Mechanisms underlying immune effects of dietary oligosaccharides¹–⁴

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ABSTRACT
The WHO refers to human milk as the nutritional gold standard for term infants. Human milk contains many immunomodulatory compounds, including oligosaccharides. Human-milk oligosaccharides can serve as prebiotics because the nondigestible oligosaccharides present in human milk show a clear bifidogenic effect on the gut microbiota. Dietary oligosaccharide structures that have prebiotic effects similar to human-milk oligosaccharides include galacto-oligosaccharides, fructo-oligosaccharides, and pectin-derived acidic oligosaccharides. Both animal studies and human clinical trials showed that dietary intervention with these dietary oligosaccharides in early life could lead to the prevention of atopic dermatitis, food allergy, and allergic asthma. The immune-modulating effects of these oligosaccharides are likely assisted via alteration of the intestinal microbiota or in a microbiota-independent manner by direct interaction on immune cells or both. In this review, an overview of the prebiotic role of dietary oligosaccharides on the microbiota and the microbiota-independent immune modulation by these prebiotics is provided. In addition, recent publications that report on the pathways by which the oligosaccharides might exert their direct immunomodulatory effect are summarized. Am J Clin Nutr 2013;98(suppl):572S–7S.

INTRODUCTION
Hippocrates (460–377 bc), considered as the father of Western medicine, stated, “Let medicine be thy food and food be thy medicine,” which indicates that the medicinal properties of various nutritional components have been appreciated since ancient times. Hippocrates also documented medicinal properties of a powder made from willow bark and leaves containing salicylic acid, which is now the most well-known painkiller found in almost every home (aspirin). This shows how nature delivers a chemical structure for medication such as aspirin. To date, almost 70% of available drugs originate from chemical compounds found in vegetables, plants, and fruit. In addition, there is a large body of evidence that indicates that human health is also modulated through interactions with microbes present in the intestine. The beneficial effect of specific microbes was postulated by the Nobel Prize winner Elie Metchnikoff (suppl):572S–7S. In 1901, he stated that certain lactic acid bacteria present in yogurt were beneficial to health” (1), whereas probiotics according to the FAO/WHO are “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” (2).

In this review, an overview of the prebiotic role of dietary oligosaccharides on the microbiota as well as the microbiota-independent immune modulation by these prebiotics is provided. In addition, recent publications that report on the pathways by which pre- and probiotics might exert their immunomodulatory effect are summarized.

HOST HOMEOSTASIS
The gastrointestinal tract is the largest immune organ in the body. It contains ~65% of the overall immunologic tissues and ≥80% of the immunoglobulin-producing tissues of the body. These immunologic areas in the gastrointestinal tract are involved in managing explicit pathogenic threats and in preventing the induction of inflammation by damage-associated molecular patterns. Recently, it has become clear that many gut-related disorders are correlated with an imbalance in the microbiota and the immune system. Well-known examples include inflammatory bowel diseases such as Crohn disease and ulcerative colitis (3, 4). In addition, impaired immune functioning is frequently associated with irritable bowel syndrome, which is characterized by the frequent occurrence of gut discomfort symptoms such as diarrhea, constipation, or bloating (5–7). It is also recognized that in other disease pathologies such as chronic heart failure (8), autism (9), allergy (10–12), and HIV (13), the microbiota and general gut integrity play a role.

The composition and development of the intestinal microbiota in infants are influenced by genetic factors, exposure to microbes, and mode of delivery but also to a large extent by the composition of human milk, which also contributes to the maturation of the infants’ immune system (14–16). Clinical data show that

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breastfed children have high numbers of the health-promoting lactobacilli and bifidobacteria, whereas children that are bottlefed with a cow-milk formula have significantly lower numbers of these intestinal bacteria (17). This large impact of human milk on an infant’s microbiota is important to reestablish the immunologic balance that is skewed during pregnancy to avoid rejection of the fetus by the mother’s body (15) and to set the metabolic homeostasis (16).

