Prof. Akira Kobata

Born in 1933, graduated from Faculty of Pharmaceutical Science, School of Medicine, University of Tokyo in 1956, received his Ph. D. in Biochemistry in 1962. In 1967, he joined the Section of Biochemistry, Laboratory of Biochemical Pharmacology, NIAMD (Section Chief, Victor Ginsburg), NIH. During that time, he elucidated the whole biosynthetic pathway of ABH and Lewis antigenic determinants. In 1971, he moved to Kobe University as Professor of the Department of Biochemistry, School of Medicine. There, he developed a series of sensitive methods to investigate the structures of the N-linked sugar chains. On moving to University of Tokyo as the Professor and Chairman, Department of Biochemistry, Institute of Medical Science in 1982, he expanded his research to the structures, metabolism, functions and pathology of the N-linked sugar chains. He was awarded the Prize for the Promotion of Young Scientists from Pharmaceutical Society of Japan for 1963, Science and Technology Prize from Toray Science Foundation for 1985, Claude S. Hudson Award of American Chemical Society for 1992, PSJ Award from Pharmaceutical Society of Japan for 1992, and also the 1992 Japan Academy Prize. He was a Fogarty Scholar-in-Residence from 1985 to 1987, Auckland Foundation Visiting Professor in 1988, and also served as the Director of Institute of Medical Science from 1990 to 1992. In 1993, he was appointed as the Director of Tokyo Metropolitan Institute of Gerontology, and became a Professor Emeritus of University of Tokyo. In this last carrier as a scientist, he developed a new glycobiology area in the field of aging research. From 2000, he has been the Director Emeritus of Tokyo Metropolitan Institute of Gerontology. Currently, he is the scientific advisor of the Noguchi Institute, a non-profit institution established for the study of carbohydrate chemistry in Japan.

Possible Application of Milk Oligosaccharides for Drug Development

Akira Kobata, PhD

By applying a finger-printing method to the analysis of human milk oligosaccharides, several oligosacchartides were found to be deleted in the milk of non-secretor or Lewis negative individual. This finding afforded a clue to elucidate the enzymatic basis of blood types in humans. Furthermore, disappearance of some major oligosaccharides led to the finding of five novel minor oligosaccharides, which were hidden under the major oligosaccharides. Later on, structures of more than seventy oligosaccharides were elucidated. These oligosaccharides are derived from eleven core oligosaccharides by sialylation and/or fucosylation. All these oligosaccharides contain lactose at their reducing termini. This evidence, together with the deletion phenomena found in the milk of two blood type individuals, suggested that the oligosaccharides are formed by the concerted action of glycosyltransferases, which are responsible for formation of the sugar chains of glycoproteins on the surface of epithelial cells constructing the mucous membrane. The elongation may start by the action of iGnT. This enzyme is responsible for the addition of a β-*N*-acetylglucosamine residue to the C-3 position of the galactose moiety constructing the *N*-acetyllactosamine group of the sugar chains of glycoconjugates. Therefore, oligosaccharides in human milk may include many structures, starting from the *N*-acetyllactosamine residues in the sugar chains of various glycoproteins.

Many evidences, which indicate that virulent enteric bacteria and viruses start their infection by binding to particular sugar chains of glycoconjugates on the surface of their target cells, were presented recently. Therefore, milk oligosaccharides are expected to be useful to inhibit the infection of these bacteria and viruses. *(Chang Gung Med J 2003;26:620-36)*

Key words: milk, human, oligosaccharides, enteric bacteria, blood types, β3-*N*-acetylglucosaminyltransferase

Milk contains many glycoproteins, glycopeptides, and oligosaccharides, which are found almost exclusively in this secretion. These components occur especially in large amounts in the milk, obtained at early stages of lactation. Some of them, such as casein and lactose, are definitely produced in the mammary gland as important nutrients, and IgA presents in milk is a component of transfer immunity for suckling babies. However, the physiological significance of other minor components is mostly unknown. Among mammalian species, the human is noteworthy because its milk contains various kinds of oligosaccharides.

In 1933, Polonovski and Lespagnol[1] found a new nitrogen-containing oligosaccharide in human milk, and named it "gynolactose". This substance could not be crystallized, undoubtedly because it was a mixture of several oligosaccharides. In 1954,

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Polonovski and Montreuil[2] reported based on a paper chromatographic investigation that the gynolactose is, in fact, a mixture of more than ten oligosaccharides, some of which contain nitrogen. This finding was also supported by independent attempts to identify the so-called *Bifidus factor*.

It had been known since 1900 that the stools of breast-fed babies are more acidic than those of artificially nourished ones.[3,4] In 1935, it was reported by Grulee, et al.[5] as a well documented study that breast-feeding was strongly associated with a lower incidence of diarrhea, otitis media, and respiratory diseases. Paul György, then a professor of Paediatrics in the University of Pennsylvania, considered that this is because *Lactobacillus bifidus* becomes a predominant intestinal flora of babies fed with human milk. This bacteria has the capacity to digest lactose, and produces large amounts of lactic acid and acetic acid. The acidic condition, thus produced in the intestine of babies, supresses the growth of many other microorganisms, and may protect babies from harmful intestinal infection.

Schönfeld[6] already found that the whey fraction of human milk contains a growth-promoting factor for *Lactobacillus bifidus* varietus pennsylvanics, and named it *Bifidus factor*. Richard Kuhn, in the Max-Plank Institute at Heiderberg, started a systematic investigation of *Bifidus factor*, in collaboration with Paul György.[7-9] This study led Kuhn to the structural studies of human milk oligosaccharides. Until 1964, structures of fourteen oligosaccharides, as shown in Table I, were determined by the independent studies of Kuhn's group and Montreuil's group.[10-22]

These oligosaccharides have a common feature that they all contain lactose at their reducing termini. Interestingly, some of these oligosaccharides showed haptenic activities of blood group determinants.[18,23] For example, 2'-FL and LNF-I showed haptenic activity of H determinant, LND-I showed activity of Le^b determinant, and LNF-II, and LND-II showed activity of Le^a determinant. Because of this, human milk oligosaccharides played key roles in elucidating the structures of H and Lewis blood group determinants.[24]

Correlation of the Occurrence of Oligosaccharides Containing Fucose Residues and the Blood Types of the Donors.

In 1967, Grollman and Ginsburg[25] found an interesting evidence that 2'-FL was not detected in the milk samples, obtained from individuals with non-secretor blood type. These people express ABO blood types on the surface of their erythrocytes according to their genetic background of ABO locus, but not in the glycoproteins, secreted from the epithelial cells of mucous glands. In order to extend this interesting finding further, Kobata, Tsuda and Ginsburg[26] devised a new technique to obtain oligosaccharide patterns by using small amount of milk samples. The method was the combination of gel-filtration and paper chromatography, which enabled them to finger-print the fourteen oligosaccharides by using approximately 10 ml of milk samples as shown in Fig. 1.

An interesting evidence, revealed by the analysis of the milk samples obtained from 50 individuals, was that three different oligosaccharide patterns were obtained by this analytical method. Approximately 80% of the milk samples gave the spots of all fourteen oligosaccharides as shown in Fig. 1.

