Human Milk Oligosaccharides and Lewis Blood Group: Individual High-Throughput Sample Profiling to Enhance Conclusions From Functional Studies

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ABSTRACT

Human milk oligosaccharides (HMO) are discussed to play a crucial role in an infant’s development. Lewis blood group epitopes, in particular, seem to remarkably contribute to the beneficial effects of HMO. In this regard, large-scale functional human studies could provide evidence of the variety of results from in vitro investigations, although increasing the amount and complexity of sample and data handling. Therefore, reliable screening approaches are needed. To predict the oligosaccharide pattern in milk, the routine serological Lewis antigens on erythrocytes. However, the actual HMO profile of the individual samples does not necessarily correspond to the serological determinations. This review demonstrates the capabilities of merging the traditional serological Lewis blood group typing with the additional information provided by the comprehensive elucidation of individual HMO patterns by means of state-of-the-art analytics. Deducing from the association of the suggested HMO biosynthesis with the Lewis blood group, the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry profiles of oligosaccharides in individual milk samples exemplify the advantages and the limitations of sample assignment to distinct groups. Adv. Nutr. 3: 440S–449S, 2012.

Introduction

Free lactose-derived oligosaccharides in human milk (HMO) are present at concentrations ranging approximately from 10 to 20 g/L; hence, several grams of these unique components pass through the breast-fed infant’s gut daily (1–3). Various health protective actions have been deduced from in vitro investigations, i.e., prebiotic, anti-infective, or immune effects (4,5), which might partially be associated with the presence of fucosylated oligosaccharide structures. Those are determined by the expression of the secretor (Se) and Lewis (Le) genes in the mammary gland. Hence, distinct patterns of milk oligosaccharides according to the Le/Se types Le(a+b–) non-Se, Le(a–b+) Se, and Le(a–b–) Se or non-Se genes have been described by several investigators (2,6,7). The prevalence in white individuals averages 22%, 72%, and 6%, respectively (8), whereas 80% of the Europeans secrete ABH substances in saliva and other secretions; thus, they are typed as Se (9,10). Observational studies indicate that certain HMO from Secretor are associated with various preventive effects, such as reducing diarrhea and promoting intestinal maturation in preterm neonates (11,12).

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The final evidence of the functionality of the Le-related HMO compounds in humans has to be confirmed by intervention studies with large numbers of participants. Therefore, reliable high-throughput screening methods are needed to determine the oligosaccharide profiles in individual milk samples. Due to high inter- and intrindividual variations in HMO expression, the relationship between the serologically detectable Le blood group and the corresponding oligosaccharide pattern in milk can only be used for a crude milk classification, even though serological detection as a routine method is rapid and highly practicable. Nevertheless, detailed information on the HMO composition in milk samples provides a more accurate indication of the substances actually ingested by the infant. For this purpose, new developments in the field of glycomics have great potential for facilitating the handling of large sample and data sets from follow-up investigations on the correlation of Le-active components in infants’ feeding and their health (13–15).

In this review, we demonstrate the correlation of the serologically detected Le blood group and the expressed HMO pattern in the milk of the donors and show how this information can be enhanced by recent high-throughput HMO screening methods. On the basis of the suggested biosynthesis of HMO, specific variances in the HMO pattern are taken as examples to highlight the necessity of careful individual milk sample analysis.

**Biosynthesis of Le and Se gene-related oligosaccharides in milk**

In the past years, the structures of the major HMO have been thoroughly characterized (16,17). From these data, some structural rules have been deduced and the biosynthetic pathways of the neutral HMO have been proposed (Fig. 1). Unfortunately, experimental data on the biosynthesis of HMO are lacking to date.

Because the reducing end of the unbound oligosaccharides from milk consistently contains lactose, which is the major macronutrient in human milk, this disaccharide is assumed to be the initial substrate for HMO synthesis. Lactose is formed in the Golgi apparatus by the action of the lactose synthase complex containing α-lactalbumin and β1–4-galactosyltransferase (18). UDP-activated galactose is attached to Glc-1-P with high affinity due to the presence of α-lactalbumin, which is only expressed in the lactating mammary gland of mammals.

