Environmental Influences on the Development of the Immune System: Consequences for Disease Outcome

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Abstract

Early T cell responses to external antigens and autoantigens are subject to a variety of regulatory mechanisms. A unifying link between the increase in both Th1-dependent autoimmune disease and Th2-linked atopic allergy would be a disturbed immune regulation involving T regulatory cells. There is a strong global correlation between childhood wheezing and diabetes. It is increasingly recognized that microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in disease prevalence throughout the world. These suggestions are supported by observations that germ-free mice do not develop tolerance in the absence of a gut flora. The potential effects of environmental stimuli on immune function is greatest in early life including fetal life when systems and responses are developing, and the maternal influences during fetal life could be particularly important for the development of immune regulation and tolerance induction. In recent years, focus has switched from searching for environmental risk factors towards an interest in factors that could induce and maintain immune regulation and tolerance to allergens and autoantigens. Currently evaluated strategies include the use of immunomodulatory factors, such as probiotics, prebiotics, and dietary nutrients, although data are still insufficient to make specific recommendations.

Introduction

The prevalence of allergies, diabetes, inflammatory bowel disease and other ‘immunologically mediated diseases of affluence’ has increased progressively, particularly over the last 50 years. Two distinct, but rapidly converging, areas of research, i.e. the hygiene hypothesis and the study of probiotic/prebiotic
effects, have emphasized the need to understand, and ultimately to manipulate, our physiological interactions with commensal microbiota. The story began with allergic disorders but now type 1 diabetes and inflammatory bowel disease are increasingly involved.

Here the influence of environmental factors on the development of immunological mechanisms governing host responses to allergens and autoantigens will be discussed. Considerably more is known about the environmental impact early in life on allergy than about the interaction between the environment and the immune system in relation to the development of autoimmune disease. The reason is that the much higher incidence of allergies appearing early in life has made it possible to conduct prospective observational and intervention studies during the first years of life and thus to better understand how immune regulation to allergens develop. The focus in this presentation will therefore be on allergy. It is reasonable though that the major lines indicated here are also relevant for the understanding of why diabetes, inflammatory bowel disease and some other diseases are increasing in many parts of the world.

**Immunological Background**

It is clear that T cells responsive to both dietary and inhalant allergens, as measured by lymphoproliferation and cytokine secretion, are present in cord blood from virtually all subjects [1, 2]. Additionally, T cell cloning and subsequent genotyping studies indicate that the responsive cells are of fetal origin and exhibit a Th2-polarized and/or Th0 cytokine profile. It has been suggested that these T cells may have been primed by processed antigen crossing the placenta, perhaps bound to maternal IgG. Evidence showing the presence of detectable levels of allergen in complex with IgG antibodies in cord blood supports this suggestion [3]. However, it is also feasible that these T cell responses may be directed against cross-reacting antigens or anti-idiotypic antibodies.

These early T cell responses are subject to a variety of regulatory mechanisms postnatally, which are driven by exposure of the infant immune system to environmental antigen. A broad range of regulatory mechanisms are involved, which are dictated by the concentration, frequency and route(s) of antigen (allergen) exposure and developmental status of the individual at the time of exposure. The relevant immunoregulatory mechanisms involved are likely to span the full range from classical low zone tolerance to high zone tolerance phenomena (anergy and/or deletion via apoptosis), and will include contributions from subsets of T regulatory cells.

Cross-sectional and prospective studies indicate that, in atopic children, consolidation of Th2-polarized immunity against inhalant allergens is initiated in early infancy [1, 4] and may be completed by the end of the preschool years in children who do not develop clinically manifest allergy [4], or even earlier.
In contrast, in infants who develop allergic manifestations, low level Th1 responses are established. Prospective studies from Estonia with a low and Sweden with a high prevalence of allergy indicate that the regulatory mechanisms are established more rapidly in Estonia [1]. It is possible that a traditional life style is associated with an early induction of a general regulation of T cell immunity. This notion is supported by the close correlation globally between the prevalence of wheezing and type 1 diabetes [5]. Thus, a unifying link between the increase in both Th1-dependent autoimmune disease and Th2-linked atopic allergy would be a disturbed immune regulation involving T regulatory cells, rather than merely either Th1 or Th2 immunity.