HUMAN MILK

The WHO refers to human milk as the nutritional gold standard for term infants. From a nutritional point of view, human-milk compounds are therefore interesting targets for immunomodulatory research. Several studies have shown the antinfective properties of human milk, which reduces the incidence of gastrointestinal and nonenteric infections in infants (15). In addition to these antinfective properties, antiinflammatory properties of human milk have been described (15). This is mainly of importance during the bacterial colonization of the mucosal surfaces of newborns. For example, in the skin and gut, large quantities of microbial components are in direct contact with the sterile neonate. Coordination of the inflammatory response occurring after this first contact is vital. The epithelial layer, together with the intraepithelial and immune-competent cells in the lamina propria, is the most important player in regulating the recognition of microorganisms and the maintenance of gut homeostasis. As described in more detail by Oozeer et al (14) elsewhere in this supplement, this gut-ecophysiologically is important for metabolic, immunologic, and even neurologic development, which emphasizes the diverse activity of human-milk compounds.

Human milk contains many immunomodulatory compounds, including IgG, IgM, and isoforms of immunoglobulins (secretory IgA), nucleotides, specific amino acids (taurine, polyanines), PUFA (EPA, DHA), monoglycerides, leucic acid, linoleic acid, cytokines and chemokines, soluble receptors (CD14, Toll-like receptor 2), antibacterial proteins/peptides (lactoferrin, lysozyme, \( \beta \)-lactoglobulin, casein), intact immune cells, and carbohydrates. Carbohydrates in human milk, such as lactose, glycoconjugates, and oligosaccharides, display a multifunctional activity spectrum as described in detail by Stahl (18) elsewhere in this supplement. In addition to being an energy source, human-milk oligosaccharides (HMOS)\(^5\) can also serve as prebiotics because the nondigestible HMOS present in human milk show a clear bifidogenic effect on the gut microbiota. However, the HMOS composition of human milk cannot be generalized because the mother’s secretor status and Lewis blood type determine the fucosylation pattern and thereby the types of HMOS present in human milk. Furthermore, Albrecht et al indicated that the composition of human milk shows considerable differences in the carbohydrate content during different phases of lactation (19). The literature on the effect of maternal diet on the nutrient composition of human milk is scarce, although it has been suggested that maternal factors such as diet have a larger influence on human-milk composition in later lactation stages than in the first few months (20).

MICROBIOTA-INDEPENDENT IMMUNE MODULATION

Apart from their prebiotic effect related to microbiota changes, HMOS can act as receptor analogs to inhibit the adhesion of pathogens on the epithelial surface (21). There is increasing evidence that HMOS act directly on the immune system, most likely via specific sugar receptors on immune cells (22–25). HMOS have been shown to interfere with in vitro leukocyte recruitment to sites of inflammation and to inhibit cell-cell interactions of lymphocytes via selectins (24), whereas other HMOS inhibit adhesion to endothelial cells and leukocyte rolling (22). Moreover, oligosaccharides have been shown to interfere with Th helper (Th1/Th2 skewing in cord blood–derived mononuclear cells (more interferon- \( \gamma \) producing CD4\(^+\) T cells) and to affect the Th2-type immune response of allergen-specific T cells from peanut-allergic individuals (24, 25). These publications support the in vitro evidence for epithelial transport of HMOS (24). Interestingly, Naarding et al (23) showed that HMOS are able to bind specifically to the lectin receptor DC-SIGN [dendritic cell (DC)–specific intercellular adhesion molecule-3-grabbing non-integrin] expressed by DCs. DC-SIGN interacts with a variety of pathogens, including HIV-1, and binding of HMOS inhibited the transfer of HIV-1 to CD4\(^+\) T lymphocytes. The above-described data suggest that oligosaccharides act systemically and are thereby modulating the immune response in a microbiota-independent manner.

DIETARY OLIGOSACCHARIDES WITH FUNCTIONS SIMILAR TO HMOS

In human milk, the proportion of prebiotic carbohydrates is substantial [12–15 g/L (26)], whereas prebiotic oligosaccharides in cow milk are present only in trace amounts. In addition, the HMOS composition is very complex because >200 different HMOS structures have been determined (26). The HMOS structures are often divided into 2 main categories: neutral and acidic oligosaccharides (27). Within the neutral core structures and fucosylated carbohydrates, 4 molecular masses represent \( \approx \)70% of the total molecules, including isomers of lacto-N-tetraose, lacto-N-neotetraose, lacto-N-hexaose, monofucosyl-lacto-N-hexaose, and difucosyl lacto-N-hexaose (26). The acidic oligosaccharides (AOS) contain sialic acids or sulfate groups, are present in human milk in relatively low amounts (1.5–3.3 g/L), and mainly consist of N-acetyl-neuraminic acid (28).