In contrast, approximately 15% of the milk samples gave the oligosaccharide pattern as shown in Fig. 2. The characteristic feature of this pattern is that four oligosaccharides are missing as shown by the spots indicated by dotted lines in the pattern. The small grey spots, detected at the positions of missing oligosaccharides, are minor oligosaccharides hidden under the major oligosaccharides. Written by white letters are the names assigned to the newly found minor oligosaccharides. An important evidence was that all mothers, whose milk gave this oligosaccharide pattern, were non-secretors, who express neither the ABO blood group determinants nor Le^b determinant in their secretory glycoproteins. The structures of the four missing oligosaccharides indicated that the deletion phenomenon, found in 2'-FL, can be extended to all milk oligosaccharides containing the Fucα1-2Gal group. Namely, the secretory organ of non-secretor individuals lack the fucosyltransferase responsible for formation of the disaccharide group.[27]

The remaining 5% of the milk samples gave the oligosaccharide pattern as shown in Fig. 3. The characteristic feature of this pattern is that the three oligosaccharides, shown by dotted lines, are missing in the pattern. Like in the case of non-secretors, presence of three new minor oligosaccharides were

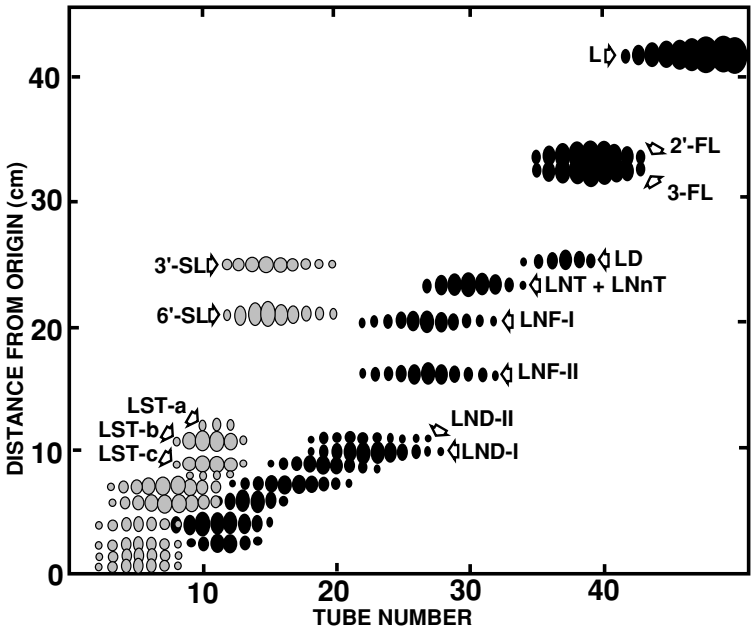

Fig. 1 Fingerprinting of the oligosaccharide fraction obtained from milk samples of Le^{a+b+} individuals. Fraction numbers as indicated by "TUBE NUMBER" in abscissa were obtained by Sephadex G-25 column chromatography of human milk oligosaccharide fraction. Aliquots of the fractions were spotted at the origin of a sheet of a filter paper, and subjected to chromatography using ethyl acetate/pyridine/acetic acid/water (5:5:1:3) as solvent. Black spots represent oligosaccharides visualized by alkaline-$AgNO_3$ reagent, and hatched ones encircled by black line represent those detected by both alkaline-$AgNO_3$ reagent and thiobarbituric acid reagent.

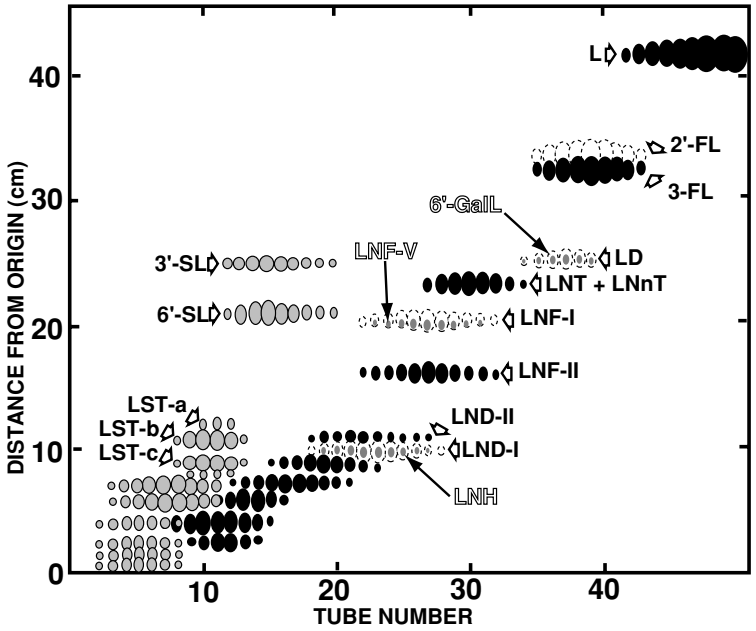

Fig. 2 Fingerprinting of the oligosaccharide fraction obtained from milk samples of non-secretor individuals. The condition of fingerprinting was the same as written in the legend for Fig. 1. Spots shown by dotted lines were missing in the pattern. Grey spots detected at the positions of missing oligosaccharides are minor oligosaccharides hidden under the major oligosaccharides.

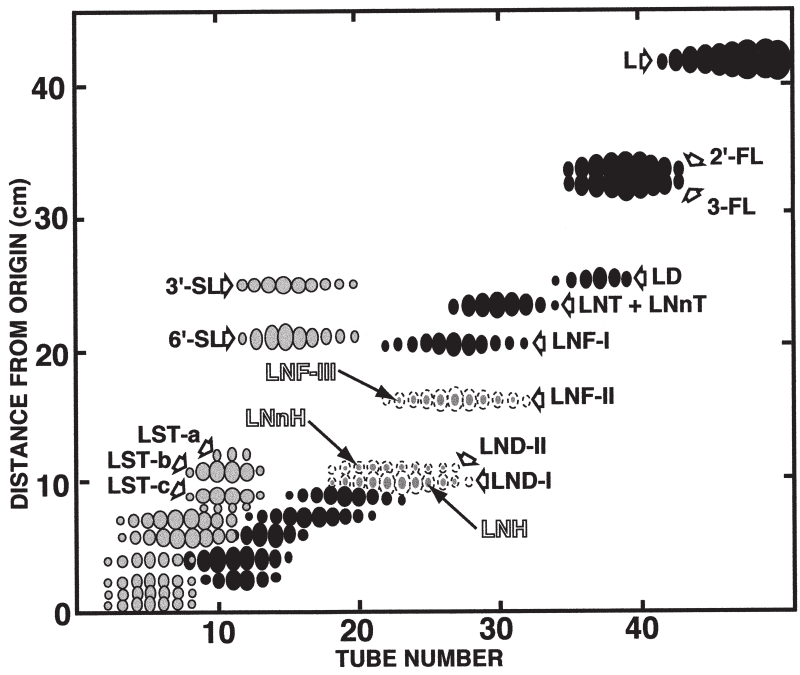

Fig. 3 Fingerprinting of the oligosaccharide fraction obtained from milk samples of Le^{a-b-} individuals. The condition of fingerprinting, and the descriptons of various spots were the same as written in the legend for Fig. 2.

found as shown by the small grey spots in Fig. 3. Examination of the blood types of the donors, whose milk gave this pattern, revealed that they are all Lewis-negative, who lack both Le^a and Le^b antigens in their secreted glycoproteins and on their erythrocytes.[28]

A common feature of the three oligosaccharides, which were missing in the milk of Lewis-negative individuals, is that they all contain the Fucα1-4GlcNAc group. Based on this finding, it was concluded that another fucosyltransferase, which is responsible for the formation of the Fucα1-4GlcNAc group, is not expressed in the epithelial cells of the secretory organ of the Lewis negative individuals. This estimation was later confirmed by enzymatic study.[29]

Enzymes Which are Responsible for the Formation of Blood Group Determinants A and B.