It is recognized that interaction with the normal microbial flora of the gastrointestinal tract is the principal environmental signal for postnatal maturation of T cell function (in particular the Th1 component) [6, 7]. Recognition of these signals is mediated by a series of Toll-like receptors (TLRs) expressed on cells of the innate immune system, and other receptors such as CD14, and it is noteworthy that a polymorphism in the CD14 gene has been associated with high IgE levels [8].

Microbial colonization of the gastrointestinal tract, linked with lifestyle and/or geographic factors, may be important determinants of the heterogeneity in disease prevalence throughout the world [6] and ongoing cohort studies are focusing in detail on this complex question. These suggestions are supported by observations that germ-free mice do not develop tolerance in the absence of a gut flora [9, 10] and by the demonstration of differences in the composition of the gut flora between infants living in countries with a high and a low prevalence of allergy and between healthy and allergic infants [for a summary see, 6].

The Prenatal Environment

The frequent appearance of allergic symptoms in the first months of life suggests that disease pathways are initiated very early in life, possibly even before birth. This has lead to interest in the role of environmental exposures in pregnancy. Although there is growing evidence that maternal exposures, including microbial products [11], smoking [12] and dietary factors [13], can influence infant immune development, experience of prevention strategies are still limited in pregnancy.

The adverse effects of maternal smoking in pregnancy on infant lung development are well recognized. There are also strong associations between maternal smoking in pregnancy and reduced lung function in later childhood [14]. The adverse effect of antenatal smoke exposure on lung function was much greater than subsequent postnatal effects. More recent studies also suggest that maternal smoking could have additional immune effects, which could contribute to allergic risk [12].
Very recent studies with probiotics suggest that the maternal influences may be more pronounced in tolerance induction than previously appreciated. There are now at least three studies trying to prevent food allergy and infantile eczema with lactobacilli. In the study with a negative outcome [15], the bacteria were given only to the babies, while in the two studies with some protective effect [16–18] they were also given to the mothers during the last month of gestation.

There is growing interest in potential proinflammatory changes in Western diets, including the specific role of dietary components with recognized immunomodulatory effects such as antioxidants and polyunsaturated fatty acids. As discussed in recent comprehensive reviews, the potential effects on immune function could be greatest in early life, including fetal life, when systems and responses are developing. Maternal dietary antioxidant intakes (vitamin E) have been associated with neonatal immune responses to allergens [19], justifying further studies on the effects of antioxidants on early immune function. So far, there has only been one intervention study in pregnancy to examine the effects of dietary nutrients on immune function. This study demonstrated that maternal n-3 polyunsaturated fatty acid (fish oil) supplementation had effects on neonatal immune function [13].

**Postnatal Environmental Influences**

There is consensus that breastfeeding has multiple health benefits and should be encouraged. This is particularly true in developing countries where the protection against infections may be a matter of life or death. Human milk affects the host defense and immunity of the infant in several ways [for review see 20, 21]. It provides passive protection against infections through numerous components of innate immunity and IgA antibodies, but it also provides the baby with components that enhance the development of the immune system. It is well established that human milk often contains food antigens that may induce IgE antibody formation. Less is known regarding the immunologic consequences of introducing foreign antigens while the infant is still breastfeed-ing. As indicated by studies on immunity to infectious agents, it is possible that this represents a mechanism by which immune responses are modulated. In the early 1990s, there was a pronounced increase in the incidence of celiac disease among Swedish infants [22]. Prior to the increase in celiac disease, gluten was gradually introduced while the baby was still being breastfed. Then, gluten was avoided for the first 6 months and then more or less abruptly introduced in large amounts. When the national recommendations were changed back to a gradual introduction of gluten while the babies were still partly being breastfed, the incidence of celiac disease dropped rapidly.

