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A dietary intervention with non-digestible oligosaccharides and partial hydrolysed whey protein prevents the onset of food allergic symptoms in mice

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ABSTRACT

Strategies to prevent food allergy to common food such as cow's milk are important because there is no causal treatment available yet. Oral tolerance induction is of great importance for allergy prevention which is strongly dependent on allergen exposure and proper immune environment. Improving the efficacy of oral tolerance with adjuvants is a promising new strategy. In the current study, we investigated non-digestible oligosaccharides (NDO) on their capacity to enhance oral tolerance induced by partial hydrolyzed whey protein (pWH). Mice were treated orally with PBS, pWH, NDO or pWH + NDO for 6 days and subsequently fed a control diet while sensitized to whey protein. Acute allergic skin responses and mast cell activation were measured after whey challenge. pWH + NDO prevented acute allergic skin responses, mast cell activation and induced lower whey-specific IgE compared to NDO fed mice. In mice supplemented with either pWH or NDO, respectively Foxp3+ regulatory T-cells or galectin-9 were enhanced. Increased CD103+ dendritic cells percentages were measured in the mesenteric lymph nodes of mice treated with pWH + NDO. These data show the capacity of NDO, if combined with a pWH, to prevent the onset of allergic symptoms. NDO could act as adjuvant in preventing allergic responses to harmless food proteins.

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1. Introduction

Food allergy develops when the body fails to develop oral tolerance, which allows allergic sensitization and subsequently the production allergen-specific IgE. Allergies to cow's milk and hen's egg are the most common food allergies in children and prevention strategies for food allergy have been mainly focused on avoidance of the offending food. This changed during the last decade to strategies aiming at supporting oral tolerance by early introduction of solid foods. In children suffering from severe atopic dermatitis or allergic to hen's egg, it was shown that early introduction of peanut protected against the development of peanut allergy [1–3]. For food proteins like hen's egg or cow's milk proteins, the effects of early introduction on oral tolerance induction and the prevention of allergic symptoms remains uncertain and early introduction might lead to unwanted and dangerous allergic reactions [4–6]. The possibility of severe side effects is one of the major drawbacks of using whole protein for oral tolerance induction. Many processing techniques are used for reducing the allergenicity of food proteins. For cow's milk allergy hydrolyzation processes are used to reduce the allergenicity of cow's milk proteins. It has been demonstrated that pWH retained the capacity to induce allergen specific oral tolerance to the native whey protein. Protective effects coincided with increased Foxp3+ regulatory T-cells that could confer the protective effects to naïve recipient mice [7,8]. Formulas containing hydrolyzed proteins are commonly used for infants with a high risk of developing allergic diseases.

CD103+ dendritic cells (DC) are present in the small intestine and they migrate to the mesenteric lymph nodes (MLN), where they initiate and support oral tolerance [9]. CD103+ DC are described as important regulators of oral tolerance by driving gut homing of

Abbreviations: NDO, non digestible oligosaccharides; pWH, partial hydrolysed whey protein; MLN, mesenteric lymph nodes; DC, dendritic cells.

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Foxp3+ regulatory T-cells [10–13]. Evidence suggests that environmental factors like dietary components, mucosal epithelium, luminal bacteria and other immune cells can condition CD103+ DC [9]. The contribution of CD103+ DC in oral tolerance induction to food proteins like hen's egg has been shown recently [14].

Breastfeeding is considered to be the gold standard for the prevention of allergic disease. It provides a unique combination of lipids, proteins, carbohydrates, vitamins and minerals. Furthermore, there are numerous bioactive compounds present in human milk with immunological properties. One of the potential protective mechanisms of breastfeeding is the activity of oligosaccharides which are abundantly present in human milk [15,16]. To mimic some of the health and immune promoting properties of human milk oligosaccharides, a mixture of neutral and acidic non-digestible oligosaccharides was developed resembling the structure of human milk oligosaccharides. This mixture of non-digestible oligosaccharides (NDO) exerts immune modulatory effects in several experimental disease models [17–19]. Based on these data, it can be hypothesized that NDO fulfill a supportive role in preventive strategies for food allergy. NDO could act as adjuvant by supporting allergen-specific oral tolerance to harmless food proteins.

In the current study, it was hypothesized that the introduction of pWH + NDO before sensitization is more effective in preventing the onset of allergic symptoms than pWH alone. In a mouse model for cow's milk allergy, we analyzed the effects of NDO in oral preventive protocols on the development of allergic symptoms and effects on immune regulatory mechanisms, such as mucosal epithelium derived galectin-9, Foxp-3+ regulatory T-cells and CD103+ DC to support oral tolerance induction.

