

Review Series: The “Long and Winding Road” to Our Goal of Primary Prevention of Allergic Diseases

Microbial Exposure and Onset of Allergic Diseases - Potential Prevention Strategies?

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ABSTRACT

Chronic inflammatory diseases are a major health problem with global dimension. Particularly, the incidence of allergic diseases has been increased tremendously within the last decades. This world-wide trend clearly indicates the demand for new approaches in the investigation of early allergy development. Recent studies underlined the basic postulate of the hygiene hypothesis that early exposure to microbial stimuli plays a crucial role in the prevention of chronic inflammatory conditions in adulthood. There is ample evidence that, both, exogenous microbes and endogenous microbial communities, the human microbiota, shape the developing immune system and might be involved in prevention of pathologic pro-inflammatory trails. According to the Barker hypothesis, epidemiological studies pointed to transmaternal transmission from the mother to the offspring already in prenatal life. Experimental data from murine models support these findings. This state of the art review provides an overview on the current literature and presents new experimental concepts that point out to future application in the prevention of allergic diseases.

KEY WORDS

allergy prevention, bacterial stimuli, Barker hypothesis, hygiene hypothesis, microbiota

ABBREVIATIONS

CID, chronic inflammatory disease; GF, germ-free; IgE, immunoglobulin E; IL, interleukin; IFN- γ , interferon- γ ; LPS, lipopolysaccharide; NO₂, nitrogen dioxide; SO₂, sulfur dioxide; TH, T-helper; TLR, toll-like-receptor; TNF- α , tumor necrosis factors α .

ALLERGIES - A HEALTH PROBLEM OF GLOBAL DIMENSION

Chronic inflammatory diseases (CID) represent a complex of multi-faceted non-communicable medical conditions manifesting in nearly all organs. Though the pathogenetic trails of CID differ between the organ-specific manifestations, these conditions share the same basic principle - a deviation of the immune response. As a result of a persistently dysbalanced immune defence chronic inflammation appears within

a characteristic window of aging, depending on the specific outcome. The major part of CID, e.g. inflammatory bowel diseases or multiple sclerosis, is initially manifested in late childhood, puberty and adolescence or later, while allergies uniquely appear already in early childhood. In spite of different onsets and courses, a global increase of incidence could be noted for all in the last decades. This dramatic increase in incidence cannot be explained solely by the presence of genetic variants (e.g. polymorphisms) in chronically diseased individuals which lead to a dys-

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balanced immune response. Such genetic variants have been identified in a high number of genes in various phenotypes of patients suffering from allergies or autoimmune diseases.¹⁻⁴

A general current concept to explain the increasing health burden of CID is a close and intimate interaction between genes of disease risk and environmental factors. It is of major importance to identify and characterize environmental components which are closely associated with disease development and manifestation, since this could be a starting point not only for a better understanding of the pathogenesis of these conditions, but also for the development of (primary) prevention strategies.⁵

Environmental factors may contribute to a development of allergies in two different and antagonistic ways: On the one hand environmental components could be defined as risk factors for the development of allergies. Particularly in the last century major research efforts were undertaken to characterize and define such risk factors. Hence, these efforts led to the identification of passive and active tobacco smoke, environmental pollutants such as SO₂ and NO₂, psychological factors (e.g. chronic stress), nutritional components and others as risk factors for the development and/or exacerbation of allergic inflammation.⁶⁻¹¹

More recently an alternative approach has been undertaken by several groups including our own research laboratory that is the search for protective environmental components. Based on three fundamental hypotheses the hygiene hypothesis, the microbiota hypothesis and the Barker hypothesis, these efforts led to the development of a new paradigm focussing around the role of environmental and barrier microbes as early determining factors of immune development. This article summarizes and highlights recent findings which are discussed and presented in a broader way in two recently published review articles by the authors.^{12,13}

