The Hygiene Hypothesis: Do We Still Believe in It?

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Abstract

Numerous epidemiological studies suggest that there is an inverse relationship between allergic diseases and infections in early childhood, but there are also several well-conducted epidemiological studies that seemingly contradict this relationship. The maturation of the immature immune regulation after birth is largely driven by exposure to microbes. Germ-free animals manifest excessive immune responses when immunized and they do not develop normal immune regulation. The controversy regarding the role of infections for subsequently developing allergy is partly due to varying clinical definitions of 'allergy'. Thus, wheezing and asthma have often been included as outcomes. The hypothesis that commensal microbes are the normal stimulants for the maturation towards a balanced immune response is relevant for IgE-mediated disease manifestations, rather than recurrent bronchial obstruction per se. Epidemiological, clinical and animal studies taken together suggest that broad exposure to a wealth of commensal, non-pathogenic microorganisms early in life are associated with protection, not only against IgE-mediated allergies, but also conceivably against type-1 diabetes and inflammatory bowel disease. This has little relationship with 'hygiene' in the usual meaning of the word. The term 'hygiene hypothesis' is unfortunate, as it is misleading. A better term would be 'microbial deprivation hypothesis'.

Introduction

There is a well-established relationship between affluence and allergy prevalence [1]. From numerous studies of migrant populations it is also clear that the differences between countries and populations within a country are caused by environmental factors and not genetically determined, despite the fact that heredity plays a major role in the allergy risk of an individual. For many years it was thought that the increasing prevalence of allergy was due
to increasing pollution and it became an issue for the environmentalists. At the time it was known that pollutants, such as tobacco smoke and car emissions, could trigger symptoms in asthmatic individuals, but it was not until the 1980s that allergists and other members of the medical community began more intensively to study environmental factors as possible causes of allergy. It was assumed by inference and ‘common sense’ that pollution and exposure to allergens early in life would cause allergy, and thus that avoidance measures would prevent allergies from developing.

These assumptions were never properly supported by clinical studies though. In 1976, the Canadian pediatrician John Gerrard, noting a low allergy prevalence among indigenous populations in northern Canada, suggested that the high prevalence of allergies among white Canadians were a consequence of a few infections during childhood [2]. His paper raised little interest, however. In 1989, the British epidemiologist David Strachan observed that children who had elder siblings were less likely to manifest hay fever as adults, as compared to firstborn children [3]. His suggestion was that this could be due to a protective effect of infections brought home by the elder siblings. The paper evoked great attention and the term ‘hygiene hypothesis’ (HH) was coined. Since then, many studies have confirmed an inverse relationship between the number of elder siblings and a propensity to develop allergy [4]. Numerous subsequent studies have also confirmed an inverse relationship between various measures of hygiene and allergy prevalence. Significant support of the HH came from studies showing that allergies were less common among children growing up in rural as compared to urban environments, particularly if growing up on a farm [5]. Among children growing up on a farm, exposure early in life to animals was protective. Further analyses revealed an inverse relationship between allergy and drinking unpasteurized milk as well as exposure to high levels of endotoxin in house dust during infancy [5].

The observations were interpreted as a protective role against allergy of respiratory infections during early childhood. This notion was clearly against solid clinical experience, as it is well known that respiratory tract infections not only trigger infant wheezing but also asthma attacks in older children and adults. Furthermore, the term ‘hygiene’ could lead one to think that poor hygiene in itself would be protective. Several studies questioned the HH based on studies showing that asthma and wheezing were common in many poor, ‘unhygienic’ environments [6]. This review discusses various aspects of the so-called HH in the light of how it has been understood over time and tries to reconcile the numerous epidemiological and experimental studies.

**Immune Regulation in Infancy**

The newborn infant is immunologically naïve and the immature immune system has not fully developed a balanced immune response and immune
regulation [7]. Early T-cell responses are postnatally subject to a variety of regulatory mechanisms which are driven by exposure of the infant immune system to environmental antigens. 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.