We speculate that analogous to the O-glycosylation of proteins in the Golgi of submammary and gastrointestinal secreting cells, the glycosyltransferases for HMO synthesis might occur as membrane-bound glycoproteins and process the oligosaccharide sequentially by the addition of a single monosaccharide from sugar nucleotides. Those are synthesized in the cytosol and conveyed to the Golgi lumen via specific membrane antiporters, e.g., GLUT1 for monophosphorylated glucose (19–21).

Thus, elongation (a), branching (b), and fucosylation (c) of lactose and derived structures might be performed by the concerted action of (a) β1–3-N-acetylgalactosaminyltransferase and β1–3- and β1–4-galactosyltransferase for type 1 and type 2 chains, respectively, and (b) β1–6-N-acetylgalactosaminyltransferase as depicted in Figure 1. Following the suggested rules of HMO synthesis, no further elongation is observed for a terminal type 1 chain (indicated by a no entry sign in Figure 1) (7,22,23). The final fucosylation and Le antigen formation is achieved by the consecutive action of α1–2-, α1–3-, or α1–3/4-fucosyltransferases (FucT)(c), as summarized in Figure 2.

The presence of at least 1 functional allele of the Le gene results in the expression of an α1–3/4-FucT (FucTIII), which is able to attach GDP-activated fucose (Fuc) in the O-4 position to N-acetylgalactosamine in type 1 (Galβ1–3GlcNAc) chains, resulting in Le epitopes, as shown in Figure 1. The same enzyme forms O-3-Fuc units at the N-acetylgalactosamine residue of type 2 (Galβ1–4GlcNAc) chains yielding Leα and, in Secretors, Leβ epitopes, however with lower specificity than for type 1 substrates due to steric aspects (24). The O-3-fucosylation of the reducing glucose residue is known to be accomplished by the Le-gene–dependent FucTIII as well (25). Furthermore, the formation of Leα and Leβ epitopes can also be performed by different α1–3-FucTs, i.e., FucTIII–VII and FucTIX (26).

Although secretory tissues and fluids have predominantly FucTII activity encoded by the Se gene, i.e., milk (27–29), saliva, or stomach tissue (30), in human serum, both FucTII and the H gene–controlled FucTI are present (31). Both enzymes transfer GDP-activated Fuc in α1–2–position to β-D-galactosides prior to the formation of Leα and Leβ epitopes (Fig. 1).

The Le and Se gene–encoded FucTs compete for the substrates so that in Secretors Leβ structures are also found in milk and other secretions, but not on erythrocytes or in plasma (29,32).

Individuals with mutations resulting in the nonfunctional FucTs FucTIII and FucTII are usually typed as non–Se and Le negative or Le(a–b–), respectively (26,33) and therefore should not secrete α1–2- and/or α1–4-fucosylated structures into milk.

**Functional aspects of Le blood group–related HMO**

Despite the fact that the Le histo-blood group system was discovered more than half a century ago, our knowledge about its biological functions is based mainly on speculations. In contrast, the role of the α1–3-FucTs IV and VII, which synthesize Leα and Leβ epitopes, seems to be proven because their corresponding genes are highly conserved among mammals and contribute to the formation of selectin ligands (34).

Considering that the Le and Se genes are mainly expressed in secretory tissues, which are in contact with the environment and, therefore, with a large number of various microorganisms, the manifold carbohydrate antigens in secretions and epithelial cells might provide protection against pathogens (32,34). Marionneau et al. (34) suggested that providing different cell surface receptors for several pathogens, Leα antigens accomplish resistance against Leα-binding pathogens and vice versa. Microbial lectins recognize host glycans in
the gut, which are presented by mucins and glycolipids, enclosing ABH and Le blood group recognition sites. This promotes colonization, which may have adverse health effects in case of pathogen adhesion (35). HMO bearing Le epitopes and other recognition sites attach to the pathogens and inhibit their adherence to intestinal cell surfaces. Subsequently, the pathogen-HMO complex can be excreted (36,37).