THE HYGIENE HYPOTHESIS

It was in the late 1980s that David Strachan published the observation that children with older siblings and/or frequent early childhood infections showed a consistent and significant protection, particularly against respiratory allergies.¹⁴ In line with Strachan's observations, increased exposure to early childhood pathogens was suggested as the major determinant that protects children attending day care early in life against the development of respiratory allergies.^{15,16} A similar allergeo-protective effect was observed in children living under unique lifestyle conditions e.g. as defined by the term "anthroposophical" lifestyle based on the Steiner concept,¹⁷ among the Amish population in Northern America¹⁸ and in emerging countries in populations with a poor hygienic standard.^{19,20} These findings were substantiated by nu-

merous studies conducted in traditional farming sites such as traditional farms in the Alpine region of central Europe.²¹ As a common thread, these living conditions are characterised by a highly enriched microbial environment. In the original sense of the hygiene hypothesis, a frequent confrontation with environmental microbes particularly in early childhood shapes normal immune responses closely linked to clinical and immunological tolerance. A number of environmental microbes have recently been identified and further functionally characterized with regard to their capacity to modulate immune responses (see below).

THE MICROBIOTA HYPOTHESIS

Regardless whether environmental microbes are primarily exposed to the respiratory, the gastrointestinal of the skin mucosal surface, these microorganisms may closely interact with the epithelial barrier and the innate immune system. Some of them will successfully colonize these surfaces and will make up the mucosal microbiota, a sustainable microbial community established in symbiosis with the host. It has recently been shown that these barrier microbes play a pivotal role in shaping immune responses, particularly early in life. These microbes apparently play a decisive role in either generating (mucosal) tolerance or leading the way to the development of inflammatory conditions.²²

The gastrointestinal microbiota represents the by far best studied microbial community coexisting with human subjects. This system consists of more than 100 trillion microorganisms, most of which are bacteria. Two of about 55 known phyla (*Firmicutes* and *Bacteroidetes*) dominate the human intestinal tract. This illustrates the relative exclusivity of the human intestinal microbiota, indicating the special relationship between us and them. The individual microbiome consists of approximately 15% of the much more than 1.000 thus far identified species.^{23,24}

It has been just recently recognized that there is a close bidirectional interaction between these microbiota and our immune system.²⁵ Some microbes provide us with essential non nutrient factors such as vitamins. We also benefit from their energy machinery, which increases our harvest of nutrients in the gut. Furthermore, of great importance is the fact that microbial triggers are necessary for the development and maturation of our immune system, as recently summarized in several review articles.^{26,27} In this regard germ-free mice represent an important model system to study the effect of (controlled and organized) colonization on the development of the innate and adaptive immune system.^{28,29}

Sudo *et al.*³⁰ discovered that GF mice were not capable of developing oral tolerance against a model allergen ovalbumin because of abrogation of TH1-mediated responses. In consequence, GF mice had

stronger T-helper (TH)2-responses, as demonstrated by increased production of interleukin (IL)-4. Reconstitution of the intestinal flora with the predominant intestinal bacterium *B. infantis* completely restored oral tolerance induction to a TH2-promoting allergen. Interestingly, this mechanism was only effective when the reconstitution was performed in neonates but not in adult mice. A similar observation has been found using sensitization to cow's milk protein.³¹ Furthermore there seems to be a close interaction between the intestinal immune responses and the lung. Missing or altered intestinal colonization has an impact on respiratory immune responses. Herbst *et al.* could demonstrate that GF mice exhibited exaggerated allergic immune responses in the lung after intranasal allergen challenge of sensitized animals. Such animals showed increased eosinophil and basophil numbers, which correlated with augmented local TH2 cytokine production, systemic IgE levels, decreased alveolar macrophage plasmacytoid dendritic cell numbers and an altered phenotype of conventional dendritic cells. Also, recolonization with the complex flora of normoflora mice could completely reverse the exaggerated and augmented allergic responses found in germ-free (GF) mice.³² Intact toll-like-receptor (TLR) signalling pathways, together with triggering of these pathways via microbial recognition seems to be an important prerequisite in order to shape intestinal homeostasis.^{33,34} On the other hand, GF mice could be protected from cow's milk allergy by a transfer of infant human gut microbiota with a predominant presence of *Bifidobacterium* and *Bacteroides species*.³⁵ These are just some of many examples indicating the importance of gut microbes on the development of immunological homeostasis not only on the local level, but also in terms of systemic responses and responses at other anatomical sites. Furthermore, it is important to note that there is apparently a certain "Window of opportunity" where these programming events can occur in a very efficient way. Both human and animal studies point to the perinatal environment as a very critical period in life in this regard.