In atopic children, consolidation of Th2-polarized immunity against inhalant allergens is initiated in early infancy [8] and may be completed by the end of the preschool years in children who do not develop clinically manifest allergy, 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, as well as type-1 diabetes, indicate that the regulatory mechanisms are established more rapidly in Estonia [9]. It is possible that a traditional lifestyle 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 [10]. 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 a shift towards Th2 immunity, as was originally thought to underlie the HH.

**The Role of Gut Microbiota**

The gut microbiota is the major source of microbial exposure, comprising 10^{14} microorganisms, i.e. ten times the number of cells in the entire body with a 30 times larger total genome than the human genome [11]. 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 and ongoing cohort studies are focusing in detail on this complex question. Intestinal microbiota are arguably the most abundant source of early immune stimulation, and contribute significantly to ‘microbial burden’ in early life. These suggestions are supported by observations that germ-free mice do not develop tolerance in the absence of gut microbiota and the observed differences in the composition of the gut microbiota between infants living in countries with a high and a low prevalence of allergy and between healthy and allergic infants [summarized in 12].

The gastrointestinal tract of the newborn baby is sterile. However, soon after birth it is colonized by numerous types of microorganisms. Colonization is complete after approximately 1 week, but the numbers and species of bacteria fluctuate markedly during the first 3 months of life. Once established, it
is surprisingly stable under normal conditions, although there is a continuous intense interaction between the microbial flora and the host. Environmental changes, e.g. a treatment period with antibiotics, only temporarily change the composition of the microbiota and this dynamic and interactive ecosystem.

All immune reactions have to be regulated in order to avoid damage to the host. Bacteria are the most powerful immunostimulants in the normal environment, activating the immune system through a range ‘pattern recognition receptors’ system (Toll-like receptors, TLRs).

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) [13]. Recognition of these signals is mediated by a series of 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 [14].

There is mounting evidence that commensal microbes acquired during the early postnatal period are required for the development of tolerance, not only to themselves, but also to other antigens. For example, Th2-mediated immune responses are not susceptible to oral tolerance induction in germ-free mice [15]. Oral tolerance was only induced after the introduction of components of the normal microbiota. Thus, experimental animals living in incubators under germ-free conditions display deficient regulation of their immune responses, e.g., when immunized.

The lack of immune regulation may cause immune reactions to foods eaten, enhanced production of IgE antibodies and excessive cell-mediated immune reactions that are associated with, for example, autoimmunity and inflammatory bowel disease. Several recent clinical studies lend support to the notion that microbes may play an important role in reducing the risk of both Th2-mediated allergic responses and Th1-associated autoimmune disease, such as type-1 diabetes [12, 16].

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, but the results are slightly conflicting [17]. It seems reasonable to conclude from the studies 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.

Microbial exposure arguably provides the strongest environmental signal for normal postnatal maturation of the immune system, and also induces the maturation of antigen presenting cells and T-regulatory cells, which are essential for programming and regulating the T-cell response. It appears likely that microbial activation of regulatory pathways through microbial pattern rec-
ognition molecules (TLRs) plays a central role in reducing the risk of immunologically mediated disease, including Th2-mediated allergic responses, and possibly also Th1-mediated autoimmune disease, such as type-1 diabetes. Thus, reduced microbial exposure may be contributing to the rising rates of this wide spectrum of immune diseases. This is likely to be of greatest relevance in early life when immune programming is initiated and less significant in relation to a mature immune system in older children and adults.