Many attempts have been made to investigate the functions of HMO, including Le-specific structures; however, most of these studies were conducted in vitro. A brief overview is given in Table 1. HMO have antiadhesive properties, possibly resulting in the reduction of infections with *Campylobacter jejuni*, *Escherichia coli*, *Vibrio cholerae*, *Shigella*, and *Salmonella* species or in a decrease in HIV-1 mother-to-child transmission. The bifidogenic effect of HMO, which has been known for decades, might also be assisted by the interaction of bifidobacteria with the Le epitopes because *Bifidobacterium infantis* is able to both use and bind different glycans from human milk and intestinal cells in vitro.  

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**Figure 1** Biosynthesis of neutral complex human milk oligosaccharides (HMO). The assumed biosynthetic pathway starts from the activated monosaccharides and includes the most important enzymes only \([\text{N-acetylglucosaminyltransferases (GlcNAcT)}]: \beta3\text{GlcNAcT} \text{attaches} \text{N-acetylglucosamine (GlcNAc) in the} \beta1–3 \text{position to terminal galactose (Gal),} \beta6\text{GlcNAcT attaches GlcNAc in } \beta1–6 \text{position to terminal Gal. Galactosyltransferases (GalT):} \beta3\text{GalT attaches Gal in the} \beta1–3 \text{position to GlcNAc and} \beta4\text{GalT attaches Gal in the} \beta1–4 \text{position to GlcNAc. Fucosyltransferases (FucT)}: \alpha2\text{FucT attaches fucose (Fuc) in the} \alpha1–2 \text{position to terminal Gal, secretor (Se) enzyme,} \alpha3\text{FucT attaches Fuc in the} \alpha1–3 \text{position to GlcNAc,} \alpha3/4\text{FucT attaches Fuc in the} \alpha1–3/4 \text{position to GlcNAc and in the}\alpha1–3 \text{position to Glc of the lactose core, Lewis (Le) enzyme. The no entry signs mean that no further elongation takes place. Fucosylation is indicated exemplarily for terminal type 1 and type 2 chains. Glycan structures are depicted according to the recommendations of the Consortium of Functional Glycomics using the GlycoWorkbench software tool (94).}
The Lewis (Le) and Secretor (Se) gene–related glycan epitopes. The Le and Se epitopes, which are characteristic for the Le phenotype in red blood cells and in human milk, are synthesized by the listed fucosyltransferases (FucTs). The Le and Se genes code for the active FucTs in presence of at least one functional allele (heterozygous with Lele or Sese, homozygous Se genes code for the active FucTs in presence of at least one functional allele (heterozygous with Lele or Sese, homozygous Se genes code for the active FucTs in presence of at least one functional allele (heterozygous with Lele or Sese, homozygous Se). The prevalence of the Le phenotypes is conferred to Europeans (8). Fuc, fucose; Gal, galactose; GlcNAc, N-acetylglucosamine.

Because the availability of HMO compounds adequate for interventional clinical trials has been limited to date, in vivo functional studies are rare. Nevertheless, data from observational investigations give an insight into the possible associations between the Le or Se phenotypes and diseases. α1-2-fucosylated HMO decreased Campylobacter jejuni infections in mice in vivo (38) and significantly prevented diarrhea in breast-fed infants in a dose-dependent manner (12,39). Furthermore, low or non-Se status was strongly associated with adverse outcomes in preterm infants, e.g., mortality and necrotizing enterocolitis (11), and with Crohn’s disease (40), giving further indication for the involvement of α1-2-fucosylated structures in the immune-related processes of gut development and health.