Oligosaccharide structures that display prebiotic effects similar to HMOS include galacto-oligosaccharides (GOS), fructo-oligosaccharides (FOS), and AOS. Several types of GOS with different chemical characteristics are used in immunomodulatory research. GOS produced by glycosylation of lactose, by using \( \beta \)-galactosidase enzymes, is usually referred to as \( \beta \)-linked GOS (29). Short-chain GOS (scGOS) has not been studied widely with respect to its immunomodulating effects as a single dietary agent. Although antiallergic and antiinflammatory effects were described for \( \alpha \)-GOS (30, 31) and raffinose (31, 32), more work is needed to clarify the effects of GOS and compare these with similar effects of other (nondigestible) oligosaccharides.
Definitions of fructans, such as various types of FOS and inulin, vary widely in the literature. Most described experiments were performed with unprocessed chicory inulin or fructans derived from chicory inulin. Unprocessed chicory inulin is mainly composed of fructans with a degree of polymerization (dp) ranging from 2 to 60, ending with a terminal glucose monomer. Partially hydrolyzed inulin (short-chain FOS) has a typical dp range of 2–8, and there are more molecules that end without a terminal glucose monomer compared with inulin. The physical removal of short-chain fructans from chicory inulin (33, 34) leads to a mixture of fructans with terminal glucose monomers and an approximate average dp of 22 (long-chain FOS (lcFOS)).

To stimulate the entire microbiota, a great variation of oligosaccharide structures is needed, because HMOS comprise many different oligosaccharides. Therefore, AOS such as methylated pectin–derived AOS (pAOS) are under investigation (35, 36). The addition of pAOS facilitates the required oligosaccharide diversity, because it is known that AOS act very specifically to their acidity, enabling them to interact with surfaces and to prevent the adhesion of pathogens on the intestinal epithelium.

Like HMOS, scGOS and lcFOS can complete the entire gastrointestinal passage because they are detectable in the feces of infants fed scGOS+lcFOS-supplemented formula (37). Several clinical trials showed that feeding infants a formula supplemented with a specific mixture of scGOS (Vivinal GOS) and lcFOS (Beneo HP) (ratio of 9:1; Immunofortis) resulted in an intestinal microbiota similar to that found in breastfed infants—ie, higher amounts of Bifidobacterium and Lactobacillus species (17, 38, 39). Term infants who were fed a formula containing pAOS with or without scGOS+lcFOS showed no differences in growth, crying, vomiting, and regurgitation patterns compared with the placebo group (35). Also in preterm infants, enteral supplementation of a prebiotic mixture consisting of scGOS +lcFOS+pAOS lowered the stool pH and stool viscosity with a trend toward a higher stool frequency (40). The effects of dietary oligosaccharides in infant formula on the microbiota and their influence on human health are extensively described by Ozoeer et al (14) in this supplement issue.

The first postnatal year of life seems to be a key period for programming the immune system. Feeding (ie, human milk) and other factors to which a newborn is subjected (ie, antibiotics) may have an influence on the indigenous gut microbiota, intestinal epithelial integrity, and the immune system. It is furthermore known that infants are more prone to develop Th2 disorders, which might be partly associated with the immature gut. Therefore, an imbalance in the immune system can lead to immunologic disorders, with allergies and atopic dermatitis as the most prevalent disorders (36). Chicken egg (4–5%), cow milk (2–3%), and peanut (>1%) are the 3 most important diagnosed food allergies in young children (41, 42). Although it is established that there is a strong genetic influence in the development of food hypersensitivity, the answer to the question of which factors are responsible for the rising number of allergic children might also lie in the changing westernized lifestyle, nutritional habits, higher consumption of food preservatives and additives, environmental conditions, and air pollution (43). Furthermore, the antigen type and dose, and the age at which the first exposure occurs, are all mentioned as key players in the subsequently evoked immune response (44). In case of food allergy, the allergic response most likely occurs because of either a failure in establishing oral tolerance or a breakdown of existing tolerance. In addition, food allergy and eczema are strongly correlated in children <1 y of age, and approximately one-third of them display atopic dermatitis symptoms as a consequence of sensitization to food allergens (45–47). Food allergens can exaggerate these atopic dermatitis symptoms by activation of Th2 cells in the skin because the gastrointestinal DCs are able to transport the allergen to the skin where they can degranulate mast cells (48).

