HiPP Scientific Services

HiPP Webinar Abstract

New insights in alimentary allergy prevention by improving gut health

Tuesday 18th January 2022, 7.00 to 8.30 pm

Prof. Dr. Ulrich Wahn
Berlin, Germany

New insights in alimentary allergy prevention by improving gut health

Research of the last decades has led to a better understanding of the so called „atopic march“ from infancy to adolescence including the driving genetic, environmental and lifestyle determinants. The most prevalent clinical manifestations early in life include atopic dermatitis with or without food allergy, which is the dominating phenotype in infancy, and allergic diseases of the upper (rhinitis) or lower airways, which have the highest incidence between the age of 3 and 15 years.

Eczema is strongly determined by multiple genes including mutations in the filaggrin gene. Early topical treatment may lead to sufficient skin hydration and reduce intensity of inflammatory skin manifestations.
Food sensitization and relevant allergies, which may develop in infancy, are supposed to be facilitated by infantile skin exposure to food allergens. Sensitization to cow's milk and hen's egg have been shown to be the most prevalent problems. They may persist over years, however, have a chance to lead to long term tolerance.

Allergic rhinitis is significantly more likely to develop in children with previous infantile dermatitis.
Allergic asthma has been shown to affect children with both, previous eczema and allergic rhinitis.

An increase of all atopic manifestations in infancy and childhood has been reported in many parts of the world, which suggests the involvement of environmental factors, behavioural or lifestyle changes rather than a genetic drift.

Regarding interventions, which aim at prevention at an early stage, unfortunately success so far has been rather limited and mostly disappointing.

One of the few examples, where consequent avoidance of neonatal exposure leads to effective prevention is anaphylaxis induced by latex exposure in babys born with spina bifida. These investigations have led to guidelines suggesting latex free surgical theaters in all pediatric centers, which today are known to be highly successful.

In order to prevent food and inhalant allergies initial studies assessed targeted avoidance of alimentary and environmental allergens, particularly from the domestic environment. The results in most studies turned out to be rather limited. Prolonged breast feeding for longer than 6 months as well as delayed introduction of solid food in infancy was not found to be effective.

In infants with a genetic susceptibility for atopy some of the larger trials with protein hydrolysates, (so called „hypoallergenic or HA-formulae“), found a reduction in the incidence of atopic dermatitis, whereas the effect on allergic airway diseases was at best marginal.

Epidemiological studies in defined populations like Bavarian or Swiss farming families, where children grow up in a very special environment with a special microbial domestic exposure have led to the hypothesis, that this environment has protective potential against allergy development.
Some more recent american reports on religious immigrant populations, (Hutterites, Amish), as well as in the Finnish and Russian part of Karelia are interpreted in a similar way, leading to the hypothesis that traditional lifestyle which is associated with a specific microbial exposure may prevent the infantile immune system from developing in the TH-2 dominated direction leading to allergic diseases.

During the last years the role of microbial exposure in the environment as well as mucosal microbial colonisation attracted the interest of immunologists and allergists. Some of these studies seem to find encouraging results and might provide the basis for future interventions early in life. Educating the human immune system might therefore lead to a decreased disease susceptibility already very early in life. So far conclusive mechanistic insights on how preventive interventions may work are still lacking. However, a number of studies aiming at a modulation of gut microbiota are currently been initiated in order to find out whether the susceptibility for manifestations like cow's milk protein allergy or asthma could be corrected in the years to come.

It is the aim of this webinar to review the state of research in the field of allergy prevention and give an outlook for exciting and hopefully effective future approaches.

References

Lau.S., Matricardi. PM, Wahn. U., Lee. YA, Keil T., Allergy and atopy from infancy to adulthood: Messages from the German birth cohort MAS. Ann. Allergy Asthma Immunol 2019,122, 25-32

Stein. M.M. et al. Innate immunity and asthma risk in Amish and Hutterite farm children. N. Engl. J. Med.2016, 375, 411-421

Petersen. C, Tuvey. SE Can we prevent allergic disease? Understanding the links between the early life microbiome and allergic diseases in childhood. Curr Opinion Pediatr. 2020

Nieto. A. et al. Bacterial mucosal immunotherapy with MV 130 prevents recurrent wheezing in children: A randomized, double blind, placebo-controlled trial. Am.J. Respir. Crit. Care Med. 2021

Prof. Dr. Ruldof Valenta
Department of Pathophysiology and Allergy Research,
Medical University of Vienna, Austria

Hypoallergenic potential and immunomodulation of a hydrolysed infant formula: Cow's milk allergy, mechanisms, diagnosis, treatment and prevention

Raphaela Freidl, Victoria Garib and Rudolf Valenta

Immunoglobulin E (IgE)-mediated allergy is the most common hypersensitivity disease affecting approximately 30 % of the population. Allergic patients suffer from a variety of symptoms, including respiratory symptoms (e.g. rhinitis, rhinoconjunctivitis, asthma), skin symptoms (e.g. urticaria, atopic dermatitis), gastrointestinal symptoms and life-threatening anaphylaxis. IgE-associated food allergy affects approximately up to 3 % of the population whereas respiratory allergy is more common with a prevalence of 25—30 %.