It is intriguing that microbial stimulation, in particular via the gastrointestinal tract, has also been implicated as an etiologic factor in respiratory
allergic diseases. This suggests that microbial stimuli from the gut exert effects beyond the mucosal tissue microenvironments adjacent to sites of exposure, and presumably can influence systemic precursor compartments such as bone marrow and thymus. The underlying mechanism(s) are likely to include stimulation of functional maturation of cells within the innate and adaptive immune systems during the early postnatal period, a process which may ultimately determine the overall efficiency of immune/tolerance induction during early life, with major flow-on effects into adulthood. A full understanding of the underlying mechanisms may open new venues for prevention by the modification of gut microflora, not only of local disease manifestations, such as food allergy, but also conceivably of diseases with manifestations at distant sites, such as diabetes and respiratory allergies.

While sensitization is a strong risk factor for persistent asthma, wheeze and bronchial hyperactivity, the relationship between early allergen exposure and the development of clinical symptoms has been much harder to confirm. The hypothesis that allergen avoidance early in life would prevent asthma is based on two independent observations, i.e. that exposure to high levels of inhaled allergen is associated with an increased likelihood of sensitization and that asthmatic children are often sensitized in early childhood. No studies have confirmed that the two observations are related to each other, however.

Bacteria are the most powerful immunostimulants in the normal environment, activating the immune system through a range of ‘pattern recognition receptors’ (TLRs). Although TLRs are found principally on cells of the innate immune system (including granulocytes, monocytes, and natural killer cells), they are also present on cells involved in programming and regulating ‘adaptive’ immune responses (such as antigen-presenting cells and regulatory T cells). It has been proposed that early microbial activation of both antigen-presenting cells and regulatory T cells may promote Th1 maturation and play an important role in reducing the risk of Th2-mediated allergic responses [23]. This is supported by animal studies demonstrating that bacterial lipopolysaccharide endotoxin exposure can prevent allergic sensitization if given before allergic responses are established [24]. These effects may be of greater significance in genetically susceptible individuals who appear to have weaker Th1 responses in the perinatal period [4]. Genetic studies also support a role for the CD14/lipopolysaccharide [8] and TLR [25] pathways in the development of allergic disease.

Intestinal microbiota are arguably the most abundant source of early immune stimulation, and contribute significantly to the ‘microbial burden’ in early life. A number of studies have suggested differences in colonization patterns of infants who go on to develop allergy [for review see 26]. These differences were already apparent at 1 week of age, suggesting that early colonization can influence subsequent patterns of immune development. Studies in germ-free animals confirm that a microbial gut flora is essential for the development of oral tolerance and for the induction of normal immune
regulation [10]. The controversy regarding the role of gut bacteria in allergy development thus lies in the clinical consequences of these findings and not as much to what extent they affect the immune system.

Studies investigating the relationship between early childhood infection and atopy risk have been inconsistent or difficult to interpret. The immunological effects of microbial agents differ with the type of infectious agent and the site of infection [27]. Differences are also seen in the responses to vaccine antigens compared to the wild-type infections they prevent. Furthermore, nonpathogenic colonizing organisms are also likely to play a central role in immune development [26]. A recent large Danish national cohort study including 24,341 mother–child pairs found that early infections do not protect from atopic dermatitis [28]. However, they observed that other environmental factors, sometimes taken for indirect markers of microbial exposure (such as early daycare attendance, having 3 or more siblings, farm residence, and pet keeping), were protective. It is possible though that these protective factors are due to factors other than microbial exposure. For example, the inverse relationship between the number of older siblings and allergy risk may be due to altered maternal immunity as a consequence of repeated pregnancies, and exposure to animals could possibly be explained by high zone tolerance induction. This highlights the emerging concept that overall ‘microbial burden’ rather than specific infections may be more relevant in early life [29].