A large, prospective, randomized, double-blind, placebo-controlled study in which healthy infants received a mixture of scGOS+lcFOS+pAOS (ratio of 9:1:2) showed prevention of the development of atopic dermatitis (49). Moreover, infants who were fed with scGOS+lcFOS (ratio of 9:1) showed increased fecal secretory IgA, suggesting a positive effect on the immune system (11). The direct beneficial effect on the immune system was also shown in a study in infants at risk (ie, a family history of atopy). In this study by Moro et al (50), the at-risk infants received a hydrolyzed formula either supplemented with the scGOS +lcFOS mixture or not supplemented during the first 6 mo of life. The data indicate that the incidence of atopic dermatitis and infectious diseases was reduced in infants who received the prebiotic mixture during these first 6 mo (50). This protection lasted, because the early-life intervention with these prebiotics also resulted in significantly lower allergic symptoms at 24 mo of age (51). Even after 5 y, preliminary observations in a subset indicated that scGOS+lcFOS consumption early in life has a protective effect on atopic dermatitis and allergic rhinitis (52). It is therefore anticipated that intervention with prebiotics early in life induces a prolonged preventive effect on the development of atopic disorders.

In addition to the effect on the allergic response, van Hoffen et al (53) showed that scGOS+lcFOS supplementation of high-risk infants reduced the total immunoglobulin response, modulating the allergic response away from the symptom-inducing IgE, whereas the vaccination response to DTP (diphtheria, tetanus, and polio) remained equally effective. Several other studies also showed a reduced incidence of infections in combination with a reduction in allergic disease outcomes after supplementation with scGOS+lcFOS-containing nutrition (54–56). In HIV–infected individuals who were not receiving highly active antiretroviral therapy, dietary scGOS+lcFOS+pAOS application resulted in increased bifidobacteria amounts; reduced numbers of Clostridium histolyticum, Eubacterium rectale, and Clostridium coccoides; reduced CD4+ T cell activation; and improved natural killer cell cytotoxicity (57).

ROLE OF REGULATORY T CELLS IN IMMUNE RESPONSES

The maintenance of a proper balance within the immune responses requires a well-orchestrated regulation. In food allergy, the subtle balance between hypersensitivity and oral tolerance is arranged by regulatory cells, including IL-10–producing Tr1 cells, natural killer T cells, and transforming growth factor β–producing Th3 cells (58–60). Moreover, naturally occurring CD4+CD25+Foxp3+ regulatory T cells (Tregs) have shown their importance in the maintenance of peripheral tolerance and the suppression of CD4+ and CD8+ T cell proliferation (61, 62).
Interestingly, these Tregs can be generated de novo in, eg, the intestinal mucosa (63). Approximately 5–10% of the CD4+ T cell population are regulatory T cells, and most of them display the transcription factor forkhead box p3 (Foxp3). Foxp3 is considered to be a necessary gene for Treg generation and maintenance (63). However, naturally occurring Tregs are plastic, which means that they are capable of losing Foxp3 and their regulatory function and, conversely, capable of acquiring various effector functions (64). In addition to their crucial role in oral tolerance development, Tregs are also increasingly prominent in vaccine strategies because they serve to limit activation, trafficking, and/or effector functions of both CD4+ and CD8+ T cells (65, 66). During a primary immune response, the expansion and attraction of conventional and Treg populations occur in synchrony (67). More important, the relative accumulation of Tregs at peak response significantly exceeds that of conventional T cells, reflecting an extensive relation between Tregs and conventional T cells, which control the magnitude of the response (68). Little is known about the intracellular factors and external cues for the differentiation and function of distinct Treg populations. Recently, it has been shown that both Foxp3 as well as T-bet (a transcription factor both necessary and sufficient for Th1 differentiation) act jointly within Tregs to produce unique functional outcomes in Th1 type of inflammation (69), suggesting the possibility of specific immune response–related Treg development.