The correct diagnosis of IgE-mediated food allergy is very important, because non-IgE-mediated forms of food intolerance are much more common than food allergy. Accordingly, it is important to confirm IgE sensitization to the culprit food allergens by serological testing in addition to performing anamnesis and food provocation. For this purpose, molecular IgE testing with purified allergen molecules allows to detect IgE sensitization to food allergen molecules which can induce severe anaphylactic reactions and quantification of specific IgE levels is useful to predict severe reactions. The measurement of allergen-specific IgG is not suitable for the diagnosis of IgE-mediated food allergy and can lead to incorrect diagnosis and incorrect treatment such as wrong diet recommendations.

Cow's milk is a very important source for food allergy affecting especially children early in life. Patients with IgE sensitization to cow's milk allergens not only suffer from gastrointestinal symptoms upon milk consumption, but also experience skin manifestations, respiratory allergy and severe life-threatening anaphylactic shock. Prevention and treatment of cow's milk allergy can be achieved by avoidance (i.e. diet), allergen-specific immunotherapy (mostly given as oral immunotherapy which can induce severe side effects) and by symptomatic treatment with drugs and biologics which however lack sustained treatment effect.

Hypoallergenic milk formulas based on cow's milk which is hydrolysed to a different degree (e.g. partially, extensively and amino acid formulations) have potential for treatment of allergic children, may be used early in diet to prevent the development of IgE sensitization and eventually may even induce tolerance by preventive immunomodulation.

In this context, the degree of hydrolyzation is important because if cow's milk allergen peptides are too large, they may induce allergic reactions or IgE sensitization but when they are too short, they will not be able to induce T cell tolerance. Furthermore, the presence of pre/probiotics may influence the capacity of a particular formula to prevent IgE sensitization.

We have investigated the properties of HiPP HA COMBIOTIK® an extensively hydrolysed milk infant formula regarding IgE reactivity and allergenic activity by biochemical techniques, with cow's milk allergen-specific antibody probes and using sera from clinically well characterised cow's milk allergic children regarding IgE reactivity and basophil activation.

Furthermore, we studied T cell activation and induction of inflammatory and tolerogenic cytokines in peripheral blood mononuclear cells of cow's milk allergic children. Our results show that HiPP HA COMBIOTIK® lacks intact cow's milk allergens, has no relevant IgE reactivity and strongly reduced allergenic activity, in particular with addition of *L. fermentum CECT5716*. Importantly, HiPP HA COMBIOTIK® induced significantly less inflammatory cytokine production but comparable induction of the tolerogenic cytokine IL-10 as compared to the intact protein formula.

Thus our results suggest the HiPP HA COMBIOTIK® may be not only used for allergy prevention in children by exhibiting less IgE-sensitizing capacity but also by induction of specific immunological tolerance. Our data also suggest that HiPP HA COMBIOTIK® is hypoallergenic and may be tolerated by already sensitised children when introduced into their diet.

Marta Calatayud Arroyo, Ph.D.
Institute of Agrochemistry and Food Technology (IATA-CSIC),
National Research Council, Valencia, Spain

Promotion of a healthy gut barrier for allergy prevention Extensively hydrolyzed infant formula improves intestinal epithelial barrier function and modulates innate immune response in a triple co-culture *in vitro* model

Marta Calatayud Arroyo, Laura Olivares and Maria Carmen Collado

The intestinal epithelium plays a critical role in regulating nutrient uptake and maintaining gut homeostasis by modulating innate immune function, regulating antigen responses, and establishing a cross-talk between intestinal cells, microbiota, and the underlying immune system.

Infancy is a sensitive period for immune programming and oral tolerance induction. During early life, dietary antigen exposure and macromolecular passage over the intestine establish a sensitive window for environmental triggers.