Although oligosaccharides containing blood group determinants A [GalNAcα1-3(Fucα1-2)Gal] and B [Galα1-3(Fucα1-2)Gal] were not included among the milk oligosaccharides listed in Table 1, finding of the close correlation of the oligosaccharide deletion phenomena in milk and the blood type background of the donors led to investigation of the glycosyltransferases in milk, which are related to the biosynthesis of blood group determinants. It was already reported that a large amount of the β-galactosyltransferase, responsible for the formation of *N*-acetyllactosamine group in the epithelial cells of mammary gland, is excreted as a soluble form in milk.[30] This evidence suggested that other glycosyltransferases of the epithelial cells of mammary gland may also be excreted as soluble forms in milk. Actually, the α-fucosyltransferase responsible for formation of the Fucα1-4GlcNAc group did exist in the milk obtained from Lewis positive individuals.[29]

When UDP-[^{14}C]GalNAc was incubated with a mixture of human milk oligosaccharides and defatted milk obtained from a woman with A blood type as an enzyme source, two radioactive oligosaccharides were produced in the presence of Mn^{2+} ion. Incubation with each milk oligosaccharide revealed that 2'-FL and LNF-I work as acceptors of the [^{14}C]

Table 1. Structures of Milk Oligosaccharides Found until 1964

Names of oligosaccharides	Structures	References
2'-Fucosyllactose (2'-FL)	Galβ1-4Glc 2 | Fucα1	(10)
3-Fucosyllactose (3-FL)	Galβ1-4Glc 3 | Fucα1	(11)
Lactodifucotetraose (LD)	Galβ1-4Glc 2 3 | | Fucα1 Fucα1	(12)
Lacto-*N*-tetraose (LNT)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc	(13)
Lacto-*N*-neotetraose (LNnT)	Galβ1-4GlcNAcβ1-3Galβ1-4Glc	(14)
Lacto-*N*-fucopentaose I (LNF-I)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 2 | Fucα1	(15)
Lacto-*N*-fucopentaose II (LNF-II)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 4 | Fucα1	(16)
Lacto-*N*-difucohexaose I (LND-I)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 2 4 | | Fucα1 Fucα1	(17)
Lacto-*N*-difucohexaose II (LND-II)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 4 3 | | Fucα1 Fucα1	(18)
3'-Sialyllactose (3'SL)	Galβ1-4Glc 3 | Neu5Acα2	(19)
6'-Sialyllactose (6'SL)	Galβ1-4Glc 6 | Neu5Acα2	(20)
LST-a	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 3 | Neu5Acα2	(21)
LST-b	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 6 | Neu5Acα2	(21)
LST-c	Galβ1-4GlcNAcβ1-3Galβ1-4Glc 6 | Neu5Acα2	(22)

N-acetylgalactosamine residue.[31] Other milk oligosaccharide, so far known, could not accept the radioactive *N*-acetylgalactosamine. This evidence strongly suggested that the oligosaccharides, containing the Fucα1-2Gal group, became acceptors of the *N*-acetylgalactosaminyltransferase, which is responsible for the formation of the blood group A determinant. Accordingly, production of radioactive oligosaccharides from 2'-FL by using many milk samples obtained from individuals of different blood types were investigated.

As summarized in Table 2, milk samples, obtained from women of blood type A or AB, all showed the enzymatic activity to form the radioactive oligosaccharides from both 2'-FL. However, none of the milk samples, obtained from donors of

Table 2. Activities of *N*-acetylgalactosaminyltransferase and Galactosyltransferase in Human Milk as Determined by Using 2'-FL as an Acceptor

Donors of milk	ABO and Lewis blood types	Uptake of *N*-acetylgalactosamine	Uptake of galactose
		(mμmoles/5hr, 130 μl milk)	
J.K.	A_1, S*, Le (b+)	27	0
M.K.	A_1, S, Le (b+)	12	0
D.C.	A_1, S, Le (b+)	15	0
J.B.	A_2, S, Le (b+)	5	0
E.K.	A_2, S, Le (a-b+)	67	0
J.C.	A_1B, S, Le (b+)	40	4.5
L.M.	A_2, NS, Le (a+)	16	0
B.B.	A_2, NS, Le (a+)	7	0
G.H.	B, S, Le (b+)	0	1.7
R.S.	B, S, Le (b+)	0	4.5
N.C.	B, S, Le (b+)	0	4.2
M.M.	B, S, Le (b+)	0	7.1
F.W.	B, NS, Le (a+)	0	3.8
L.N.	O, S, Le (b+)	0	0
C.D.	O, S, Le (b+)	0	0
J.S.	O, NS, Le (a+)	0	0
D.R.	O, NS, Le (a+)	0	0

*: S indicates secretor, and NS indicates non-secretor

blood type B or O, showed the enzymatic activity. An important evidence is that the milk samples, from non-secretor individuals of blood type A, showed the enzymatic activity at almost the same level as secretor individuals. This evidence indicated that the absence of the blood type A antigenic determinant in the glycoproteins, secreted by the epithelial cells of non-secretor individuals, takes place not because they lack the α-*N*-acetyl-galactosaminyltransferase in the epithelial cells of their secretory glands, but because the cells lack the fucosyltransferase responsible for the formation of the Fucα1-2Gal group, which is an essential substrate for the α-*N*-acetyl-galactosaminyltransferase. The distribution of the enzyme activity, in addition to its acceptor specificity, made it likely that the α-*N*-acetyl-galactosaminyltransferase actually forms blood group A determinant, and was named A-enzyme.[31]

By using the same assay system, but replacing UDP-[^{14}C]GalNAc by UDP-[^{14}C]Gal, an α-galactosyltransferase activity, which adds a galactose residue to the C-3 position of the galactose residue of the Fucα1-2Gal group of 2'-FL and LNF-I, was also detected in human milk, and named B-enzyme. As shown in Table 2, the enzyme activity was detected in the milk samples obtained from blood type B and AB donors, but not in those from other blood types. A non-secretor with blood type B showed the enzyme activity.[32]

Characterization of A-enzyme.[33]

A-enzyme was partially purified from defatted milk, obtained from a donor of blood type A, by ammounium sulfate fractionation, and gel permeation chromatography using a Sephadex G-200 column.

Investigation for the ability of various sugars to act as acceptors for *N*-acetylgalactosamine by replacing 2'-FL in the standard assay revealed that LNF-I, 2-*O*-α-L-fucopyranosylmethyl-β-galactopyranoside, and 2-*O*-α-L-fucopyranosyl-galactose worked as active acceptors. Sugars, which did not work as acceptors were D-galactose, D-glucose, D-mannose, α- and β-methyl galactopyranoside, *N*-acetylglucosamine, 4-*O*-α-L-fucopyranosylmethyl-β-galactopyranoside, 6-*O*-α-L-fucopyranosylmethyl-β-galactopyranoside, lactose, 2-*O*-α-L-fucopyranosyl-D-talose, 3-fucosyllactose, LD, LNT, LNnT, LNF-II, LNF-III, LND-I, and LND-II.

The Km values for the four acceptor sugars, 2'-FL, LNF-I, 2-*O*-α-L-fucopyranosylmethyl-β-galactopyranoside, and 2-*O*-α-L-fucopyranosyl-galactose

are 4.0×10^{-4}M, 3.5×10^{-4}M, 5.0×10^{-4}M and 7.4×10^{-4}M, respectively. The highest Vmax value was obtained with 2-*O*-α-L-fucopyranosylmethyl-β-galactopyranoside, which was twice as high as the other acceptors.

Accordingly, A-enzyme transfers an *N*-acetylgalactosamine residue to the galactose residue constructing the Fucα1-2Gal group. Galactosyl residues without the Fucα1-2 substitution are not acceptors. An interesting aspect of the specificity of the enzyme is that LD and LND-I are not acceptors in spite of having the Fucα1-2Gal group.