The growing awareness of the potential importance of early microbial exposure for early immune development has prompted speculation about the role of antibiotics and other antimicrobials in the first year of life. Several authors have subsequently assessed the possibility that antibiotics may be a risk factor for the development of asthma and other allergies and the results are slightly conflicting. It seems reasonable to conclude, however, that usage of broad-spectrum antibiotics but not penicillin in the first year of life is associated with an increased risk of allergic disease, although the data are conflicting.

It is logical to explore the benefits of probiotics earlier when immune responses are still developing, and there are now a number of studies addressing the role or probiotics in primary allergy and diabetes prevention (in Australia, Finland, New Zealand, Singapore, Sweden and the United Kingdom), examining the effects of various probiotic strains using direct infant supplementation. Some are still in progress.

As it appears increasingly unlikely that supplementation with a single probiotic strain will be sufficient to overcome the high environmental pressure to develop allergic disease, there has been a shift in interest to dietary substrates that could potentially have a more global effect on gut flora, namely prebiotics. Prebiotics are non-digestible but fermentable oligosaccharides (food starches) which specifically stimulate the growth of bifidobacteria and lactobacilli species. Altering the intake of foods containing these products can directly influence the composition and activity of intestinal microflora. This could
explain some of the protective effects of grains and cereals that have been seen in epidemiologic studies [30]. At this stage there are still very little data to directly confirm the immunological or therapeutic effects of prebiotic supplements, although one recent study has reported encouraging results [31].

**Potential for Prevention**

Most previous approaches to prevention were based on avoiding candidate factors which could be implicated in the development of disease. These studies have not been successful. In recent years, focus has switched from searching environmental risk factors towards an interest in factors that could induce and maintain immune regulation and tolerance to allergens and autoantigens. Thus, current research is more directed towards an understanding of how immune regulation develops and how tolerance could be induced early in life. More recent strategies include the use of immunomodulatory factors such as probiotics, prebiotics, and dietary nutrients (such as n-3 polyunsaturated fatty acids) although data are still limited and there is still insufficient evidence to make specific recommendations.

Until the 1970s textbooks in pediatrics did not discuss prevention strategies, or only mentioned them in passing. The increasing awareness of environmental pollution and the continuing increase in the prevalence of allergies, diabetes and other immunologically mediated diseases brought public attention to environmental factors that could explain the increase. The lesson learnt from 20 years of epidemiological analyses, observational studies and recent intervention studies, has so far not been successful in developing strategies for prevention.

Recommended preventive measures should be based on scientific documentation that is evaluated equally as strictly as for medical treatment, because even seemingly innocent advice may have a profound impact on a family. The World Health Organization has defined certain principles for decisions on preventive measures. First, the disease should be common and have potentially serious consequences. Second, the causes should be known and measures should be effective, safe and acceptable. There should also be resources for implementing the measures. Finally, the health economic consequences of the measures should be known.

It could be argued that it is never harmful to give health-promoting advice regarding, for example, the value of breastfeeding, ‘good’ nutrition, and the harm of tobacco smoking. Even seemingly innocent advice may have negative consequences, however, e.g. parental guilt feelings that not enough was done if the child develops disease. Advice on diet may interfere with optimal nutrition, customs and family economy; cleaning procedures may be taken so far that they interfere with daily life; ‘good’ ventilation may come in conflict with energy conservation, and ‘no air pollution’ may prevent a family from painting
the house. Advice on pet avoidance may profoundly affect a family with a loved pet, and visits to grandparents who keep pets and may even force people to move from a farm. Thus, the consequences of advising preventive measures should always be considered, including how advice may be interpreted by those receiving it.

References


Discussion

Dr. Walker: You did a great job for the last lecture since everything has previously, presumably at least, been mentioned. The concern I have is that your study used a different probiotic than was used in the Finnish study; it is like comparing apples and oranges. Probiotics may function differently in different situations.

