The First Prebiotics in Humans

Human Milk Oligosaccharides

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Abstract: The development of intestinal microflora in newborns is strictly related to the kind of feeding. Breast-fed infants, unlike the bottle-fed ones, have an intestinal ecosystem characterized by a strong prevalence of bifidobacteria and lactobacilli. Data available so far in the literature show that, among the numerous substances present in human milk, oligosaccharides have a clear prebiotic effect. They are quantitatively one of the main components of human milk and are only partially digested in the small intestine, so they reach the colon, where they stimulate selectively the development of bifidogenic flora. Such results have been recently proved both by characterization of oligosaccharides in breast-fed infant feces and by the study of intestinal microflora using new techniques of molecular analysis, confirming that human milk oligosaccharides represent the first prebiotics in humans.

Key Words: prebiotics, human milk oligosaccharides, intestinal microflora

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In the field of pediatrics, the first studies about intestinal microflora date back to the beginning of the 20th century, when it was demonstrated that bifidogenic flora prevails in the breast-fed newborn compared with the bottle-fed one.[1,2]

Later it was observed that the bifidogenic flora had a beneficial effect not only on the newborn and baby's health, but also on the adult. In fact, the data available so far show that the bifidogenic flora makes the absorption of nutrients and minerals easier, prevents the development of pathogenic flora protecting from intestinal infections, and stimulates the immune system and the synthesis of vitamins.[3–5] This is why, during the last few years, particular care has been turned to modulate the composition of the intestinal ecosystem in children as well as in adults.

Different strategies have been used to achieve such an end. The first includes the probiotic approach. According to the latest definitions, it consists of the administration of live microorganisms of human origin in a quantity suitable to survive the acidic environment of the stomach and the action of bile salts to reach and colonize the large intestine and to modify the composition of microbic flora.[6,7] The prebiotic approach consists of the administration of substances that are not hydrolyzed or absorbed in the first part of the digestive system, and reach the colon to stimulate selectively the proliferation of resident beneficial strains (bifidobacteria). The symbiotic approach consists of the contemporary administration of probiotics and prebiotics to obtain the beneficial effects described earlier.

THE MODEL OF HUMAN MILK

The microbic colonization of human intestine begins at birth, when from a sterile state the newborn is exposed to an external environment rich in various bacterial species. Experimental studies have proved that the kind of delivery has an important influence on the composition of the intestinal microflora in the very first days of life. In fact, in babies born by vaginal delivery the first colonizing species are largely represented by vaginal and fecal bacteria of maternal origin,[8–10] whereas in babies born by Caesarian section the prevailing bacteria are those commonly found in an external environment.[8,11,12]

After the first week of life, irrespective of the type of delivery, the development of intestinal flora is mainly influenced by the kind of feeding. In fact, breast-fed infants show a microbic flora characterized by a definite predominance (about 90%) of bifidobacteria and lactobacilli (bifidogenic flora). On the contrary, the bottle-fed infant develops mixed flora with a lower number of bifidobacteria (40–60%) and the presence of other germs that, in some situations, may also have pathogenic effects (*Clostridium*, *Staphylococcus*, *Bacteroides*).[13–15] These historic data obtained with classic culture methods have also been confirmed recently by using the most

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up-to-date techniques of molecular biology,[16–18] which do not depend on the bacterial viability and growth techniques in plating (culture-independent methodologies). On the basis of the characteristic flora found in breast-fed infants, the research of bifidogenic substances in human milk has been taken up, because it does not contain bifidobacteria.

The bifidogenic effect has been, until now, ascribed to oligosaccharides, lactoferrin, and nucleotides. Regarding the last 2 substances, only sporadic reports document an increase of bifidogenic flora, which has to be ascribed to an inhibitory effect of the pathogenic flora rather than to a direct stimulus to the development of bifidobacteria.[19–22] There is, instead, copious literature in support of the clearly prebiotic effect carried by human milk oligosaccharides. In fact, Schonfeld[23] was the first, in 1926, to show that the bifidogenic effect of human milk was the result of a "nonproteic fraction." Later, Gyorgy et al[24] showed that the prebiotic effect was related to a mixture of oligosaccharides, and Kuhn[25] explained that it was a matter of oligosaccharides containing N-acetyl-glucosamine.