Then, the structures of the two radioactive oligosaccharides, obtained by using 2'-FL and LNF-I as acceptors, were investigated. These oligosaccharides were named P_I and P_{II}, respectively. By analysis of monosaccharides composition, it was revealed that only one *N*-acetylgalactosamine was added to both acceptor oligosaccharides. The position, where the *N*-acetylgalactosamine residue was added, was confirmed to be the C-3 position of the galactose residue of the Fucα1-2Gal group by carefully investigating the radioactive disaccharide, liberated from P_I and P_{II} by partial acid hydrolysis. Only GalNAcα1-3Gal was obtained as the radioactive disaccharide from both P_I and P_{II}. The positional assignment of the added *N*-acetylgalactosamine residue was further confirmed by investigating the structures of the products obtained by Smith-degradation of P_I and P_{II}. That the newly added *N*-acetylgalactosamine residue is linked to the galactose by α-anomeric configuration was confirmed by the evidence that the radioactive *N*-acetylgalactosamine residues of P_I and P_{II} are completely released by incubation with α-*N*-acetylgalactosaminidase, which was purified from bovine liver, but not with various β-*N*-acetylhexosaminidases.

Based on these results, the structures of P_I and P_{II} were confirmed as shown in Fig. 4.

That both P_I and P_{II} have the haptenic activity of blood type A was confirmed by the following study. To 300 μl of anti-A serum, 50 μl of phosphate buffered saline containing various amounts of P_I and P_{II} were added. After being left for 15 min at room temperature, 5 μg of soluble blood group substance A was added, and incubation was continued for 30 min at 37 ˚C, and then left overnight at 4 ˚C. The incubation mixtures were then centrifuged for 15 min at 10,000 rpm. The supernatant solutions were

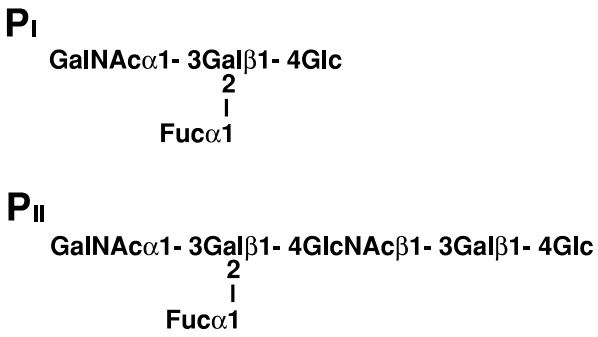

Fig. 4 Structures of oligosaccharides P_I and P_{II} formed by the addition of *N*-acetylgalacosamine to 2'-FL and LNF-I, respectively.

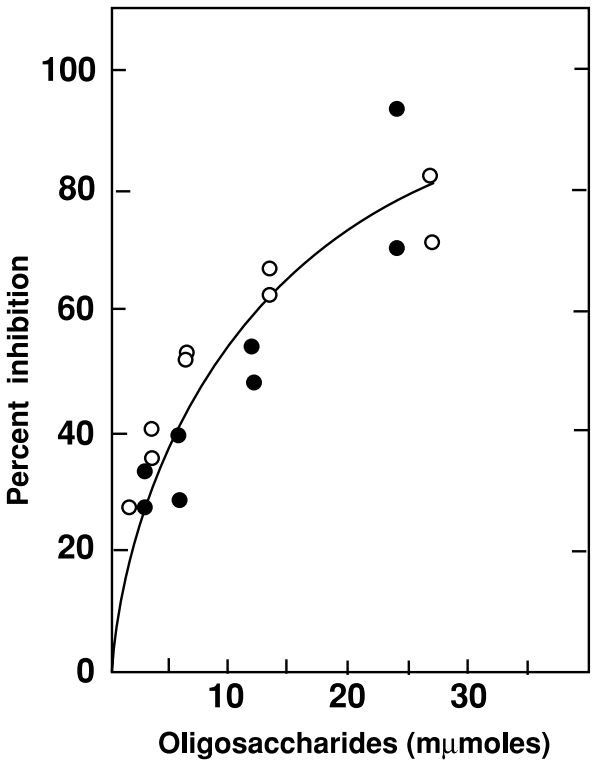

Fig. 5 Inhibition of A, anti-A precipitin reaction by oligosaccharides P_I and P_{II}. Condition of the assay are described in the text. Open circles are the data obtained for P_I, and closed circles are those obtained for P_{II}. The figure was taken from reference 33.

decanted, and the precipitates were washed twice with 0.3 ml aliquots of cold phosphate-buffered saline, and recentrifuged. The protein content of the precipitates were then determined colorimetrically. As shown in Fig. 5, both oligosaccharides showed almost the same haptenic activity of blood type A on molecular basis.

Biosynthetic Pathway of the ABO and Lewis Blood Group Determinants.

Based on the oligosaccharides patterns, and the enzymatic studies, the biosynthetic scheme of blood group A, B, H, Le^a and Le^b determinants were elucidated as shown in Fig. 6.[34]

In the mucous epithelial cells of non-secretor individuals, the Fucα1-2Gal group is not formed on the sugar chains of their secretory glycoproteins, because H-enzyme in Fig. 6, is not expressed. Since the Fucα1-2Gal group, formed by the action of the fucosyltransferase, is the substrate of A and B enzymes, blood group A and B determinants could not be expressed in the sugar chains of the secretory glycoproteins of non-secretor individuals, even if they have A and B genes. Expression of H gene, the structural gene for H-enzyme, in mucous epithelial cells was originally considered to be regulated by *Se* gene.[34] However, recent gene cloning study by Ball et al.[35] indicated that *Se* gene is also a structural gene that codes for H-enzyme specifically in the secretory organs.

The *Le* gene is the structural gene of Le-enzyme in Fig. 6. Therefore, individuals, who are of *le*,*le* genotype, lack both Le^a and Le^b antigenic determinants.

The sugar chain containing the structures of A-blood group determinant and Le^b determinant was reported to occur in the blood group substances purified from ovarian cysts by Elvin Kabat's group.[36] Since LND-I is not an acceptor for A-enzyme, Le^b determinant cannot be an acceptor for the A-enzyme in spite of having the essential Fucα1-2Gal group. Therefore, sugar chains containing the two determinants can only be made from H-determinant by the sequential action of A-enzyme, and then Le-enzyme (Fig. 7).

Occurrence of P_I and P_{II} in the Milk Samples Obtained from Secretors with Blood Type A.

Whether P_I and P_{II} shown in Fig. 4 occur in human milk or not was investigated by measuring the haptenic activities of oligosaccharide fractions.[33] The oligosaccharide fractions, obtained from the milk of donors with various blood types, were fingerprinted by combination of gel-permeation and paper chromatography, and the oligosaccharides at the area of P_I and P_{II} were recovered by elution with water.

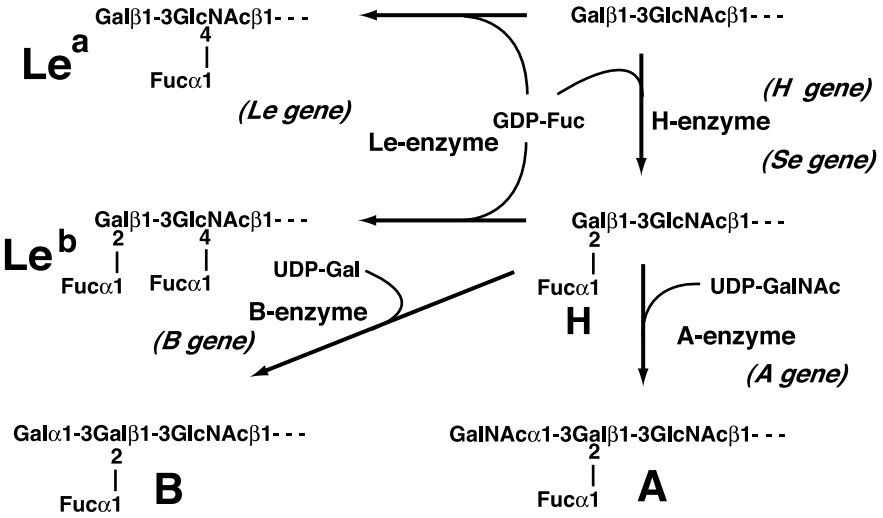

Fig. 6 Biosynthetic pathway of ABO and Lewis blood group determinants. The figure was revised from that in Glycoconjugate J. 2000;17:443-464.