Clinical Aspects of Allergy

Some of the controversy regarding the relevance of the HH may be explained by the selection of clinical phenotypes. The hypothesis suggests that exposure to a broad range of microbes would modify immunity towards suppression of IgE antibody formation and thus, as a consequence, result in less allergies. Manifestations of IgE-mediated allergy include eczema, rhinoconjunctivitis, asthma, urticaria, gastrointestinal reactions to foods, and anaphylactic reactions. None of these clinical symptoms are specific for an IgE-mediated allergy, however. For example, recurrent wheezing during the first years of life is mostly a consequence of respiratory tract infections and the role of inhalant allergy is minimal. In older children, asthma may or may not be the consequence of an allergic reaction. Indeed, studies have shown that asthma, recurrent bronchial obstruction and bronchial hyper-reactivity may be more prevalent among children living in less affluent countries with a low prevalence of positive skin prick tests, as shown by a study comparing lung function in schoolchildren in Estonia with a low and Sweden with a high prevalence of allergies [18]. In the recent phase-II studies of the International Study of Asthma in Children (ISAAC), the association between atopic sensitization and asthma symptoms in children differed strongly between populations and increased with economic development [19]. Different causes of wheezing in children in affluent and less privileged areas in USA [6] and a high prevalence of asthma in some Latin American countries [1] may possibly be explained by this.

Eczema is the major clinical symptom of IgE-mediated allergy in infants, but even then at least two distinct forms of eczema can be distinguished, i.e. atopic and non-atopic [20]. Thus, children having itching flexural dermatitis may not necessarily manifest an IgE-mediated allergy [21]. Questionnaire-based studies of prevalence also have an inherent problem in the interpretation of the words in different cultural contexts [21].

In regions with a temperate climate and distinct seasonal variations and pollen seasons, rhinitis and conjunctivitis during the pollen season are arguably the most specific clinical symptoms associated with IgE-mediated allergy. It is of interest to note that among clinical manifestations associated with allergy, rhinitis and conjunctivitis show the strongest inverse relationships with vari-
ous environmental exposures during infancy. This is particularly obvious if symptoms during the pollen season rather than 12-month prevalence are employed as outcome [22].

**Microbial Deficiency Syndromes of Affluence**

There is an interesting high correlation between the prevalence of allergic diseases and autoimmune disease, such as type-1 diabetes in different countries [10]. Furthermore, there is surprising similarity in risk factors for the two diseases identified in epidemiological studies [23]. The immunological mechanism underlying the HH as developed in the 1990s is that infections, including respiratory tract infections, would skew the immune system towards Th1-skewed immunity. Conversely, the lack of recurrent infections would lead to excessive Th2 responsiveness and thus to allergic disease. The similarities in prevalence, epidemiological risk factors and increase over the past 40 years strongly suggest that this explanation was incorrect. As previously discussed, current hypotheses suggest that the development of immune regulation, including Treg cells are stimulated by a diverse exposure to microbes.

This notion was suggested in some detail in 1998 but it had limited impact on the interpretation of allergy epidemiology and the general understanding of the HH [24]. However, with a broadened understanding of the seminal role of diverse microbial stimulation caused by the myriads of different non-pathogenic commensal microorganisms, rather than infectious diseases, the understanding of the HH has been modified. Today, not only IgE-mediated allergic disease manifestations, but also type-1 diabetes, inflammatory bowel disease and perhaps also obesity [25] should be included among diseases of modern society that may have a relationship to changes in the diversity of microbial microbes, notably the gut microbiota, associated with an affluent lifestyle [26].

**Concluding Remarks**

The development of the HH shows a pattern that seems to be typical for the evolution of novel concepts. When it was first suggested that allergy was the price to be paid by members of an affluent society for the relative freedom from infections [2], this went by more or less unobserved as it was not considered to be in accordance with current dogmas about the causes of allergies. When this was again suggested 13 years later [3], the time was ripe for acceptance and the suggestion was acknowledged with enthusiasm, particularly as numerous subsequent studies seemed to suggest that infections, including those in the respiratory tract, were indeed protecting against allergy. This enthusiasm was gradually weakened by studies that failed to support the hypothesis. Today it seems that the HH, although rightly pointing out a protective role of microbial
exposure early in life, was oversimplified. Respiratory infections, particularly severe infections early in life remain risk factors for developing asthma, while a diverse microbial exposure is still accepted as a major stimulus for the development of immune regulation. Unfortunately, the term ‘hygiene hypothesis’ is misleading and potentially harmful for public health, as it has led to questioning childhood vaccinations and other major public health measures. No one would seriously question the enormous health gains by improved hygiene and nobody would argue for more childhood infections, intestinal infections and serious infections, such as tuberculosis merely to hypothetically reduce the incidence of allergies. A better term than ‘hygiene hypothesis’ would therefore be ‘microbial deprivation hypothesis’, as this would point towards the possibility of preventing, or perhaps even treating, several immunologically mediated diseases with cocktails of non-pathogenic microorganisms and antigen mixtures derived from them.

