences in immune response, with responses noted to the antigen during infancy but not later in life (early has one, late does not [3]); elucidating whether these clinical observations are due to physiologic properties of gut absorption, temporal defects in the concept of oral tolerance, or reflect disruption of normal immunologic development will be very helpful in understanding the impact of early exposures on the immune system.

Exposures during pregnancy, labor and delivery and their immunologic impact

A number of studies have tried to look at a variety of early-life risk factors for the development of atopy. Many have examined how specific antigenic (allergic and infectious) histories during early life have affected subsequent specific and general allergic histories [4–7]. Others have looked at specific events, such as transient and prolonged labor (perhaps due to exposure to stress hormones) [8^{••}], and caesarian section (perhaps due to the altered gut flora from infants not exiting via the birth canal) [9], both of which were associated with atopy. Moving beyond atopy, other pathology related to the immune system may occur from even brief oxygen exposure during routine neonatal resuscitation. Such exposure can increase inflammatory mediators (usually free radicals) for months [10]. Some even suggest that exposure to as few as 3 minutes of 100% O₂ increased the risk for childhood cancers [11,12].

A recent case-control study attempted to see how a variety of infectious episodes, pharmaceutical interventions and uterine difficulties affected the risk for atopic and non-atopic asthma risk beyond age 3 as measured by a clinical diagnosis of asthma with and without positivity to any of 10 chosen skin-prick antigens [6]. Maternal febrile episodes treated with antibiotics were associated with increased risk for atopic asthma only, while influenza infection was associated with increased risk for non-atopic asthma. The tentative nature of these reports and the contradictory state of the literature are illustrated by another recent prospective study of febrile episodes in infants within the first year of life, which demonstrated protection from atopy for each febrile episode [13^{••}].

Transient exposure to cows'-milk-based formula

There has been much study attempting to understand how formula feeding can affect the risk of atopy and specific food allergies. While breast-feeding appears to be protective [14], the data are not consistent. Furthermore, the practice of delaying the introduction of certain foods in at-risk individuals does not appear to change the risk for development of food allergies [5] and may, in truth, be more valuable for simply bypassing the age window when any food allergies tend to occur.

While long-term exposure to cows'-milk-based formula has been extensively examined, a group of studies particularly relevant to the topic of transient neonatal exposures can be found in investigating the common practice of very briefly supplementing otherwise exclusively breast-fed newborns with formula when breast milk supply appears insufficient. A number of non-randomized prospective studies have been performed, with mixed results. Some support the notion that brief feedings have no effect either clinically or in measuring cows'-milk-specific IgE [15,16], some indicate that brief feedings were a risk factor for cows'-milk allergy and/or increased cows'-milk-specific IgE with decreased IgG A and M to cows' milk [17–19], and one actually shows a decrease in allergic symptoms by 5 years of age (particularly eczema) in infants given brief feedings with cows'-milk-based formula [20]. One confounder in these studies is that infants who appear to clinically require such supplementation may have other underlying processes going on which are affecting the development of their immunologic machinery. The only large randomized controlled trial showed no differences in atopic diseases, food allergies or IgE RAST to cows' milk by 5 years of age in placebo versus brief cows'-milk exposure during infancy [21]. Whether this study is the definitive answer on this issue is unclear. Nonetheless, it provides an excellent example of the study of prolonged immunologic consequences to very transient exposures. It also serves as an example of strong statistical associations not being borne out in randomized study.

The role of dietary vitamins and other micronutrients in the risk for atopy

While the above-mentioned variables have yielded potentially promising data, many of them are very difficult to study due to their complex nature, which leads to a large number of confounding possibilities. Dietary micronutrients have discrete effects on the immune system *in vitro* and their supplementation in a variety of clinical scenarios could be very easy to randomize. Below is a brief and certainly incomplete summary of studies already ongoing and other potential candidates for future study.

N-3 polyunsaturated fatty acids

N-3 and n-6 fatty acids have opposing effects on the formation of prostaglandin E-2 – an eicosanoid implicated in the pathogenesis of atopy. Study of Western diets shows steadily decreasing n-3 intake with a concomitant increase in n-6 intake over the period of time that allergies have increased. The role of n-3 polyunsaturated fatty acid (PUFA) supplementation in the prevention of atopy has been reviewed previously in this *Journal* [5]; however, the ongoing studies have begun to yield significant amounts of data in the past few years.