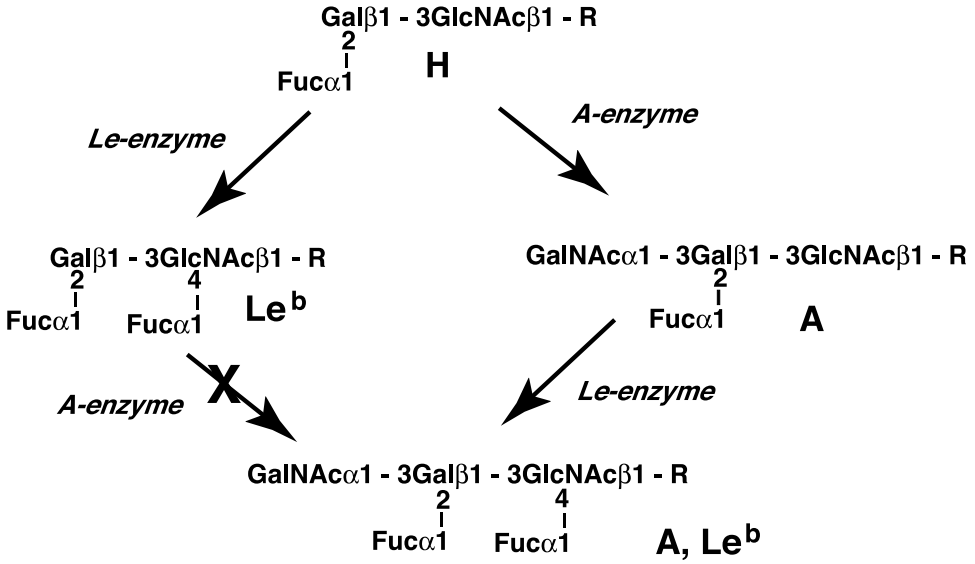

Fig. 7 Biosynthetic scheme to produce A, Le^b structure.

The haptenic activity of the two eluates were tested by the same method described for P_I and P_{II}, and the amounts of inhibitors were estimated from the curve shown in Fig. 5.

As summarized in Table 3, it was confirmed that only the oligosaccharide fractions, obtained from individuals of blood type A, secretor, showed the presence of both P_I and P_{II}. Milk samples from A, non-secretor, and from other blood types did not contain P_I and P_{II}. The amount of both haptenic oligosaccharides varied by individuals as low as 0.2 mμmole up to 13 mμmole per ml of milk. These values are from one-hundredth to one-thousandth amounts of LNT and lacto-*N*-fucopentaoses I and II.

Table 3. Natural Occurrence of A-haptens in Human Milk

Dono	Blood type	Secretor status	Haptenic oligosaccharides	
			P_I	P_{II}
			(mμmoles/ml milk)	
B.J.	A_2	secretor	13	4
E.K.	A_2	secretor	3	2
E.W.	A_1	secretor	0.4	0.2
M.C.	A_1	secretor	8	4
M.R.	A_1	secretor	0.2	0.3
K.C.	A_1	secretor	6	1
B.B.	A_1	non-secretor	<0.1	<0.1
H.S.	O	secretor	<0.1	<0.1
M.S.	B	secretor	<0.1	<0.1

Structures of the Novel Minor Oligosaccharides Found in the Milk Samples Obtained from Non-secretor Individuals and Le^{a-b-} Individuals.

Structures of the three novel minor oligosaccharides found in the milk of non-secretors, and two additional novel minor oligosaccharides found in the milk of Lewis negative individuals were elucidated as shown in Table 4. Lacto-*N*-fucopentaose III later served as an important haptenic oligosaccharide to investigate the functional role of Le^x antigen.[41] Lacto-*N*-hexaose and lacto-*N*-*neo*hexaose were used as the models of the core portions blood group determinants in the later studies of many blood group related antigens.[42,43]

Before the structures of these oligosaccharides were elucidated, lactose, LNT and LNnT were considered as the cores of human milk oligosaccharides. Branched hexasaccharides: LNH and LNnH were newly added as cores, and a large number of fucosyl and sialyl derivatives of these newly added cores were found in human milk.[39,40-49]

For the structural studies of these novel milk oligosaccharides, many new sensitive analytical techniques, such as tritium-labelling[50], sequential exoglycosidase digestion[51], and sensitive methylation analysis suitable for the aminosugar-containing oligosaccharides[52] were established.

Table 4. Novel Oligosaccharides Isolated From Milk of Non-secretor and Lele Individuals.

Names	Structures	References
6'-Galactosyllactose (6'-GalL)	Galβ1-6Galβ1-4Glc	37
Lacto-*N*-fucopentaose V (LNF-V)	Galβ1-3GlcNAcβ1-3Galβ1-4Glc 3 | Fucα1	38
Lacto-*N*-fucopentaose III (LNF-III)	Galβ1-4GlcNAcβ1-3Galβ1-4Glc 3 | Fucα1	28
Lacto-*N*-hexaose (LNH)	Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-3GlcNAcβ1 ⟋ 3 Galβ1-4Glc	39
Lacto-*N*-neohexaose (LNnH)	Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-4GlcNAcβ1 ⟋ 3 Galβ1-4Glc	40

Other New Core Oligosaccharides Found in Human Milk.

Later on, seven larger oligosaccharides were further added as new cores of milk oligosaccharides (Table 5). Sialylation and/or fucosylation of these core oligosaccharides will produce a large number of different oligosaccharides.

So far, over seventy of oligosaccharides are currently known to occur in human milk.[45-49,53-59]

As in the case of other milk oligosaccharides already reported, all these oligosaccharides contain lactose at their reducing termini. This evidence, together with the deletion phenomena found in various blood type individuals, suggested that human milk oligosaccharides are formed by concerted action of glycosyltransferases, which are responsible for the formation of the sugar chains of glycoproteins and glycolipids of epithelial cells of mammary gland.

The elongation of lactose may start by the action of the β3-*N*-acetyl-glucosaminyltransferase (iGnT).[60] This enzyme is responsible for the addition of a β-*N*-acetylglucosamine residue to the C-3 position of the galactose moiety constructing the *N*-acetyllactosamine group of the sugar chains of glycoconjugates, and may use lactose as a false acceptor because of their structural similarity (Fig. 8).

Therefore, oligosaccharides in human milk are expected to include many sugar chain structures, which are derived from the *N*-acetyllactosamine groups in the sugar chains of various glycoconjugates. These sugar chains may work as various recognition signals on the surface of epithelial cells.

Table 5. Various Core Structures Found in Human Milk Oligosaccharides.