During this critical window, exclusive breastfeeding is the desirable goal and it is recommended for at least 6 months, but it is not possible in some cases. Human milk contains human milk oligosaccharides, maternal antigens, micro-organisms and other bioactive molecules involved in gut maturation and development, microbial colonization and immune maturation.

The healthy gut environment is characterised by a homeostasis between dietary antigen exposure, gut barrier function, microbial shifts and orchestration of innate and adaptive immune responses. Disturbances of this homeostasis during the weaning process can lead to increased susceptibility to allergy and inflammation.

Especially for infants with a high risk of allergy development, hydrolysed infant formulae promoting gut barrier function and reducing the risk of undesired inflammatory responses are required. Partially or extensively hydrolysed infant formula might help in reducing the risk of different allergy manifestations such as atopy in intervention studies, but mechanisms behind these effects are not fully characterised.

In the current study, we used a triple *in vitro* model of gut inflammation and homeostasis, including enterocyte-, goblet- and macrophage-like cells in a Transwell setup. The effect of extensively hydrolysed (eHF) infant formula containing the prebiotic fibre GOS (Galactooligosaccharide) combined or not with heat-inactivated *Limosilacto-bacillus fermentum CECT5716* on the intestinal barrier (mucus, tight junction proteins, Lucifer Yellow transport) and cytokine production was assessed.

Under LPS-inflammatory trigger, eHF and co-exposure of eHF and inactivated *L. fermentum CECT5716* increased mucus production and *MUC2* mRNA expression, restored the epithelial barrier integrity, and increased secretion of regulatory TGFβ, when compared to control condition. Overall, eHF had a significant effect on barrier function and immunomodulation of the simulated epithelium.

These results suggest a beneficial role of eHF combined with GOS and *L. fermentum CECT5716* in recovering intestinal homeostasis and modulating pro-inflammatory responses.

Gabriela Grigorean, Ph.D.
Proteomics Core Facility at
University of California Davis, USA

Bioactive peptides in human milk and infant formula

Gabriela Grigorean, Xiaogu (Tina) Du and Bo Lonnerdal

Human milk contains proteins with biological activities and during digestion peptides are formed that are also known to exert bioactivities. Infant formulae also contain bioactive proteins and bioactive proteins can be formed during gastrointestinal digestion. Extensively hydrolysed formulae contain a multitude of peptides some of which may exert bioactivities.

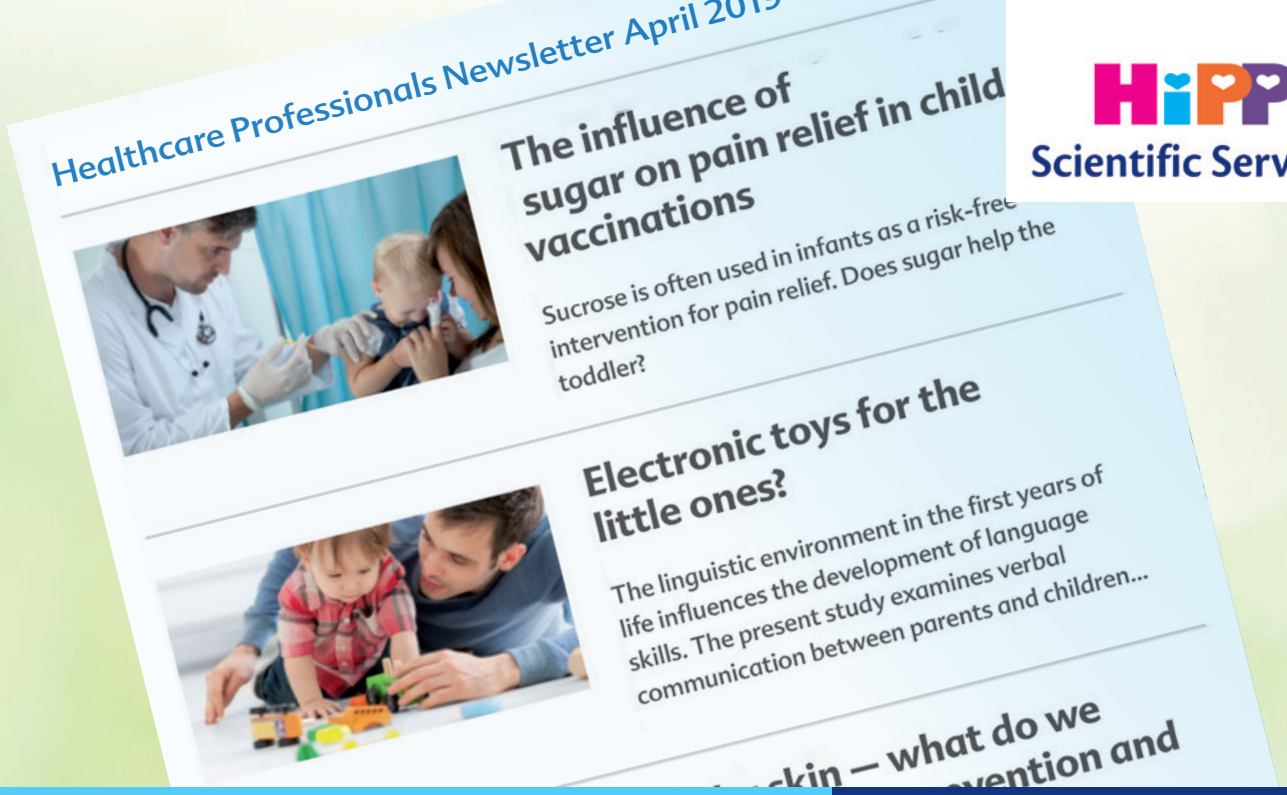
Infant formulae preparations were therefore analysed via liquid chromatography/mass spectrometry (LCMS) to survey them for potential biologically active peptides. LCMS is a sophisticated detection methodology, which provides many aspects of information, such as presence, specificity, relative quantity, all subjected to thorough statistical analysis vetting. The HiPP formulae subjected to LCMS were shown to contain peptides with potential biological activities for several pathways involved in beneficial immunological activity. Standard infant formula (manufactured from intact cow's milk protein, iPF) and HiPP HA COMBIOTIK® formula (manufactured from extensively hydrolysed protein, eHF) were analysed. For each of the two formulae (iPF and eHF), one liquid (L) and one powdered (P) product were analysed. All of them contain a prebiotic fibre (Galactooligosaccharide, GOS) and in addition, the powdered formulae contain a probiotic strain (*L. fermentum CECT5716*).