Considering the potentially important role of Le and Se epitopes in the infant’s digestive tract, it is noteworthy that infants are typed Le(a−b−) in the first months of life, as discussed in the following section. This could be due to a reduced Le and Se antigen expression in the immature gut of neonates because gastrointestinal epithelial cells are suggested to be the main source for Le-specific glycolipids in blood after reabsorption (41,42). Nevertheless, strong Lea activity has been detected consistently in the fecal samples collected after birth (meconium) as well as at the 6-mo follow-up. Leb reactivity, if present, was complementary to Lea activity. Even though the investigation was not representative, it is striking that the feces of the formula-fed neonates exhibited slightly lower Le reactivity than their exclusively breast-fed counterparts (43).

Because several grams of HMO pass through the breast-fed infant’s gut daily, they may compensate for the initial lack of Le and Se antigens in the neonate’s intestine.

In addition to the lower production of Le and Se antigens in the newborn, there are also observations of decreased Fuc content of fecal glycans in younger infants (44) and formula-fed infants compared with older and breast-fed infants, respectively (45). Fucosylated HMO in milk of Se but not in Lea non-Se decrease steadily in the first 3 mo of lactation (2), which might be due to an adaptation of the oligosaccharide composition in milk to the infant’s gut maturity. Interestingly, HMO seem to be involved in the infant’s intestinal cell maturation (46). In a recent study, the fucoligosaccharides of mixed-fed infants resembled the breast milk oligosaccharide patterns with few modifications. In contrast, the fucoligosaccharide profiles from exclusively breast-fed preterm infants were substantially different from those of their mothers’ milk, showing an intense metabolism in the digestive tract. Interestingly, the authors reported the additional modification of the HMO with ABH epitopes several weeks postpartum (47), a phenomenon first described by Lundblad (48) as well. Albrecht et al. (47) explained these time-dependent variations in the infant’s individual gastrointestinal adaptation to enteral food.

Le-specific HMO might also contribute to the protective effect of breastfeeding against urinary tract infections (49) because uropathogenic E. coli has been found to attach to glycolipids in non-Se women, presumably causing more urinary tract infections than in Se, in whom the receptor is masked by the additional α1-2-Fuc (50). Some of the Le and Se-specific HMO structures have been detected in the urine of lactating women (51,52) and, more recently, also in the urine of breast-fed infants. The data suggest that the intact compounds reach the circulation after absorption in the infant’s gut and might, therefore, display systemic and local effects in the infant (3,53).

Identification of Le blood group–related compounds

Traditionally, Le blood group determination is performed by serological methods, which can become a challenging task. Cross-reactions of the commonly used antibodies are described as well as the presence of side products leading to misinterpretation of results (54). For example, healthy Le(a−b−) and Le(a+b−) individuals can show a slight expression of Leb epitopes in plasma, which may be due to mutation with incomplete inactivation of FucTIII and FucTII, respectively (55–57). The saliva of Lea non-Se can also contain Leb antigens as a result of slight FucT activity in the salivary glands (58).

Red blood cells (RBC) do not synthesize Le antigens themselves, but acquire them secondarily from tissues (59,60). Therefore, the serological phenotyping of RBCs does not necessarily describe the Le genotype of a donor because the Le epitope expression may differ in various tissues (61–63). Genetic factors and several conditions such as diseases, infections, transfusions, and bone marrow transplantations can lead to alterations in the Le phenotype, hence,
leading to misinterpretation of the blood group determination (64–68).

In pregnancy, the prevalence of Le(a$-$b$-$)-typed women can increase threefold, which is most likely due to an increased attachment of Le-active glycolipids to plasma lipoproteins with a subsequent decrease in the antigen quantity on erythrocytes (63,69). In these individuals, discordant Le phenotypes can be detected on RBC and saliva.

A similar situation can be observed in neonates. Most of them are typed Le(a$-$b$-$) by serological detection on RBC, whereas in saliva, Le- and Se-related epitopes are already expressed, according to the genotype of the infant (70). Several weeks after birth, Le$^a$ antigen can also be detected on erythrocytes, whereas Le$^b$ antigens are fully present in blood only at the age of 6 y due to delayed activation of the Se gene-controlled FucTII (71).