References

Discussion

Dr. B. Koletzko: Thank you for a very stimulating presentation. I am a bit confused by the proposal to call this the gut hypothesis. Obviously the gastrointestinal (GI) tract has an enormous surface and is one of the prime routes of interaction with microbes. There are a number of studies pointing to respiratory infections, for example, in daycare settings, and there are some other studies showing that respiratory infections in the first months of life are also associated with reduced atopic disease. So is it appropriate to limit this to the gut only?

Dr. Björkstén: Yes it is. Of course I am aware of the daycare center studies, but they are indirect. Before the hygiene hypothesis was launched, there were several studies showing an increased incidence of allergies if children were in daycare centers early in life. Those studies were even used against the introduction of daycare centers in Scandinavia. It is interesting that there is a difference in the composition of the gut flora between children who spend time in daycare centers and those who do not. We have separated the influence of daycare centers from a history of infections. Daycare centers early in life were protective but respiratory infections were risk factors. I think that the search for infections may be misleading. Obviously if a child spends time in a daycare center, then he/she is more likely to get respiratory infections, but that may have little to do with the induction of the immune system.

Dr. Zwiauer: According to your hypothesis, do you think that we could protect children by giving them a germ in different ways, for instance one strain of lactobacillus or bifidobacteria? Do you think that this would improve diversity?

Dr. Björkstén: No.

Dr. Salminen: I would like to ask you about the situation in Estonia. Having worked partly with the same people in Estonia as you, I am a little puzzled by the situation with infections. You didn’t have any information about infections, and the situation was quite different between Finland and Estonia at the time in terms of factors that certainly will influence the infection rate, such as milk quality and the way milk...
was treated. Prior to and during the Socialist time there was no pasteurization, which has been mandatory in most Nordic countries since the Second World War. So exposure to infections was quite different. Do you have any information about pediatric infections? You didn’t show that.

**Dr. Björkstén:** We could not relate differences between the two countries to infections. In our two prospective studies in Estonia and two similar cohorts in Sweden, there was no major difference in the incidence of respiratory infections.

I should add that that antibiotic treatment during the first 2 years of life was not a risk factor in Sweden, but it was indeed a risk factor for allergy in Estonia. We looked carefully into this and found that in Sweden penicillin is prescribed, while most of the treated Estonian children received broad-spectrum antibiotics. The rate of respiratory infections was rather similar.

**Dr. Isolauri:** I have some epidemiological evidence to support your conclusion. First the epidemiological evidence [1] suggests that Th1- and Th2-type diseases most likely also coexist in the same individuals, so patients who have diabetes have a higher risk of asthma and so on, in support of your conclusion. Second, protective exposure should occur very early, as the first expression of the atopic responder type frequently occurs within the first months of life. Thus the establishment of the gut microbiota in providing an initial and massive source of microbial stimuli may be a good candidate for ‘infection’ [2].

**Dr. Haschke:** I want to follow up on the question whether the continuous administration of one probiotic bacterial strain has an effect. You showed one slide where lactobacilli were applied and had an effect. When Dr. Zwiauer asked you about the clinical effect, you said there was none. Can you explain this discrepancy?

**Dr. Björkstén:** Why I provocatively agreed with the question about one bacterium is that I think that we are discussing a very complex ecology. I think it would be a severe oversimplification to believe in the panaceas of a single microbe. Even the small effects seen in clinical studies with only one single strain are encouraging, however. I think that the future probably lies in an ecologic approach with a combination of different strains. I realize that it is difficult to do such studies and that we have to go through with various single strains. My point also relates to Dr. Kasper’s presentation suggesting that polysaccharide A would be the panacea. I think this is too simplistic.