Names	Structures	References
Para-Lacto-*N*-hexaose	Galβ1-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	53
Para-Lacto-*N*-neohexaose	Galβ1-4GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	53
Lacto-*N*-octaose	Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-3GlcNAcβ1 ⟋ 3 Galβ1-4Glc	54
Lacto-*N*-neooctaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-4GlcNAcβ1 ⟋ 3 Galβ1-4Glc	54
iso-Lacto-*N*-octaose	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-3GlcNAcβ1 ⟋ 3 Galβ1-4Glc	55
Para-Lacto-*N*-octaose	Galβ1-3GalNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glc	44
Lacto-*N*-decaose	Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-3GlcNAcβ1 ⟋ 3 Galβ1-4GlcNAcβ1 ⟍ 6 Galβ1-3GlcNAcβ1 ⟋ 3 Galβ1-4Glc	44

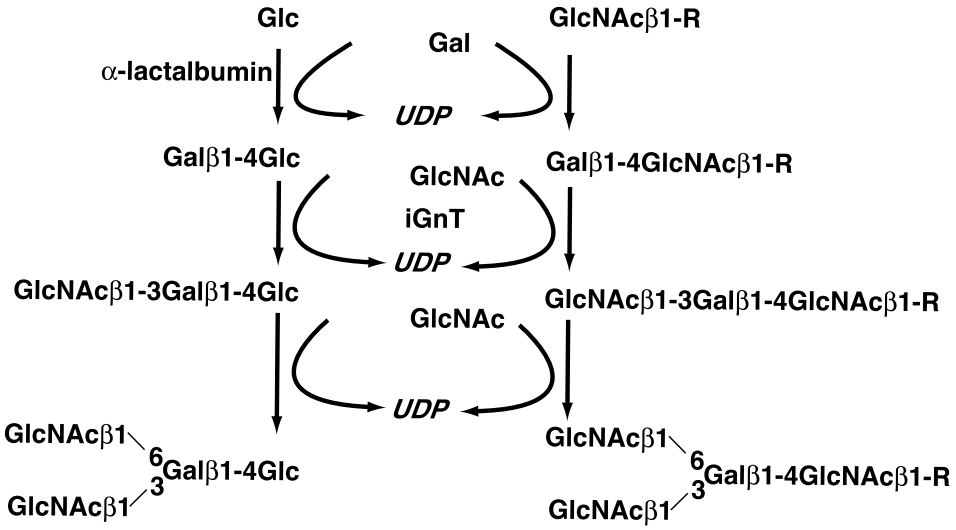

Fig. 8 Elongation of lactose by the action of iGnT.

Application of Human Milk Oligosaccharides as Drugs for Prevention of Bacterial and Viral Infection.

It was known that the infection of many bacteria and viruses starts by binding to particular sugar chains of glycoconjugates on the surface of epithelial cells, which are constructing the mucous epithelium of digestive and respiratory tracts. Therefore, it may be possible to elucidate the structure of the target sugar chain of each bacteria or virus by investigating the structures of milk oligosaccharides.

At this moment, we are now coming back to the situation of the original motivation in the field of paediatrics to elucidate the superiority of breast-feeding over artificial-feeding. As was already introduced at the beginning of this review, Grulee et al[5] reported that breast-feeding was strongly associated with a lower incidence of diarrhea, otitis media, and respiratory diseases. Unfortuantely, the *Bifidus* factor hypothesis ended to find that *Lactobacillus bifidus, varietas pennsylvanicus* is a mutant, which requires aminosugars as its essential nutrient. Accordingly, only milk oligosaccharides, containing *N*-acetylglucosamine residue, worked as *Bifidus* factor. However, most recent data[61,62] still support the conclusion that breast-feeding is associated with less disease in the infant than artificial-feeding.

As summarized by David Newburg,[63] oral administration of various oligosaccharides and glycoconjugates were found to protect babies against pathogens.[64-75] If the non-reducing termini of the sugar chains, which work as the receptor for a bacteria on the surface of epithelial cells of digestive tracts, is printed in the non-reducing termini of human milk oligosaccharides, these oligosaccharides may work as haptenic inhibitors of adhesion of bacteria to the receptor sugar chain of intestinal epithelial cells, and protect babies from their infection. Therefore, these oligosaccharides may be useful for the development of new drugs for suckling babies to protect from bacterial infection.

The idea that oligosaccharide mimics of the receptors of pathogens will work as effective drugs for preventing infection by the pathogens was recently found to be quite useful for the development of drugs to prevent infection with influenza virus. Whether the same strategy works for developing a new drug to prevent from bacterial infections has not been confirmed yet. However, recent reports of Crane et al[76] indicated the rationale of such an idea in the field of bacterial infection also.

One of the toxins produced by enterotoxigenic *E. coli* is called stable toxin (STa), because of its stability to organic solvents and heat. By activating guanylate cyclase of the surface of intestinal epithelial cells, STa induces secretory diarrhea. T84 cell is a cell-line, which will differentiate in culture, and express many features of intestinal epithelium,

including formation of microvillae and transmembrane guanylate cyclase, whose enzymatic activity is intra-cellularly located, but whose STa binding domain is extra-cellularly located.

By using this cultured cell, Crane et al[76] found that a crude fraction of human milk oligosaccharides showed a dose-dependent inhibitory activity for the STa-induced cGMP activation. The crude oligosaccharide fraction was then fractionated into non-fucosylated oligosaccharides fraction and fucosylated oligosaccharides fraction by subjecting to the affinity chromatography using an immobilized *Ulex europeus* lectin column. The fucosylated oligosaccharides fraction inhibited the increase of guanylate cyclase activity by stimulation with STa, whereas the non-fucosylated oligosaccharide fraction did not.

Based on this finding, they have been studying hard to determine the structure of the oligosaccharide with the inhibitory activity.

Future Prospects.

The stories so far introduced indicate that studies of human milk oligosaccharides are expected to be useful for the development of drugs, which are effective for protection of babies from harmful intestinal infections. The key to lead to the success of this line of studies is how one will be able to develop an effective method to pick up minor but useful oligosaccharides from the mixture of a large number of different milk oligosaccharides.

Kitagawa et al. reported the structures of a series of oligosaccharides, which were retained by an immobilized column of anti-CA19-9 antibody.[77] As shown by **A-D** in Fig. 9, they are different sizes of oligosaccharides containing the sialyl-Le^a determinant in common.

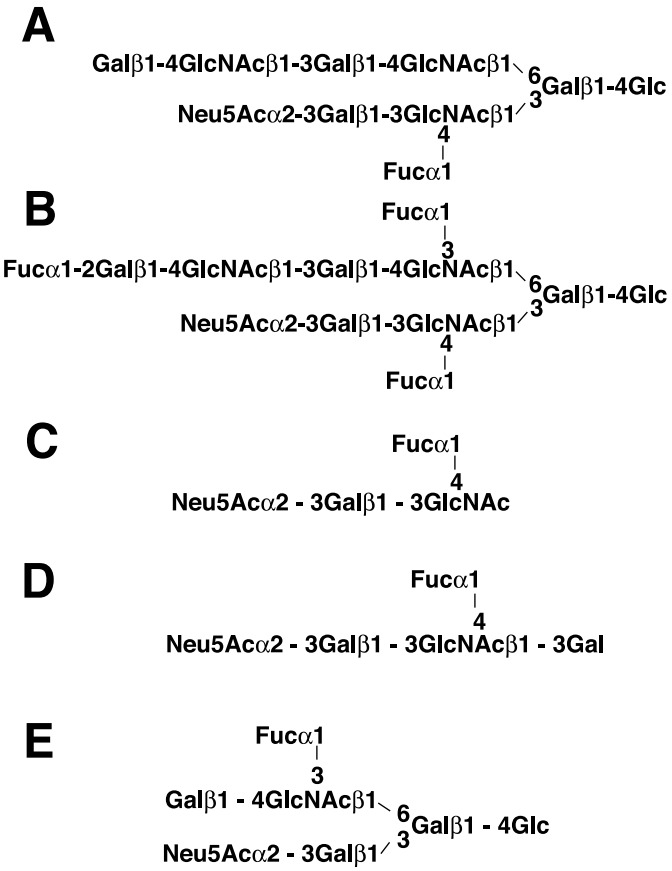

Fig. 9 Structures of milk oligosaccharides containing the sialyl-Le^a group (A-D), and three human milk oligosaccharides (C-E) which do not contain the known core oligosaccharides.