A number of more recent cross-sectional and cord blood studies have continued to show an association between increased n-3 intake/levels and reductions in risk for various laboratory and clinical atopic outcomes for atopy [22–25]. Results from randomized control trials have not been consistent. One randomized controlled trial of n-3 PUFA supplementation of pregnant mothers showed lower tendencies toward allergen-specific cytokine production in the intervention group with a tendency for lower prick test positivity and severe atopic dermatitis by 1 year of age [26]. In contrast, another randomized study providing n-3 supplementation of formula (for those participants who were formula-fed) prior to 6 months and supplementation of all participants diet after 6 months showed no effects on wheeze, skin prick atopy or eczema, and a mild improvement in cough rates for the intervention group [27••]. Larger sample sizes with long-term follow-up will be needed to determine the importance of PUFAs in the development of atopy. Future studies need to evaluate whether supplementing pregnant mothers has a comparable impact to supplementing infants.

Vitamins

Vitamins have been shown to have a multitude of effects on immune function. Of particular interest is the ability for certain vitamins to tend to skew towards either Th1 or Th2 phenotypes. Most studies have shown that vitamins A and D tend to skew towards Th2, while C and E skew towards Th1 phenotypes [28]. The clinical relevance of these observations is far less clear. Several major epidemiologic studies have shown that vitamin D supplementation may prevent insulin-dependent diabetes mellitus – a disease thought to be mediated by Th1-associated autoimmunity, although the exact mechanism was not pursued [29,30].

Vitamins C and E

Significant interest in the role of dietary antioxidants such as vitamins E and C in the prevention or reduction of asthma also exists. Data are usually cross-sectional, providing only a snapshot of the nutritional or health status of the subjects. Results have been mixed, with no clear

evidence that any vitamins reduce asthma symptoms in those already with disease, and that perhaps vitamin E could prevent asthma or atopy [31–35]. A recent prospective observational study of lactating women showed that in breast-fed infants, elevated vitamin C found in breast milk is associated with reduced risk of atopic dermatitis and allergen sensitivity at 12 months of age [36]. Without randomization, and without further follow-up, it is difficult to reach any conclusions; however, the results are intriguing. It may also serve as a cautionary note that dietary vitamin C intake correlated with breast milk vitamin C levels far better than supplemental vitamin C intake. To best understand the roles of these vitamins, we will have to pick apart whether the micro-nutrient exerts its effects on oxygen-free radicals, the adaptive immune system itself or other aspects of the pathogenesis of atopy or asthma.

Vitamin D

Vitamin D's role in the pathogenesis of atopy has been examined on a limited basis in a few recent studies. Matheu *et al.* showed that regular calcitriol (1,25-[OH]₂-D3) injections during allergic sensitization increased total and allergen-specific IgE, while injection during challenge actually decreased pulmonary eosinophilia, suggesting a complicated and divergent role for vitamin D in allergen sensitization and end-organ pathogenesis in asthma [37]. It should be pointed out that this system is problematic, as calcitriol is the active form of vitamin D – not the nutrient commonly ingested – and its formation is tightly regulated [38]. Adding to the complexity of vitamin D's interaction with atopic models in mice, a recent study showed that the vitamin D receptor null mouse has increased GATA-3 and serum IgE production, but cannot undergo allergen-induced bronchoconstriction [39]. Vitamin D receptor polymorphisms were recently demonstrated to be associated with serum IgE and asthma risk in humans [40]. Given that formula tends to have significantly higher amounts of vitamin D than breast milk, and given our ever-changing practices of sun exposure, especially to infants, which directly impacts vitamin D levels, vitamin D should be a very important nutrient to study in the pathogenesis of atopic disease, and could serve as a model for study of early-life exposures having long-term impact.

Vitamin A

In-vitro data showing Th2 skewing by retinoids have caused some to theorize that increased vitamin A may either systemically or locally increase the risk for asthma [41]. Existing data seem to indicate otherwise, as vitamin A levels in children who were severe asthmatics were lower than those without in a cross-sectional study [42]. Also, the interaction of vitamin A and vaccination has been studied extensively in children, as, in some countries, the two are given together [43,44]. Little impact of

vitamin A on clinical vaccine responses exists. There have been no prospective studies of vitamin A and its influence on allergy or atopy.

Infant multivitamins

One of the authors of this review recently demonstrated in a retrospective cohort study based on the National Maternal–Infant Health Survey and Longitudinal Follow-up that infant multivitamin supplementation for more than 1 month prior to 6 months of age was associated with a mildly increased risk for parental report of physician-diagnosed asthma and/or food allergy by age 3 [45]. African-American infants had about a 30% increased risk for asthma by age 3 if supplemented prior to 6 months of age, while formula-fed infants, regardless of race, had about a 60% increased risk for food allergies by age 3 if supplemented over and above their formula prior to 6 months of age. While a number of confounders were controlled for, family history of atopy, amongst others, was not. Longer follow-up and more careful histories (which are unavailable for this particular database) are needed, but this too could serve as a model for study of exposures during early infancy.