Because of the discrepancies and the numerous influencing factors of the Le phenotype determination in various tissues and body fluids, the data obtained from RBC phenotyping for Le blood group identification need to be regarded with suspicion (72). Nevertheless, as routine method in the clinical sector, it is convenient for screening purposes. For the assignment of milk samples to the distinct Le groups, the serological RBC-based typing of the milk donors remains a valuable tool to roughly estimate the HMO pattern expressed in milk. The link between the Le blood group and the oligosaccharide profile in milk is explained by the correspondence between the biosynthesis of the Le-active glycans present on RBC and the formation of free fucosylated oligosaccharides in the mammary gland (see previously).

However, because of the described variations in serological phenotyping as well as individual and lactation time-specific alterations in HMO expression, serology by itself is not sufficient to predict the relative amount of single Le-type oligosaccharides in milk. Detailed information on the expressed oligosaccharide patterns might be a more appropriate basis for future research on HMO, especially for functional clinical studies and investigations on the metabolism and the biological activity of HMO.

Table 1. Effects of Lewis and secretor gene–related factors

<table>
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<tr>
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<td>HMO, i.a. Lewis-epitope bearing</td>
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1 CFU, colony-forming units; Fuc, fucose; HMO, human milk oligosaccharides; Lac, lactose.
Variation of the HMO pattern
Automated MALDI-TOF-MS/(MS) as an HMO screening method enables the Le blood group correlation of a large milk sample set and delivers, at the same time, the distinct HMO pattern of each milk sample with high reliability (74).

Recent findings in the oligosaccharide pattern of single milk samples show the necessity of individual sample monitoring. The discrepancy described between the serologically detected blood group and the expression of Le antigens in other body fluids and tissues seems to be conferrable to Le-type oligosaccharides in human milk.

From the genetic point of view, a more consistent oligosaccharide pattern might be expected within a distinct Le blood group (83). However, several studies showed that the proportion of distinct oligosaccharide structures can vary greatly among individual milk samples, depending on the lactation period and/or Le status (2,4,74,75,81,84).

The results of the MALDI-TOF-MS/(MS) screening of 40 milk samples from mothers with serologically determined Le blood group underlined these findings. Figure 3 displays mass spectra from 2 Le(a−b−) and 2 Le(a−b+) donors, which differ markedly in their HMO pattern. The 95% CIs calculated for each signal within an Le blood group are indicated by shaded and open bars. In case of coincidence between the measured signal intensity and the predicted interval, the bar is shaded; otherwise, the CI is shown by an open bar. Each signal represents a distinct oligosaccharide composition with several structural isomers.

The HMO pattern of a serologically Le(a−b−)-typed woman is depicted in Figure 3 A. A strong overexpression of lacto-N-(neo)tetraose [LN(n)T] (m/z 730), as well as the monofucosylated lacto-N-(neo)hexaose [LN(n)H] (m/z 1241) was apparent. The multiply fucosylated species, such as difucosyllactose (m/z 657), difucosylated LN(n)T (m/z 1022) and the trifucosylated LN(n)H (m/z 1533) were substantially lower than expected or even absent. All of these 3 structures should contain a remarkable amount of α1–2-Fuc, whereas monofucosylated and difucosylated LN(n)H might contain mainly α1–3/4 fucosylated compounds. This leads to the conclusion that the donor might have an inefficient α1–2-FucT in the mammary gland, which is Se-gene dependent. Two explanations are possible for the anomaly in the depicted mass spectrum: i) this donor might belong to the rare subgroup of Le(a−b−) non-Se and ii) the donor’s Le phenotype is actually Le(a+b−) because the HMO profile resembles that of an Le(a+b−) pattern with regard to the calculated CIs (data not shown).