**Dr. Renz:** I would like to follow up on this issue of how to apply and how to use this knowledge which we have gained from all these epidemiological and also experimental studies for possible prevention. In this regard your point to discriminate between infection and exposure is very important because exposure is probably a non-inflammatory response of the immune system and that makes it very difficult to study because we don’t know where to look to analyze the mechanism of this non-inflammatory response in the gut or even in the airways, and I agree that the airways are probably also important mucosal surfaces for this. So really if we know and can delineate the mechanism of this, then we might be able to interfere and prevent by intelligent manipulation of this tissue.

The second point I would like to make is with regard to the clinical phenotype to be manipulated or studied. Many of the farming studies, including those we are also involved in, indicate that the preventive effect is more for respiratory allergies and not so much for eczema, at least no major effect on atopic dermatitis was seen. On the other hand, the clinical effects seen with probiotics are on eczema and there is very little evidence so far that if probiotics are used for prevention that respiratory allergies are helped in this regard. So I think we must be very careful when looking for phenotypes in the clinical outcomes of all these issues.

**Dr. Björkstén:** I think you are right. I would like somebody to carefully study polymorphonuclears in this context. There are studies from the 1970s and 1980s reporting
differences in polymorphonuclear function between allergic and non-allergic children. These observation have been forgotten because of modern immunology, but I think we will be hearing more about innate immunity. I believe that it is time to look prospectively also at granulocyte function.

Dr. Exl-Preysch: May I go back to the question of infections, going a little bit along with what Dr. Renz was saying. Earlier we were always putting everything into one box and didn’t make a difference between respiratory allergies, GI allergies, and atopic eczema. We are talking about a lot of different entities, and you said that infections are not helping. What would be your hypothesis concerning the difference between respiratory infections or GI infections? Is there a difference in terms of your studies? Respiratory infections don’t seem to make a difference, but what about GI infections?

Dr. Björkstén: I can’t answer that because we have such a low incidence of diarrhea and gastroenteritis in our cohorts in Sweden due to hygiene, so we can’t do that type of analysis. The best answer I can give is the study by Matricardi et al. [3], who showed an inverse relationship between hay fever and GI infections, toxoplasmosis and hepatitis A, but no relationship with antibody levels against respiratory agents. But again, why does someone get hepatitis and toxoplasmosis? It could be an effect of the entire microbial flora rather than those agents by themselves.

I should also add that we very recently observed differences in the composition of the gut microbiota between those who have and those who do not have older siblings. So again, if you believe in the gut hypothesis, I think we can now explain the sibling story also by gut microbiota. Also for immunological reasons I question the role of respiratory infections.

Dr. Hernell: We have discussed before whether starting to give probiotics before birth is necessary. As you know we have done a randomized study where a probiotic was given during weaning and we found virtually the same results [4]. With regard to the effect on the switch from Th2 to Th1, we can also see the same effect at 1 year, so is it really necessary to start giving it before birth?

Dr. Björkstén: Obviously you are answering the question yourself, Dr. Hernell.

Dr. Tobin: I think that epidemiology in Scandinavia is fantastic, and we have not been able to accomplish that yet in Australia. But one of the things I think we are potentially missing if we just talk about microbes in the environment is that it is environmental factors that have changed, and we need to be careful not to narrow ourselves too much. Another thing that is obvious is that being further away from the equator has lead the way with this increase in allergy and autoimmune diseases; an interaction with vitamin D may of course be very relevant to that. People think that in Australia we have a balanced lifestyle but actually that is not the case, we are spending more time indoors now, and the fact that we are now catching up to you in allergy and autoimmune diseases just suggests that there is an interaction between the microbial content on one hand and on the other hand the regulation of the immune system by adding adequate or inadequate levels of vitamin D as the case may be.