Dr. Björkstén: Yes, theoretically. Going through the literature, however, it seems that all probiotic lactobacilli actually work in the treatment of infantile gastroenteritis. They also seem to reduce lactose intolerance and prevent diarrhea induced by broad-spectrum antibiotics. The effects have been similar for several strains, provided that you give reasonable amounts of live bacteria. But you are quite right and as I showed, there are differences in the capacity of different lactobacilli to induce T regulation. Three allergy prevention studies show some effect. The only negative study was the one in which treatment started after birth.

Dr. Wilson: I too would like to thank you for an informative and entertaining talk, highly useful at this time of the day. I have a comment that may be relevant to the lactobacilli studies you and others have been doing. This is a gram-positive bacterium. Thus, the major TLRs through which it will act are 2+1 or 2+6. Soon to be published are studies showing allelic differences in TLR1, both alleles have a frequency of approximately 30–35% in North American populations of European ancestry. One of these two alleles results in hyporesponsiveness to TLR2+1 ligands. You will need to control for that kind of variability if you are using an organism that is principally going to be acting through those TLRs.

Dr. Björkstén: You are right. I would expect different outcomes in different populations for the reasons you have given. We do not even know if gram-positive bacteria should be used, as there are several reasons why the gram-negative flora actually could have more of an impact on immune regulation.
Dr. Wilson: I think that the state-of-the-art of looking at the diversity of microbial flora is really sequencing – essentially sequencing is the only way to determine what the true diversity of microbes is, as Dr. Gordon has shown. I wonder whether any of the samples that you or other studies have accrued are stored so that such an analysis could be done retrospectively? You pointed out extremely clearly that it is likely that some of the bacteria you are finding are surrogates for a more complex situation. Given where we are now technologically, I would think that that kind of information is obtainable.

Dr. Björkstén: That is a valid comment and I am happy to say that if you have the methods, I have the samples stored, as this is precisely what we hoped to do. Crude bacterial cultures were the only available methods when we started 12 years ago. We collected consecutive samples in the freezers and we had an 11-year follow-up of the first birth cohort. We could go back and look at diversity with modern technology of samples stored from Estonian and Swedish children who are either allergic or not by age 11 years. I also want to make it clear to the audience that I never said clostridia are bad, nor that lactobacilli are good. Our observations could be surrogate markers for other microbes. The fact that we have shown differences between groups in prospective studies does not prove that these bacteria are directly involved. I am only suggesting that the internal environment is more interesting than shooting dogs and strangling cats for allergy prevention.

Dr. Bier: As a person who doesn’t have any stake in this, it is very hard to convince me that any of these things have meaning until they are molecularly typed. I don’t know what it means if half the bacteria is thrown away. I think all of the data that have been collected over time really have to be redone in the context of us knowing what the true bacterial population is. In relation to the genotypes, alleles and polymorphisms that may exist in the regulatory genes, or in Estonia compared to Sweden, and until they are known, it is also very hard to understand whether these are differences due to environment or they are differences due to genotype.

Dr. Björkstén: These are ethnically quite similar populations so, at the population level, there is no reason to believe that there would be differences. Obviously there would be individual differences. There has not been a shift in our genetic set up over a 50-year period therefore the increase has to be due to environmental factors. I previously cautioned during the discussions that there may be a problem with a traditional reductionist molecular approach.

Dr. Ogra: Has anyone looked at idiotypes and idiotypic regulation of immune responses in these settings? Is there anything which we can identify at the regulatory level before we see Th1 and Th2 responses which might explain the differences between Estonians and the Finnish? For example, alcohol may be a very important modulator of the flora between the two populations. Furthermore there are many other variables which might affect the responses in these two population settings.

Dr. Björkstén: I don’t want to be interpreted as saying that molecular studies should not be done, obviously they should. What I am saying is that we have also to adapt a more holistic approach when asking our research questions. In a way Estonia in the 1990s was a time capsule of Scandinavian life some decades back. The country is ethnically and culturally Finnish or Swedish. The lifestyle when I started to work there in the early 1990s really threw me back to my childhood after the Second World War. The diet comprised locally produced foods, no vegetables coming from the southern hemisphere in the winter. Fermented cabbage, carrots and beets were the main vegetables. Apples were available in autumn. In Sweden in spring I can buy an apple in a supermarket that has been transported from Tasmania. The low allergy prevalence cannot be explained by infections, breastfeeding, exposure to allergens, pollution, or tobacco smoking. All those external environmental factors have been
controlled for. What we obviously cannot say is whether differences in the diet would explain the differences in allergy prevalence between Estonia and Sweden.