The second Le(a−b−) sample showed the opposite phenomenon (Fig. 3 B). The nonfucosylated precursor for the complex HMO, LN(n)T (m/z 730) and its monofucosylated form redundant (m/z 876) were underexpressed, whereas the multiply fucosylated species difucosylated LN(n)T (m/z 1022) and trifucosylated LN(n)H (m/z 1533) were overexpressed. Moreover, unusually extensive signals up to a mass of 2500 Da were detected in high abundance in this specific sample. This suggests that this donor expressed several highly efficient glycosyltransferases as well as FucTs forming...
a diverse HMO pattern also in the higher mass range, con-
forming to an Le(a−b+) HMO pattern rather than the ex-
pected Le(a−b−)-specific one. Because the HMO spectra 
shown in Figure 3A and B are atypical Le(a−b−) and re-
semble those characteristic of Le(a+b−) and Le(a−b+), re-
spectively, the suspicion is raised that the milk HMO profiles 
may also reflect the partial discordance of the serological 
RBC- and saliva-based Le phenotyping in pregnant women, 
as discussed previously.

Figure 3C shows the HMO spectrum of an Le(a−b+) do-
nor expressing an atypical HMO pattern, which cannot be 
assigned to any Le blood group. All signals from m/z 657 
to 1022 were expressed in substantially lower intensity 
than expected, whereas the signals in the mass range from 
m/z 1095 to 1533 were overexpressed. Hence, a shift to 
high molecular weight HMO can be observed for this partic-
ular sample. As already discussed for Figure 3B, also in this 
example highly efficient glycosyltransferases might be re-
ponsible for the observed variation.

Only slight deviations in the Le(a−b+) spectrum are seen in 
Figure 3D, except for the unexpectedly high proportion of 
difucosyllactose (m/z 657).

The demonstrated variations in the MALDI-TOF-MS 
profile spectra confirm the conclusion of Thurl et al. (2) 
that each lactating woman expresses an individual HMO 
pattern, even though an assignment of the HMO profiles 
to the distinct Le blood groups was applicable for the major-
ity of the milk samples in our investigations (see later).

The importance of screening methods to detect the indi-
vidual oligosaccharide profile is further emphasized by the 
tandem mass spectrometry analysis of the precursor m/z 
1022 in a milk sample from a woman typed Le(a+b−) (Fig. 
4). As a major isomer, an LN(n)T core bearing 1 Fuc at the 
reducing end and the LeN epitope at the nonreducing end 
is expected in a milk sample from an Le(a+b−) donor. The 
most intensive fragment signals at m/z 730 (Y4B4a) and m/ 
z 876 (Y4a) result from the dissociation of 1 and 2 Fuc resi-
dues, respectively [fragment ions are designated in accordance 
with the nomenclature of Domon and Costello (85)]. In addi-
tion, the signals m/z 696 (B2a), m/z 511 (Y2a), and m/z 365 
(Y2aB2a) underline the presence of the likeliest precursor 
structure. Strikingly, an indication for the presence of an 
LeN epitope is given by the signal m/z 680 (B2a), a difucosyl-
dated N-acetyllactosamine unit. The serologically detected 
Le blood group does not explain the presence of an LeN epitope 
because of the lack of the Se gene–dependent FucTI in Le 
(a+b−) individuals. Nevertheless, the difucosylation of either 
a terminal type 1 or 2 N-acetyllactosamine unit in this milk 
sample is an indication for α1–2-FucT activity. This finding 
confirms the unexpected presence of α1–2-fucosyl HMO in 
2 serologically typed Le(a+b−) donors by Newburg et al. 
(12). These findings might be explained by a slight activity 
of the H gene–controlled FucTI, which has been detected at 
least in the saliva of Le(a+b−)-typed individuals (58).

The application of the fragmentation analysis of individ-
ual oligosaccharides for structural characterization is de-
scribed for various MS techniques (17,73,82,86–93). Hence, 
tandem MS analysis can also provide additional structural 
information for individual HMO compositions in the case of 
HMO screening.