More recently, in a study based on the 1966 Northern Finland Birth Cohort, atopy, allergic rhinitis and asthma diagnosed by age 31 were associated with vitamin D supplementation in infancy with adjusted odds ratios similar to the ones seen in the previously mentioned study. Family history of allergy and asthma was controlled for and, interestingly, subjects who were suspected of having rickets (due to severe vitamin D deficiency) during infancy tended to have lower rates of atopy [46].

In understanding the question regarding the impact of very early exposures to allergic disease, it should be noted that a few studies have now shown that premature infants have a lower risk for atopy (not asthma) than their full-term counterparts [45,47]. Presumably, most premature infants are subject to far more medical and nutritional interventions than term infants are, and are exposed to the ex-utero environment at stages when term infants are of course still *in utero*. Either the underlying cause(s) and conditions of premature delivery neatly balance out the risks associated with the ex-utero exposures or, indeed, those exposures have little impact on either the fetus or the newborn. This line of research could be quite helpful in elucidating the role of many of the interventions discussed in this review in the pathogenesis of asthma and atopy.

Strategies and challenges

Untangling the complexity of the multitude of factors which potentially influence immune development and the atopic march will not be easy. Randomized controlled

clinical trials are the gold standard for determining causality. Their widespread use to untangle the early-life morass are limited, as only a small number of the factors can be randomized. Many of the reputed factors are important components of modern public health and civilization. Ethically, they cannot be randomized simply to see their impact on the development of atopy. However, certain of the perinatal practices have the potential to be randomized. O₂ supplementation at birth, especially 100% O₂ provided during resuscitation, is being questioned [48–50], and there are ongoing randomized studies to see whether it is needed and whether harm can be done. Asthma and immunomodulation are outcomes which these studies could easily address with careful, prolonged follow-up. Furthermore, vaginal birth after cesarean section (VBAC) is a field of constant research also [51] and while there have been no randomized studies yet, data from such a trial could be similarly evaluated for immunologic/allergic consequences. Including atopic outcomes in these studies will greatly contribute to our understanding. Also, clinical recommendations are constantly changing as new information becomes available. An example is the recent recommendation for vitamin D supplement for exclusively breast-feeding babies. These recommendations only are slowly implemented on a population scale. As they are implemented in certain subgroups, they can be monitored for atopic outcomes. These types of studies can make good use of existing population databases.

Many observational birth cohorts are now under way, looking at the natural history of atopy. These cohorts offer extraordinary opportunities to gain insight into early exposures and the development of atopy. A creative example was a publication from the Avon Longitudinal Study of Parents and Children – a community-based cohort studying health and development. Children who manifested peanut allergy by age 3 years were compared with controls selected from other participants in the cohort without peanut allergy. Results from additional in-depth investigations were combined with previously collected prospective data to show that application of peanut oil to inflamed skin lead to the development of peanut sensitization [52]. In addition to the aforementioned barriers to intervention studies, a number of caveats must be kept in mind as we attempt to design and interpret studies of atopy. The ‘who’, ‘what’ and ‘when’ of our study can make a profound difference in our findings.

The ‘who’ refers to the population of our study. It is unlikely that one factor acts uniformly across the population. It has been long reported that parental history of asthma increases the probability that a child will develop asthma. Other studies have shown that certain genetic variations increase the susceptibility of an

individual to environmental insults. For example, early exposure to endotoxin has been reported to prevent the development of atopy in European children [53]. Further investigations revealed that specific single nucleotide polymorphisms in the toll-like receptor-2 identified that the individual would be influenced by endotoxin [54]. Others have demonstrated gene–environment interactions for pet [55**] and smoke [56] exposure. At this point, we do not have the technologies to mass screen populations for certain genetic characteristics. General population studies will be looking at heterogeneous mixes of individuals, which will vary between studies which will contribute to the seemingly endless number of contradictory studies.

The ‘what’ is a reminder that atopy is not a single entity. For example, we are beginning to understand that asthma is not a single phenotype. Much work has focused on the different wheezing types [57]. There are the early wheezers, who tend to stop by age 3 and are less atopic, others whose wheezing begins after 3 and others who wheeze continuously. Less well reported on are those who develop their asthma later in life. No clear indication that risk factors will be the same for all of these exists.

And, finally, the ‘when’ directs us to the period of exposure. Much speculation is centered on the importance of certain periods of life. Prenatal exposures have been shown to be associated with smaller airways at birth. These reduced dimensions are present even before the first symptoms [58]. During the next several years, the size of the airways leads to increased symptoms. Other work has intimated that exposure to respiratory infections is only protective if it occurs in the first 6 months of life [59]. This period may not be as restrictive as once thought, as a recent intervention trial has reported that immunotherapy can prevent the development of asthma in at-risk children up to age 14 [60].

Conclusion

Early-life exposures have the potential to have profound influence on the development of the immune system. Despite the barriers, we must continue to creatively study this problem, to understand which environmental exposures are best avoided to decrease the onset of atopy.

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