ENVIRONMENTAL MICROBES AND ALLERGIES

One of the best studied model situations for clinical and experimental evaluation of the hygiene hypothesis is the traditional farming environment. Here it was shown that living under these conditions results particularly in a marked reduction of incidence and prevalence of respiratory allergies and atopic sensitization. This has been repeated and reproduced now in quite a number of independent studies (reviewed in²¹).

EXPOSURES ASSOCIATED WITH THE PROTECTIVE EFFECT

One important component in this regard is the exposure to animals raised on farms.³⁶ The higher the number of species exposed to the stronger is the asthma-protective effect. Particularly exposure to cows seems to be of great importance.³⁷ In addition, nutritional components have also been identified. Drinking non-pasteurised, non-homogenised milk and consuming dairy products also seems to be quite helpful.³⁸

The first bacterial component which has been linked to asthma protection is the exposure to endotoxin/lipopolysaccharide (LPS), cell wall components of gram-negative bacteria. LPS-exposure was measured in dust samples collected from mattresses as a surrogate marker for continuous chronic exposure to bacteria. An inverse correlation was found between the dose of exposure and the protective effect. The higher the natural exposure dose the stronger the protective effect or the lower the risk for the development of respiratory allergies, atopic sensitization and other phenotypes. Additional assessments were then performed using mononuclear cells collected at school age and polyclonally stimulated for 24 hours. Measurements of cytokines reveal a strong down regulation of cytokine production with increasing doses of natural LPS-exposure. This was found for proinflammatory cytokines such as tumor necrosis factor (TNF)- α , interferon (IFN)- γ , and IL-10 and others, reflecting a state of "endotoxin tolerance" at school age with increasing exposure dose.³⁹

LPS is recognized by pattern recognition receptors of the TLR-family. It was therefore the next step to analyse a TLR-expression pattern in association with endotoxin exposures. It was found that early life exposures have a strong effect on the TLR-expression as indicated by increased TLR5 and TLR9 mRNA levels at birth. The higher the number of different farm animal species the mother had contact with during pregnancy, the higher the levels of TLR2, TLR4 and CD14 at school age.⁴⁰

THE "FOETAL ORIGIN OF DISEASE" HYPOTHESIS

In the early 90th of last century the epidemiologist David Barker postulated that foetal developmental programming is modified by environmental factors which are transmitted from the mother to the progeny. Based on observations he made in large birth cohort studies Barker suggested that modification of foetal programming caused by prenatal exogenous exposures will lay the foundation for later pathogenic conditions in adulthood such as cardio-vascular or metabolic diseases.⁴¹

Recently, this concept was broadened by new evidence that microbial prenatal exposures play a pivotal

Table 1 The asthma protective effect of bacterial strains and bacterial components in experimental models of murine airway inflammation