The presented examples demonstrate the drawbacks of 
serological Le blood group classification and emphasize 
the need for individual sample mapping. Le phenotyping 
in human milk by MALDI-TOF-MS(/MS) analysis and sub-
sequent statistical data evaluation provide the opportunity 
not only to assign the specimens to definite groups, but 
also to reveal unusual tendencies for each individual milk 
sample, including its unique HMO profile (74).

Figure 5 shows the results from a discriminant analysis of 
40 individual milk samples measured threefold. Using the 
new screening approach, 95% of the samples were correctly 
assigned to the serologically detected Le phenotype in blood 
by at least 2 of 3 measurements. Specifically, 99% of all se-
rologically Le(a−b+), 100% of the Le(a+b−), and 68% of 
the Le(a−b−) typed samples were assigned to the previ-
ously determined Le blood group. Nevertheless, the partial

![Figure 4 Matrix-assisted laser desorption/ionization time-of-flight MS/MS analysis of purified human milk oligosaccharides of a Lewis (a+b−) donor. Inset shows range from m/z 650 to m/z 720 at 50× magnification. The obtained fragment ions were assigned according to the recommendations of the Consortium of Functional Glycomics using GlycoWorkbench (94). Fragment ions are designated in accordance with the nomenclature of Domon and Costello (85). In some cases, fragments may be formed by different fragmentation pathways, only 1 of which is illustrated. All fragment ions represent sodium adducts. The unexpected signal is circled in red.](Image 235x66 to 534x269)
inhomogeneity of the Le-specific HMO profiles described previously is also evident from the wide distribution of the samples within the Le blood groups. The overlapping area of the Le(a-b+) and Le(a-b-) typed samples, in particular, displays the similarity of the oligosaccharide profiles in several specimens with a different Le phenotype, most likely resulting from their Se gene activity. However, the fact that the majority of the milk samples were matched to the serologically detected Le blood group shows that a classification using both approaches can be useful despite the variations in the expression level of single oligosaccharide composition in milk. The location of each breakpoint in the coordinate plan therefore provides information about the Le phenotype tendency of a distinct milk sample and at the same time information about its actual oligosaccharide pattern. Consequently, the new MS screening approach provides a fast and material-saving option for individual milk sample mapping with detailed information on the expression level of individual oligosaccharide compositions.

CONCLUSION

Serological Le blood group determination can offer only a first indication of the expressed oligosaccharide pattern in human milk. Modern high-throughput screening methods can support the traditional serological RBC analysis and provide detailed information on the relative abundance for each oligosaccharide composition, thus, enhancing or qualifying the conclusions of functional studies. In particular, the capability of individual glycan epitope recognition and its relationship to observable, biologically relevant effects will be of great benefit. Furthermore, reliable Le phenotype screening, e.g., by the MALDI-TOF-MS approach presented here, can replace serological determination on erythrocytes if no blood sample is available.

The variations between the serologically detected Le blood group and the Le phenotype in other body fluids and tissues, which are frequently reported in literature, were also detectable in human milk using the novel screening approach. Based on the determined individual HMO patterns, some speculations about the activities of certain glycosyltransferases in the milk donors were deduced by taking into consideration the information from the suggested HMO biosynthetic pathway.

The combination of the traditional serological Le blood group detection supported by modern milk screening methods will lead to a solid glycan characterization as the basis for future research on the effects of HMO from native milk.

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Literature Cited


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Figure 5 Discriminant analysis. The results obtained for 113 single matrix-assisted laser desorption/ionization time-of-flight MS and redundant MS/MS measurements of 40 milk samples underwent discriminant analysis. Discriminant function 1 is plotted on the x-axis and discriminant function 2 on the y-axis. Open diamonds, red squares, and green triangles represent HMO samples from Le(a–b+), Le(a+b–), and Le(a+b–) donors, respectively. The distribution of each group is indicated by colored shading. Reproduced with kind permission from Springer Science+Business Media (74), Figure 7.