The samples were subjected to *in vitro* gastrointestinal digestion considering the physiological gastrointestinal conditions of an infant (0—4 months of age). In vitro digestion was performed according to our previously published work (Wada & Lönnerdal 2015). Briefly, formulas were digested at pH 4 (infant stomach pH) for 15 and 30 min, respectively. The pH was then adjusted to 7 and pancreatic enzymes were used to simulate intestinal digestion for 15 and 30 min, respectively. The reaction was stopped by heat inactivation of the enzymes. These peptides were then separated into two fractions based on mass: above and below 10 kDa. Those above 10 kDa were subjected to an additional enzymatic digestion with trypsin. This is necessary in order to cut the larger peptides into smaller size peptides that are required for LCMS analysis.

LC separation was done on C18 reverse phase analytical column. Mass spectra were collected on an Orbitrap QExactive mass spectrometer (Thermo Fisher Scientific).

Peptides produced were found to have a variety of potential bioactive properties. Osteopontin peptides were found in eHF and iPF. This protein is a Th1 cytokine and is described in literature to have a role in setting up a tolerogenic milieu (Alissafi, 2018). In addition, the detected bioactive peptides were grouped into categories according to their biological function, e.g. immune regulation and allergic response. The levels of these grouped bioactive peptides were very similar in powdered and liquid formulae, while type of protein of the infant formulae (eHF vs. iPF) had an impact on the relative quantity of some of them. The level of immune regulatory peptides was comparable in eHF and iPF, suggesting that both formulae are able to promote immuno-competence. In addition, peptides associated with allergic response were strongly reduced in eHF (L: 15.21 %; P: 14.78 %) compared to iPF (L: 100 %; P: 100 %), suggesting that eHF is not likely to trigger an allergic response and that the immune system maintains its balance.

Our results show that protein type (extensive whey hydrolysate vs. intact cow's milk protein) influences the quantity of bioactive peptides detected rather than physical form (liquid vs. powder) of the infant formula. In both types of formula, eHF and iPF, a broad spectrum of bioactive peptides was detected. Both contain bioactive peptides known to affect several pathways involved in beneficial immunological activity, while bioactive peptides associated with allergic response were strongly reduced in eHF.



HiPP Scientific Services

hipp.com/hcp

Exclusively for paediatricians, nutrition experts, midwives and related medical professionals

Have you already subscribed?

Welcome to the HiPP newsletter service for healthcare professionals

Subscribe now to this free newsletter and enjoy these benefits:

- Current studies in the fields of paediatrics, nutritional medicine and care, compactly summarised and reviewed by experts
- All the latest information every four months

Information for healthcare professionals