Postnatal exposure to:	Sensitisation with HDM							
	Alum i.p.				without Alum s.c.			i.n.
	<i>A. lwoffii</i> F78 i.n. ⁵¹	<i>L. lactis</i> G121 i.n. ⁵¹	<i>B. lichini-</i> <i>formis</i> 467 i.n. ⁵²	<i>E. coli</i> i.n. ⁵¹	<i>S. scuiri</i> W620 i.n. ⁵³	<i>A. lwoffii</i> F78 i.n. ⁵⁶	<i>L. rhamnosus</i> GG neonatal i.n. ⁶⁰	<i>S. scuiri</i> W620 i.n. ⁵³
1) BAL:								
Leukocytes	↓ ↓	↓ ↓	↓	↓ ↓	↓ ↓ ↓	↓ ↓	↓	↓ ↓
Eosinophils	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓ ↓ ↓	↓	↓ ↓ ↓
Lymphocytes	0	↓	0	↓	↓ ↓	↓ ↓	0	↓ ↓
2) Immunoglobulines:								
anti-OVA IgE	0	0	n.d.	n.d.	↓ ↓	↓	↓	n.d.
anti-OVA IgG1	↓ ↓	↓ ↓	n.d.	↓ ↓	↓ ↓	↓ ↓	0	0
anti-OVA IgG2a	0	0	n.d.	0	↑ ↑	↑ ↑	0	0
3) Airway reactivity	↓ ↓ ↓	↓ ↓ ↓	↓ ↓	↓ ↓ ↓	↓ ↓ ↓	0	0	n.d.
4) Inflammation (Histology)	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↓ ↓ ↓

As compared to OVA or house dust mite extract respectively.

B

Prenatal exposure to:	<i>A. lwoffii</i> F78 i.n. ⁵⁴	<i>L. lactis</i> G121 i.n. [†]	<i>L. lactis</i> G121 i.g. [†]	<i>LPS</i> i.n. ⁴⁸	<i>L. rhamnosus</i> GG i.g. pre- and perinatal ⁶¹	<i>L. rhamnosus</i> i.g. prenatal ⁴⁸	<i>S. scuiri</i> W620 i.n. [†]
1) BAL:							
Leukocytes	↓ ↓	0	↓	↓ ↓ ↓	↓ ↓	↓	0
Eosinophils	↓ ↓	0	↓	↓ ↓	↓ ↓ ↓	↓	↑
Lymphocytes	↓ ↓	0	↓ ↓	↓ ↓	0	↑	↑
Macrophages	↓	↑	↓	↑ ↑	↑	↑	0
2) Immunoglobulines:							
anti-OVA IgE	↓	0	0	↓ ↓	0	0	0
anti-OVA IgG1	↓	0	0	↓ ↓ ↓	0	0	0
anti-OVA IgG2a	0	0	0	↓ ↓	0	0	0
3) Airway reactivity	↓ ↓	0	↓ ↓	0	0	0	0
4) Inflammation (Histology)	↓	0	↓ ↓	↓ ↓	↓ ↓	↓ ↓	↑

As compared to OVA.

(A) Postnatal model of murine allergic airway inflammation: Female BALB/c mice were exposed to PBS (control) or bacterial strains (see below) over the whole sensitization and challenge process. After challenge the asthmatic phenotype was analyzed. Protocol for bacterial application: 1×10^8 CFU, 3 times per week starting directly after birth over the whole sensitization and challenge process. 1) Numbers of total leukocytes, eosinophils, lymphocytes (reduction of respective cells) in bronchoalveolar lavage (BAL). 2) Concentration of anti-OVA IgE, anti-OVA/HDM IgG1 (decrease of respective antibody titer) and anti-OVA/HDM-IgG2a (increase of respective antibody titer) in serum of offspring. 3) Airway responsiveness to methacholine (MCH) measured by head-out body-plethysmography. Shown is MCH50 (improvement of airway reactivity). 4) Quantification of inflammation in airways (reduction of inflammatory cells). 0 = 0%; ↑ > 0-33%; ↑ ↑ > 34-66%, ↑ ↑ ↑ > 67-100%. † own unpublished data.