Many studies on the metabolic fate of human milk oligosaccharides have shown that such substances are resistant to digestion, reach the colon, and a considerable portion of them is excreted in feces, thus representing the model of prebiotics.[26–30]

HUMAN MILK OLIGOSACCHARIDES

Oligosaccharides are carbohydrates made up of 3-9 monosaccharide units[31] and are quantitatively the third component of human milk, after lactose and lipids. They reach the highest concentration in colostrum (more than 20 g/L) and then decrease, after about 2 weeks, to approximately 12 to 14 g/L in mature milk.[32,33] On the contrary, cows' milk, commonly used to prepare infant formulas, contains less than 1 g/L oligosaccharides.[34]

Human milk oligosaccharides are synthesized in the mammary gland by specific enzymes, the glycosyltransferases, by adding sequentially monosaccharide units (galactose, fucose, N-acetyl-glucosamine, sialic acid) to the basic molecule of lactose, thus forming compounds with both linear and branched structures. A peculiar characteristic of such substances is that monosaccharides, of which they are comprised, are bound by specific bonds resistant to the enzymes present in the newborn and infant's intestinal wall (lactase, saccharase–isomaltase, maltase–glucoamylase, amylase). As a consequence, most oligosaccharides ingested with mother's milk pass through the small intestine undigested and reach the colon.

At this level, undigested oligosaccharides are used by the microflora. They cause a "biomass effect," characterized by selective development of the bifidogenic flora. Moreover, processes of bacterial fermentation lead to the production of short-chain fatty acids (acetate, propionate, butyrate), which are used with an energetic purpose but which also have a trophic effect on the mucosa[35,36] and make the reabsorption of water easier.[27] Finally, the bacterial fermentation of oligosaccharides leads to the production of gases (hydrogen, carbon dioxide, and methane), which may be sometimes responsible for abdominal distension.

The oligosaccharides unused at colic level are eliminated with feces, behaving as dietary fiber.[37] Recent studies[26,29] have shown that the pattern of carbohydrates present in breast-fed infant feces is almost the same as that of ingested milk, except for lactose which (split by intestinal lactase) is absorbed as glucose and galactose. On the contrary, only traces of oligosaccharides are present in bottle-fed infant feces. It follows that the considerable difference in the composition of the intestinal microflora between the breast-fed newborn and the bottle-fed one is strictly related to the different milk oligosaccharide content.

DIFFERENT MICROFLORA FROM DIFFERENT OLIGOSACCHARIDES?

It is useful to specify that in the composition of human milk oligosaccharides there are substantial differences in quality and quantity among the different nursing mothers. This is strictly dependent on the set of glycosyltransferases in the mammary gland, which is genetically determined and is related to Lewis blood group-dependent status.[38,39] Until now, 4 different groups of nursing mothers have been identified in the general population. Their milk is characterized by a different composition of fucosylated oligosaccharides, which affect the total content of such substances, varying from 12 to 14 g/L to 7 to 8 g/L. These important differences have never been correlated with the composition of intestinal microflora in the breast-fed newborn, even in the most recent studies.[16,18]

As a consequence, we wanted to determine whether the quality and quantity of milk oligosaccharides could affect the composition of intestinal microflora. For this purpose we analyzed samples of feces obtained from babies fed by mothers within the different groups mentioned earlier. Among the numerous bacterial families making up the intestinal microflora, we chose to study more carefully the presence and the possible fluctuations of bifidobacteria, because of their well-known probiotic properties.