The effects of scGOS+lcFOS have also been studied extensively in mouse models for influenza vaccination (70), allergic asthma (71), and cow milk allergy (12). Although the influenza-specific vaccination response and fecal bifidobacteria and lactobacilli proportions in scGOS+lcFOS-fed mice were significantly enhanced, dietary intervention with scGOS+lcFOS+pAOS was even more effective (72). Moreover, van’t Land et al (73) showed that dietary scGOS+lcFOS+pAOS clearly induces a more pronounced Th1 responsiveness, as shown by increased influenza-specific, delayed-type hypersensitivity responses and T-bet expression levels. Furthermore, CD25+ Tregs played a prominent role in the development of prebiotic scGOS+lcFOS+pAOS-induced immune modulation toward enhanced Th1 vaccine responsiveness, because in vivo depletion of CD25+ cells completely diminished scGOS+lcFOS+pAOS-induced immune modulation toward control levels.

Dietary scGOS+lcFOS+pAOS was also investigated in an experimental allergic asthma model in mice (71) and showed a significant suppression of ovalbumin-induced airway inflammation and hyperresponsiveness. A similar allergy-reducing effect of pAOS was observed in a model for cow-milk allergy to whey, in which the severity of allergic symptoms, as determined by a strongly reduced acute allergic skin response, in mice fed the prebiotic diet during whey sensitization was ameliorated (74). This reduced allergic skin response was accompanied by a slight reduction in Th2-type Ig concentrations (whey-specific IgE and IgG1) and unaltered high concentrations of Th1-type IgG2a. Because naturally occurring or de novo–induced CD25+ Tregs are important for the acquisition of oral tolerance (61–63), the contribution of these cells to the protective effect of the prebiotic diet was studied by transferring spleen cells. The beneficial diet-induced reduction in the allergic response was transferable, and ex vivo depletion of CD25+ cells from the transferred spleen cells abolished this effect (74). Similar results were obtained in a study that used another cow-milk protein, casein. Dietary scGOS+lcFOS+pAOS before and during oral casein sensitization showed a strong reduction in the allergic effector response compared with allergic control mice, which could be abrogated by in vivo depletion of CD25+ Tregs (75). Overall, these results indicate active regulation of the mucosal immune response that occurs on sensitization with casein or whey as a consequence of dietary intervention with scGOS+lcFOS+pAOS.

Flinterman et al (76) showed that there is a considerable chance of developing acute allergic reactions to cow milk after its elimination in children with atopic eczema dermatitis syndrome who previously did not experience problems after cow-milk intake. A possible explanation for this was published by van Esch (77) who showed in a mouse model for cow-milk allergy that partial and not extensive whey hydrolysates retained the putative capacity to prevent allergic symptoms when given orally before sensitization. In addition, the protective effects coincided with elevated numbers of Tregs in the intestine and could be adoptively transferred by using mesenteric lymph node (MLN) cells. In a subsequent study, they postulated that unique mucosal CD103+ DCs, present in the lamina propria and MLNs, and capable of sensing external stimuli present in the gastrointestinal tract, possess the capability to induce Tregs that are responsible for the above-described tolerance induction. Because systemic oral tolerance to food antigens may be generated via the induction of Tregs in the MLNs by antigen-presenting migratory DCs arriving from the intestinal mucosa, the effect of dietary scGOS+lcFOS+pAOS intervention in a cow-milk allergy model on CD103+ DCs and CD4+CD25+Foxp3+ Tregs in MLN cells was investigated. It was shown that scGOS+lcFOS+pAOS could improve the tolerizing capacity of a partial whey hydrolysate, and improved tolerance response coincided with increased percentages of CD11c+CD103+ DCs and Foxp3+ Tregs in MLN cells (78).

CONCLUSIONS

In this review, the prebiotic function of dietary oligosaccharides was compared with the functionality of HMOS. Both animal studies and human clinical trials show that dietary intervention with oligosaccharides in early life could lead to the prevention of atopic dermatitis, food allergy, and/or allergic asthma. It is thought that the immune-modulating effects of these oligosaccharides are mediated via modification of the intestinal microbiota and/or in a microbiota-independent manner by direct interaction on immune cells. These direct “pharmaceutical properties” of oligosaccharides are very promising and should be studied further. Because most studies to date are performed in a preventive setup (ie, applying the prebiotics before induction of the disease), studies investigating treatment protocols are required to establish the capacity of oligosaccharides to reduce, eg, established allergies. Moreover, the studies on the role of Tregs indicate that dietary intervention induces Tregs that are (at least in large part) responsible for the abrogation of allergic symptoms and tolerance induction. In addition to the role in the reduction of Th2-mediated allergic responses, Tregs also play a prominent role in the development of prebiotic-induced immune modulation toward enhanced Th1 Influenza-specific vaccination response.
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