(B) Prenatal model of murine allergic airway inflammation: Female BALB/c mice were exposed to PBS (control), LPS or bacterial strains (see below). Offspring were sensitized to ovalbumin (OVA), OVA aerosol challenged and the asthmatic phenotype was analysed. Protocol for bacterial application: 1×10^8 CFU, i.n., 3 times per week starting 10 days before mating until birth of the offspring. Protocol for LPS application: 1×10^8 CFU, 3 times per week starting 10 days before mating until birth of the offspring. 1) Numbers of total leukocytes, eosinophils, lymphocytes (reduction of respective cells) and macrophages (increase of respective cells) in bronchoalveolar lavage (BAL). 2) Concentration of anti-OVA IgE, anti-OVA IgG1 (decrease of respective antibody titer) and anti-OVA-IgG2a (increase of respective antibody titer) in serum of offspring. 3) Airway responsiveness to methacholine (MCH) measured by head-out body-plethysmography measured as MCH50 (improvement of airway reactivity). 4) Quantification of inflammation in airways (reduction of inflammatory cells). ↓ > 0-33%; 0 = 0%; ↑ > 0-33%; ↑ ↑ > 34-66%, ↑ ↑ ↑ > 67-100%. † own unpublished data.

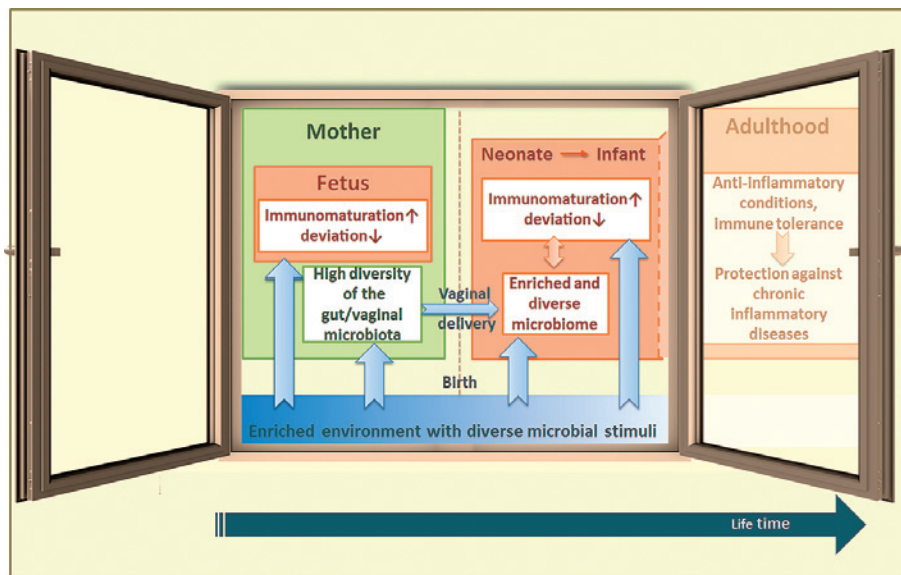


Fig. 1 Early environmental microbial stimuli contribute to immune tolerance and protection against chronic inflammatory conditions in adulthood - “window of opportunity”. Already in fetal life transmaternally submitted environmental stimuli affect the developing immune system of the offspring. After birth the microbiota seems to play a decisive role in shaping the neonate immune response with consequences for later immune homeostasis or chronic inflammatory conditions in adulthood. Within an early window of opportunity application of microbes or microbial components seems to be promising tool in preventive and therapeutic strategies against the development of chronic inflammatory diseases.

role in shaping and programming early immune responses and thereby later chronic inflammatory conditions. Maternal exposure to a diverse microbial environment (as found on traditional farming sites) during the early life window of pregnancy was shown to be associated with a TH1 balanced immune responses of the progeny at birth. Already in cord blood, a consistent upregulation and increased production of IFN- γ and TNF- α at birth has been found while no effect on TH2-cytokine levels or IL-10 or IL-12 production could be detected.⁴² Furthermore, an inverse relationship between neonatal IgE-production and cord blood IFN-gamma levels was noted.⁴³ These data have shed important light on regulation of perinatal adaptive immune responses. It is now established that the prenatal period is characterized by a marked upregulation of TH2 immune responses. Many reasons may account for this phenomenon, including the prevention of abortion and rejection for the maternal immune system, the ability to recognize very low antigen concentrations as well as the prevention of unwanted inflammatory responses.⁴⁴ However, it is an important task to stimulate the TH1-immune pathway very early on in life. This is of great importance since the type 1 immune responses within the CD4 and CD8-subsets are very critical for the defence of viral, bacterial and also fungal infections. It is, therefore, critical that the developing immune system