Dr. Björkstén: The vitamin D story has been around for about 20 years and was based on epidemiological comparisons. It is difficult to accommodate a major role of vitamin D with the outcome of the global ISAAC studies, not to mention the Baltic States versus Scandinavia which are all up in the north. You also have the differences between eastern and western Europe where the only thing that would support your thought is that northern Sweden, Finland and Norway, as compared to southern parts of those countries, have higher levels of allergies.

Dr. Tobin: When gross national income goes up people are actually spending more time indoors so you can’t just base it on the latitude of the country but you actually have to look at individual behavior.

Dr. Björkstén: I am not aware of studies actually showing that relationship, because you could argue that by having more free time, you spend more time outdoors.
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Dr. Prescott: You mentioned the importance of investigating neonatal pathways and we’ve certainly got some preliminary evidence showing that in the neonatal period the innate Toll-like receptor microbial recognition pathway is actually upregulated, but this is more exaggerated in the children who go on to develop allergic disease. So these inflammatory pathways are more active, and we could perhaps couple that with evidence from developing countries, comparisons where microbial burden may actually be downregulating these Toll-like receptors. Fagerås Böttcher et al. [5] in Sweden have made similar findings. Could you comment on that in the context of what you have presented?

Dr. Björkstén: The comments you made are correct. You have done more work along that line than we have and I think your studies fit the hypothesis.

Dr. Sinn: Regarding antibiotics in the first 3 years of life; as neonatologists we give antibiotics to many babies who are admitted to the nursery. The question is what can we do to prevent the potential increase in allergy in these babies, and secondly do we tell the parents that we may increase their infants’ allergy risk by changing the gut flora with antibiotics? Antibiotics are for infection, respiratory stress, premature infants, and any baby who has a risk factor for infection.

Dr. Björkstén: That is a situation where again I would say the most convincing indication for probiotics today may very well lie in neonatal intensive care. There is a recent meta-analysis showing that probiotics reduce the risk of necrotizing enterocolitis and the figures are rather impressive. It makes sense because under any circumstances colonization will take place. So the question is what bacteria should the baby be colonized with; hospital bacteria that are prevalent in that unit, or under controlled circumstances with probiotics?

Dr. Sinn: Premature infants seem to have less allergy long-term than term infants, even if they had antibiotics.

Dr. Björkstén: The situation with preterm babies and allergies is not that simple. It used to be said that preterm babies were less likely to develop allergies as opposed to those born by cesarean section for example where there is an increased risk. I don’t know the answer to the preterm babies, why there may be a slight reduction. Possibly you could speculate during pregnancy whether there is a difference with regard to the maternal Th2 skewing between the pregnancies ending at term or with premature birth. It could be a more complex explanation than merely a role of microbiota; I don’t know.

Dr. Tang: The rise in the prevalence of different allergic conditions has not occurred simultaneously; for example we saw that the prevalence of asthma was the first to increase and this seems now to be plateauing or even falling in some countries. Meanwhile allergic rhinitis and eczema continue to increase but the rate of rise is also slowing, and currently we are in this epidemic of food allergy and anaphylaxis. Could you comment on how the different rates of rise in asthma, eczema, allergic rhinitis and food allergy might be accounted for within the context of the microbial hypothesis? It seems to me that there are different things regulating the rise in allergic disease for different conditions. So while the hypothesis of microbial exposure seems good, what do you think the different factors actually are that are differentially influencing the prevalence of these allergic disorders?

Dr. Björkstén: To my knowledge nobody can explain why there are different expressions of the atopic genotype; why there is eczema in infants; why infants don’t have allergic asthma or hay fever, and why respiratory allergies develop later. For some reason it takes longer for IgE antibodies to develop against respiratory allergens than to foods. Over 20 years ago we showed in prospective studies that healthy infants who do not develop respiratory allergy have lower levels of food IgE in infancy than those who develop allergy. In contrast, respiratory allergens do not appear until a few years of age.
References