Among them, two oligosaccharides, **C** and **D**, which occur as very minor components in the oligosaccharides fraction retained by the column, have unusual features. They do not contain lactose, but contain either *N*-acetylglucosamine or galactose residue at their reducing termini.

Another unusual oligosaccharide (E in Fig. 9), which does not contain the core structures of milk oligosaccharides so far introduced, was isolated from human milk.[49] However, this oligosaccharide also contains lactose at its reducing termini. Therefore, the mechanism to produce oligosaccharides **C** and **D**, which lack lactose group, is totally unknown. They may be produced by an unknown degradation mechanism from larger milk oligosaccharides.

In any event, the report of Kitagawa et al. indicated that we will be able to pick up even a very minor oligosaccharide with a particular ligand specificity, if a proper affinity chromatographic method is devised by isolating receptor proteins on the surface of bacteria and viruses. Perhaps, this can be performed in the near future by collaborating with the bacteriologists and virologists, who are investigating the virulent factors by cloning the surface lectins of bacteria and viruses.

REFERENCES

1. Polonovski M, Lespagnol A. Nouvelles acquisitions sur les composés glucidiques du lai de femme. Bull Soc Chem Biol 1933;15:320-49.
2. Polonovski M., Montreuil J. Etude chromatographique des polyosides du lait de Femme. C R Acad Sci Paris 1954;238:2263-4.
3. Tissier H. Rechershes sur la flore intestinale de nourissons. PhD thesis. Univ. Paris, Paris, France.
4. Moro E. Morphologi und bakteriologisch Untersuchungen über die Darmbakterien des Säuglings: die Bakteriumflora des normalen Frauenmilchstuhls. Jahrbuch Kinderh 1900;61:686-734.
5. Grulee C, Sanford H, Schwartz H. Breast and artificially fed infants; study of the age incidence in morbidity and mortality in 20,000 cases. JAMA 1935;104:1986-8.
6. Schönfeld H. Uber die Beziehungen der einzelnen Bestandteile der Frauenmilch zur Bifidusflora. Jahrbuch Kinderh 1926;113:19-69.
7. György P, Kuhn R, Rose CS, Zilliken F. Bifidus factor I. A variant of Lactobacillus bifidus requiring a special growth factor. Arch Biochem Biophys 1954;48:193-201.
8. György P, Kuhn R, Rose CS, Zilliken F. Bifidus factor III. The rate of dialysis. Arch Biochem Biophys 1954;48:209-13.
9. György P, Kuhn R, Rose CS, Zilliken F. Bifidus factor IV. Preparations obtained from human milk. Arch Biochem Biophys 1954;48:214-24.
10. Kuhn R, Baer HH, Gauhe A. Kristallisierte fucosido-lactose. Chem Ber 1956;89:2513
11. Montreuil J. Structure de deux triholosides isolés du lai de femme. C R Acad Sci 1956;242:192-3.
12. Kuhn R, Gauhe A. Über die lacto-difuco-tetraose der Frauenmilch. Justus Liebig's Ann Chem 1958;611:249-53
13. Kuhn R, Baer HH. Die Konstitution der Lacto-*N*-tetraose. Chem Ber 1956;89:504-11.
14. Kuhn R, Gauhe A. Die Konstitution der Lacto-*N*-neotetraose. Chem Ber 1962;95:518-22.
15. Kuhn R, Baer HH, Gauhe A. Kristallisation und Konstitutionsermittlung der Lacto-*N*-fucopentaose I. Chem Ber 1956;89:2514-23.
16. Kuhn R, Baer HH, Gauhe A. Die Konstitution der Lacto-*N*-fucopentaose II. Chem Ber 1958;91:364.
17. Kuhn R, Baer HH, Gauhe A. 2-α-L-Fucopyranosyl-D-galaktose und 2-α-L-fucopyranosyl-D-talose. Justus Liebig's Ann Chem 1958;611:242-9.
18. Kuhn R, Gauhe A. Über ein kristallisiertes, Le^a-aktives Hexasaccharid aus Frauenmilch. Chem Ber 1960;93:647-51.
19. Kuhn R, Brossmer R. Uber das durch Viren der Influenza-Gruppe spaltbare Trisaccharid der Milch. Chem Ber 1959;92:1667-71.
20. Kuhn R. Biochemie der Rezeptoren und Resistenzfaktoren. Von der Widerstandsfähigkeit der Lebewesen gegen Einwirkungen der Umwelt. Naturwissenschaften 1959;46:43-50.
21. Kuhn R, Gauhe A. Bestimmung der Bindungsstelle von sialinsäureresten in Oligosaccharides mit Hilfe von Perjodat. Chem Ber 1965;98:395-413.
22. Kuhn R, Gauhe A. Über drei säure pentasaccharide aus frauen milch. Chem Ber 1962;95:513-7.
23. Watkins WM. Blood-group specific substances. In: Gottschalk A. ed. Glycoproteins. 2nd ed. Part B, Elsevier, Amsterdam, 1972;830-91.
24. Watkins WM. Blood group specific substances. In: Gottschalk, A. ed. Glycoproteins. Elsevier, Amsterdam, 1966:462-515.
25. Grollman EF, Ginsburg V. Correlation between secretor status and the occurrence of 2'-fucosyllactose in human milk. Biochem Biophys Res Commun, 1967;28:50-3.
26. Kobata A, Tsuda M, Ginsburg V. Oligosaccharides of human milk. I. Isolation and characterization. Arch Biochem Biophys 1969;130:509-13.
27. Shen L, Grollman EF, Ginsburg V. An enzymatic basis for secretor status and blood group substance specificity in humans. Proc Natl Acad Sci U S 1968;59:224-30.
28. Kobata A, Ginsburg V. Oligosaccharides of human milk. II. Isolation and characterization of a new pentasaccharide, lacto-*N*-fucopentaose III. J Biol Chem 1969;244:5496-502.
29. Grollman EF, Kobata A, Ginsburg V. An enzymatic basis