trains the type 1 pathway in a way that this is becoming the preferred pathway instead of the TH2 system.

Though microbial exposures during pregnancy might at the end alter adaptive immune responses, innate immune cells seem to act as crucial initiators of this interplay. As an important step further in understanding this mechanism it could be demonstrated that local (mucosal) administration of natural or synthetic TLR agonists trigger a cascade of immune responses which result in the protection of experimental asthma in several murine models. This has been shown for TLR2 (lipopeptides),⁴⁵ TLR2/4 (peptidoglycan),⁴⁶ TLR4 (lipopolysaccharides),^{47,48} TLR3 and TLR7 (poly I:C and R848)⁴⁹ and TLR9 agonists.⁵⁰ In most of these studies, the TH2-protective effect was related to either a marked upregulation of type 1 immune responses or increased levels of IL-12 and IL-10.

Based on these experimental data, whole bacteria were investigated instead of synthetic TLR ligands. These bacteria were derived from the analysis of the microbial content of dust samples collected on traditional farms. Tested in experimental models of asthma and demonstrated in asthma protective effect when administered repeatedly and through exposure to mucosal surfaces: *Acinetobacter lwoffii*, *Lactobacillus lactis*, *Bacillus licheniformis* and *Staphylococcus sciuri*.⁵¹⁻⁵⁴ In between a number of farm-derived bacteria

were investigated for their allergoprotective capacity. These studies have shown that microbial protection is provided pre- and postnatally and both via the inhalative and the digestive route (see Table 1). Further studies are on their way to delineate the underlying regular mechanism which lead to these protective effects on both sides, namely the maternal and the materno-fetal interface as well as events operating in the neonate itself.⁵⁵

OPPORTUNITIES FOR PREVENTION AND INTERVENTION WITH MICROBES

It was more than 10 years ago that the first reported clinical trial using probiotics was published in terms of allergy prevention. Since then most of the studies focussed primarily on early outcomes of allergic diseases such as eczema and IgE-mediated food allergies with far fewer looking at longer-term outcomes of the atopic march such as respiratory allergies. Unfortunately there is a high degree of inconsistencies between findings from different studies. Many of them can be attributed to the wide range of methodological heterogeneity which prevents direct comparison and meaningful meta-analysis. Differences include probiotic strains or combinations used in the preparation, method of delivery, dose, inclusion criteria, timing and duration of intervention and measurement of clinical outcomes. Furthermore, the results are conflicting even for those strains such as *Lactobacillus rhamnosus* GG, which are most extensively studied.⁵⁶⁻⁵⁹ This topic has been recently covered by a review article.¹² Most probably, interventions should include both ante-natal and post-natal treatment periods. Furthermore, there is still uncertainty about the ideal bacterial strains used in the preparation. Also it is still open whether the general population would benefit from such strategies or only high risk infants. Further open questions include dosing, timing of introduction and length of treatment.

Future intervention strategies might target the microbiota as a central immune compartment. The life-saving strategy of faecal microbiota transplantation in patients suffering from pseudomembranous colitis points out to novel approaches in the prevention and therapy of chronic inflammatory conditions.⁶²

In conclusion, the close and intimate relationship between man and bacteria seems to be established already in the mother's womb and lasts up until adulthood. The most critical phase for immune dysbalance and deviation seems to be the perinatal life span but this interval also offers a "window of opportunity" to intervene the early development of chronic inflammatory conditions (Fig. 1).

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