2. Methods

2.1. Cow's milk proteins and pWH

Whey was obtained from DMV International, Veghel, the Netherlands and hydrolyzed with an established mixture of endopeptidases and exopeptidases (confidential enzyme composition used by Nutricia Research) resulting in partial hydrolyzed whey protein (pWH). The enzymatic process was stopped by fast cooling. The pWH was characterized by analysis of the peptide size (85% < 1 kD, 8% < 2 kD, 4% < 5 kD, 1% < 10 kD, 0.6% < 0 kD and 1.4% > 20 kD) by means of high pressure liquid chromatography. This experimental whey hydrolysate was used in the animal studies as mentioned below.

2.2. Reagents and antibodies

Cholera toxin was purchased from Quadratech Diagnostics, Epsom, UK. Biotin labeled rat anti-mouse IgE, IgG_1, FITC-conjugated anti-CD4 (L3T4), PE-conjugated anti-CD25, APC-conjugated anti-CD103, PerCP-Cy5.5-conjugated anti-CD11c and isotype controls were obtained from Pharmingen, Alphen a/d Rijn, the Netherlands. APC-conjugated Foxp3 was obtained from eBioscience, San Diego, CA, USA. PBS was obtained from Cambrex Bio Science (Verviers, Belgium), streptavidin-horse radish peroxidase was obtained from Sanquin, Amsterdam, the Netherlands. Collagenase IV and DNAse 1 were obtained from Roche Diagnostics, Almere, the Netherlands. All other chemicals were obtained from Sigma-Aldrich, Zwijndrecht, the Netherlands.

2.3. Diets

Semi-purified cow's milk protein free AIN-93G-based diets were composed and mixed with NDO by Research Diet Services (Wijk bij Duurstede, the Netherlands). NDO of less complex structures than human milk oligosaccharides have been used as components in several dietary products to resemble the beneficial effects of human milk oligosaccharides. A specific mixture of short-chain-galacto- and long-chain-fructo-oligosaccharides enriched with acid-oligosaccharides was used in the current study. Structural aspects show similarity to human milk oligosaccharides in sugar chain length and molecular weight ranges. Moreover, human oligosaccharides start with a lactose moiety just as a prominent fraction of the galacto-oligosaccharides. The NDO mixture contained a 2 w/w% (9:1:2) mixture of short-chain galacto-oligosaccharides (scGOS, obtained by enzymatic elongation of lactose with galactose by β-galactosidase), long-chain fructo-oligosaccharides (lcFOS, derived from chicory inulin) and acidic oligosaccharides (pAOS, produced from pectin) as described previously [19] and is indicated as NDO throughout the manuscript. All oligosaccharides were exchanged for the same amount of total carbohydrates resulting in a comparable carbohydrate composition in the diets. The diets were stored at −20° C prior to use.

2.4. Animals

Three- to 4-week-old pathogen free female C3H/HeOuJ mice were purchased from Charles River Laboratories (Maastricht, the Netherlands), maintained on cow's milk protein free standard mouse chow (AIN-93G soja, Research Diets Services, Wijk bij Duurstede, the Netherlands). Animal care and use of animals were performed in accordance with and approved by the guidelines of the Dutch Committee of Animal Experiments (2010.III.02.023).

2.5. Oral tolerance induction, oral sensitization and challenge of mice

Prior to whey sensitization mice were fed a control diet and orally treated (daily; day −7 until day −2) with PBS or pWH (50 mg, once a day) using a blunt needle. Another group of mice were fed the NDO for 6 days with or without additional oral administration of the pWH. Subsequently, mice were fed a control diet and orally sensitized by gavage dosing, on day 0, 7, 14, 21 and 28 with whey (20 mg) per animal homogenized in PBS (0.5 ml) mixed with cholera toxin (10 μg), as previously described [20,21]. Non-sensitized mice received cholera toxin in PBS only. At day 33, five days after the last sensitization, the acute allergic skin response (ear swelling at 1 h) after intradermal whey challenge was measured. Mice were subsequently orally challenged with 0.5 ml of whey (100 mg/ml PBS) and 18 h later blood samples were collected, centrifuged for 15 min at 20,000*g* and stored at −70 °C until further analyses. A schematic representation of the timeline of tolerance induction, oral sensitization and challenge protocol is provided in Fig. 1.

2.6. Acute allergic skin response

An acute allergen-specific skin response was determined in all whey-sensitized mice, 1 h after intradermal challenge with whey (10 μg) in the ear pinnae. As a negative control, non-sensitized mice were challenged in the ear with whey. Ear thickness was measured in duplicate using a digital micrometer (Mitutoyo, Veenendaal, the Netherlands). The allergen-specific net ear swelling was calculated by correcting the allergen-induced increase in ear thickness with the non-specific ear swelling due to local injection in the non-sensitized mice. The ear swelling is expressed as delta μm.

2.7. Measurement of serum specific antibodies, mouse mast cell protease-1 (mMCP-1) and Galectin-9

Whey-specific IgE and IgG_1 levels were measured in serum by means of ELISA. Microlon plates (Greiner, Alphen aan de Rijn, the

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