Dr. Ogra: So how does it then fit with your observation that allergy is in fact increasing, and yet the old type of farm life was not protective but modern farm life is protective, although it is more sanitized?

Dr. Björkstén: This is the end of the day, and we may be allowed to speculate. The differences between urban and rural populations in a country are not only pollution and the presence of pets, but also a question of tradition. For example, people in rural Sweden eat differently than people in the cities these days. Whatever is now still prevailing in rural areas was also common in urban areas 50 years ago. I have no idea what it is, I have already given you what is known, so your guess is as good as mine at this stage.

Dr. Walker: Let me answer the concerns raised by Dr. Wilson and Dr. Bier; there is in fact a lot of cloning being done on the common probiotics on a mechanistic level, it is just that there are different types of studies going on. There is a lot of evidence that there is a slight 1 or 2 log difference in one organism versus another in allergy, inflammatory bowel disease and so forth. In an environment of a billion cells, how can a 1 log difference be so large in terms of expression of disease?

Dr. Björkstén: That is actually what I am trying to say and why I am cautioning against believing in one single miracle probiotic strain. Usually we are giving something like 100 million up to possibly one billion bacteria which is still 10^{14} less than the entire gut microbiota. What could be a reasonable explanation is that it actually modifies the composition of the microbiota. That is why I am careful not to say that what we see is strain specific.

Dr. Smith: How might a single probiotic continuously stimulate the immune system? In a natural environment, when a patient might be infected by many different bacteria, if an attempt is made to induce tolerance and stimulate the immune system, a single antigen might not provide that.

Dr. Björkstén: I agree. Thank you for giving me the chance to reiterate. Again we know from the germ-free animals that normal gut microbiota are essential not only for downregulation of IgE formation, but also for oral tolerance and the development of T regulatory cells. Several clinical studies do, however, indicate clinical benefit in the treatment of eczema in infants and also in prevention. There are three meta-analyses showing the shorter duration of infectious diarrhea in infants. We cannot explain how the limited number of bacteria given have an effect.

Dr. Smith: Do you think that a probiotic of the month would be needed? In the children who are on this trial, to reproduce what is in nature, they may need to be exposed to many different types. Do you think that might be better?

Dr. Björkstén: This is a reasonable suggestion from what I have said; I am not quite happy with the thought of it though. As doctors we were trained to prefer one drug for one disease rather than cocktails of compounds, but that is an obvious way to consider.

Dr. Giovannini: Speaking about lactobacillus, you said that some are effective and others not. All lactobacilli break down protein or milk protein, especially casein; in some patients it is effective but another patient may have protein milk allergy. If they are effective in some pathologies, milk should not be given because otherwise milk allergy may occur.

Dr. Björkstén: Lactobacilli have nothing to do with milk. They are predominantly present in any fresh food and we have lived with them through the evolution of man. All mammals seem to harbor lactobacilli. Anything that is fermented, whether it is vegetables or meat that has started to ferment, contains lactobacilli.

Dr. Giovannini: Is there a difference between rural and urban areas? In urban areas perhaps the lack of parasites may be a reason for the high rate of atopic manifestations also in breastfed babies.
Dr. Björkstén: We do not have parasites in our cold climate. The development of immune regulation in Estonian infants is similar to what has been shown in Africa and is suggested to be connected with parasites there. So I am suggesting that the broad microbial spectrum, possibly caused by the traditional lifestyle, has an effect that is similar to what has been reported for parasites. We looked for the possibility and the only parasites we would find in Estonia were ascaris and trichuris.