To reach such a goal, oligosaccharides contained in nursing mothers' milk were characterized by thin-layer chromatography,[40] and the groups to which they belonged were identified (Fig. 1). On the specimens of infant feces taken at the end of the first month of lactation, molecular biologic techniques were used, such as species-specific amplification of 16S rRNA, electrophoresis on polyacrylamide (DGGE; Fig. 2), and the sequence of DNA fragments.[41]

Six *Bifidobacterium* species were studied by these methods: *catenulatum, adolescentis, infantis, longum, breve,* and *bifidum*. Preliminary results outlined that the number of *Bifidobacterium* species for each sample studied was strictly cor-

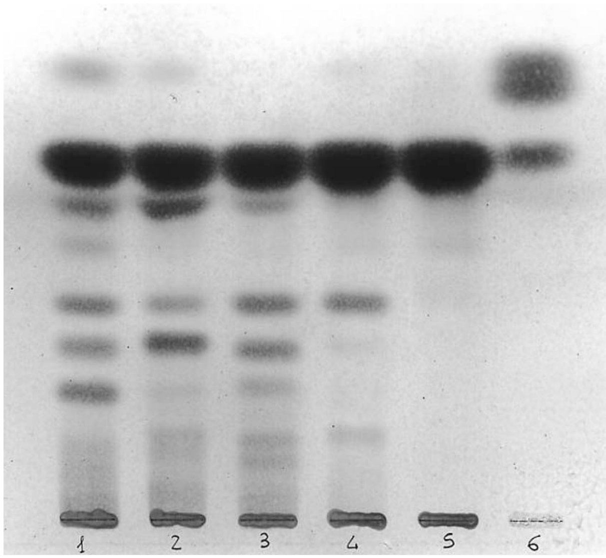

FIGURE 1. Thin-layer chromatography of milk carbohydrates. Lanes 1–4, human milk samples. The main band is lactose; all the other bands below lactose are different types of oligosaccharides. Subject groups: lane 1: secretor, Lewis a–/b+; lane 2: secretor, Lewis a–/b–; lane 3: nonsecretor, Lewis a+/b–; lane 4: nonsecretor, Lewis a–/b–. Lane 5: cow milk. Lane 6: standards. From top to bottom: glucose, galactose, lactose.

related with the content of oligosaccharides in mother's milk: Four to 5 species were found in the feces of infants fed with milk having a high content of oligosaccharides, whereas only 1 species was identified in the feces of babies fed milk with a low oligosaccharide content (nonsecretory group, Lewis a–/b–).

In conclusion, human milk oligosaccharides are undoubtedly the first and most important prebiotics for humans. It must, however, be determined whether there is a relation between quantity and quality of human milk oligosaccharides and the presence of different bacterial species in the composition of intestinal microflora.

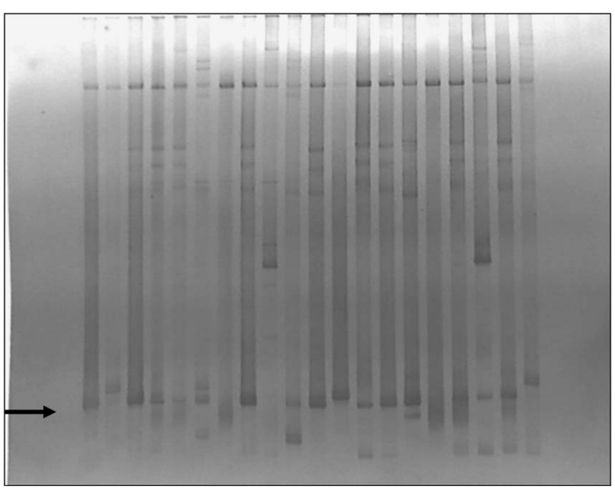

FIGURE 2. Species-specific DGGE of fecal samples of breast-fed newborns. The arrow shows the sequential band corresponding to *B. catenulatum* or *B. pseudocatenulatum.* Such a band is present, although with a different intensity.

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