for Le(a⁻b⁻) blood type in humans. J Clin Invest 1969; 48:1489-94.
30. Babad H, Hassid WZ UDP-D-Galactose: D-Glucose β-4-Galactosyltransferase from milk. Methods Enzymol 1966; 8:346-51.
31. Kobata A, Grollman EF, Ginsburg V. An enzymatic basis of blood type A in humans. Arch Biochem Biophys 1968;124:609-12.
32. Kobata A, Grollman EF, Ginsburg V. An enzymatic basis of blood type B in humans. Biochem Biophys Res Commun 1968;32:272-7.
33. Kobata A, Ginsburg V. UDP-*N*-acetyl-D-galactosamine: D-Galactose α-3-*N*-acetyl-D-galactosaminyltransferase, a Product of Gene That Determines Blood Type A in Man. J Biol Chem 1970;245:1484-90.
34. Grollman EF, Kobata A, Ginsburg V. Enzymatic basis of blood types in man. Proc N Y Acad Sci 1970;169:153-8.
35. Ball SP, Tongue N, Gibaud A, Le Pendu J, Mollicone R, Gerard G, Oriol R. Ann Hum Genet 1991;55:225-33.
36. Lloyd KO, Kabat EA, Licerio E. Immunochemical studies on blood groups XXXVIII. Structures and activities of oligosaccharides produced by alkaline degradation of blood-group Le^a substance. Proposed structure of carbohydrate chains of human blood-group A, B, H, Le^a and Le^b substances. Biochemistry 1968;7:2976-90.
37. Yamashita K, Kobata A. Oligosaccharides of human milk. V. Isolation and characterization of a new trisaccharide, 6'-galactosyllactose. Arch Biochem Biophys 1974;161: 164-70.
38. Ginsburg V, Zopf DA, Yamashita K, Kobata A. Oligosaccharides of human milk: Isolation of a new pentasaccharide, lacto-*N*-fucopentaose V. Arch Biochem Biophys 1976;175:565-8.
39. Kobata A, Ginsburg V. Oligosaccharides of human milk. III. Structure of a new hexasaccharide, lacto-*N*-hexaose. J Biol Chem 1972;247:1525-9.
40. Kobata A, Ginsburg V. Oligosaccharides of human milk. IV. Isolation and characterization of a new hexasaccharide, lacto-*N*-neohexaose. Arch Biochem Biophys 1972; 150:273-81.
41. Fenderson BA, Zehavi U, Hakomori S. A multivalent lacto-*N*-fucopentaose III-lysyllysine conjugate decompacts preimplantation mouse embryos, while the free oligosaccharide is ineffective. J Exp Med 1984;160:1591-6.
42. Yamashita K, Tachibana Y, Takasaki S, Kobata A. ABO blood group dterminants with branched cores. Nature 1976;262:702-3.
43. Yamashita K, Tachibana Y, Kobata A. Oligosaccharides of human milk:Structures of three lacto-N-hexaose derivatives with H-haptenic structure. Arch Biochem Biophys 1977;182:546-55.
44. Haeuw-Fiévre S, Wieruszeski JM, Plancke Y, Michalski JC, Montreuil J, Strecker G. Primary structure of human milk octa- dodeca-, and trideca-saccharides determined by a combination of ^{1}H-NMR spectroscopy and fast atom-bombardment mass spectrometry. Evidence for new core structure, the para-lacto-*N*-octaose. Eur J Biochem 1993; 215:361-71.
45. Kitagawa H, Takaoka M, Nakada H, Fukui S, Funakoshi I, Kawasaki T, Tate SI, Inagaki F, Yamashina I. Isolation and structural studies of human milk oligosaccharides that are reactive with a monoclonal antibody MSW 113. J Biochem 1991;110:598-604.
46. Yamashita K, Tachibana Y, Kobata A. Oligosaccharides of human milk. Isolation and characterization of three new disialylfucosyl hexasaccharides. Arch Biochem Biophys 1976;174:582-91.
47. Dua VK, Goso K, Dube VE, Bush CA. Characterization of lacto-*N*-hexaose and two fucosylated derivatives from human milk by high performance liquid chromatography and NMR spectroscopy. J Chromatogr 1985;328:259-69.
48. Grönberg G, Lipniunas P, Lundgren T, Erlansson K, Lindh F, Nilsson B. Isolation of monosialylated oligosaccharides from human milk and structural analysis of three new compounds. Carbohydr Res 1989;191:261-78.
49. Grönberg G, Lipniunas P, Lundgren T, Lindh F, Nilsson B. Structural analysis of five new monosialylated oligosaccharides from human milk. Arch Biochem Biophys 1992;296:597-610.
50. Takasaki S, Kobata A. Microdetermination of sugar composition by radioisotope labeling. Methods Enzymol 1978;50:50-4.
51. Kobata A. Use of endo- and exoglycosidases for structural studies of glycoconjugates. Anal Biochem 1979;100:1-14.
52. Tai T, Yamashita K, Kobata A. Synthesis and mass fragmentographic analysis of partially *O*-methylated 2-*N*-methylglucosamines. J Biochem 197578, 679-86.
53. Yamashita K, Tachibana Y, Kobata A. Oligosaccharides of human milk: Structural studies of new octasaccharides, difucosylderivatives of *para*-lacto-*N*-hexaose and *para*-lacto-*N*-neohexaose. J Biol Chem 1977;252:5408-11.
54. Tachibana Y, Yamashita K, Kobata A. Oligosaccharides of human milk:Structural studies of di- and trifucosyl derivatives of lacto-*N*-octaose and lacto-*N*-neooctaose. Arch Biochem Biophys 1978;188:83-9.
55. Strecker G, Fiévre S, Wieruszeski JM, Michalski JC, Montreuil J. Primary structure of four human milk octa-, nona-, and undeca-saccharides established by ^{1}H and ^{13}C-nuclear magnetic resonance spectroscopy. Carbohydr Res 1992;226:1-14.
56. Grimmonprez L, Montreuil J. Etude physico-chemique de six nouveaux oligosaccharides isole du lai de femme. Bull Soc Chim Biol, 1968;50:843-55.
57. Wieruzeski JM, Chekkor A, Bouquelet S, Montreuil J, Strecker G. Structure of two new oligosaccharides isolated from human milk:Sialylated lacto-*N*-fucopentaose I and II. Carbohydr Res 1985;137:127-38.
58. Smith DF, Prieto PA, McCrumb DK, Wang WC. A novel sialofucopentaose in human milk. Presence of this oligosaccharide is not dependent on expression of the

secretor or Lewis fucosyltransferases. J Biol Chem 1987; 262:12040-7.
59. Strecker G, Wieruszeski JM, Michalski JC, Montreuil J. Structure of a new nonasaccharide isolated from human milk: VI^{2}Fuc, V^{4}Fuc, III^{3}Fuc-*p*-lacto-*N*-hexaose. Glycoconjigate J 1988;5:385-96.
60. van den Eijnden DH, Koenderman AH, Schiphorst WE. Biosynthesis of blood group i-active polylactosaminoglycans. Partial purification and properties of an UDPGlcNAc: *N*-acetyllactosaminide b1,3-*N*-acetylglucosaminyltransferase from Novikoff tumor ascites fluid. J Biol Chem 1988;263:12461-71.
61. Feachem RG, Koblinsky MA. Interventions for the control of diarrhoeal diseases among young children: promotion of breast-feeding against infection. Bull WHO 1984; 62:271-91.
62. Morrow AL, Pickering LK, In Principles and Practice of Infectious Diseases (Long, S. S., Pickering, L. K., Prober, C. G., Eds) pp. 87-94, Churchill Livingstone, New York, 1997.
63. Newburg DS. Human milk glycoconjugates that inhibit pathogens. Current Medicinal Chemistry 1999;6:117-27.
64. Cleary TG, Chambers JP, Pickering LK. Protection of suckling mice from heat-stable enterotoxin of *Escherichia coli* by human milk. J Infect Dis 1983;148:1114-9.
65. Andersson B, Porras O, Hanson LA, Langergard T, Svanborg-Eden C. Inhibition of attachment of *Streptococcus pneumoniae* and *Haemophilus influenzae* by human milk and receptor oligosaccharides. J Infect Dis 1986;153:232-7.
66. Laegreid A, Kolsto Otnaess AB, Fuglesang J. Human milk and bovine milk: Comparison of ganglioside composition and enterotoxin-inhibitory activity. Pediatr Res 1986;20:416-21.
67. Laegreid A, Kolsto Otnaess AB, Orstavil I, Carlsen KH. Neutralizing activity in human milk fractions against respiratory syncytial virus. Acta Paediatr Scand 1986;75: 696-701.
68. Clark GF, Krivan HC, Wilkins TD, Smith DF. Toxin A from *Clostridium difficile* binds to rabbit erythrocyte glycolipids with terminal Gal alpha 1-3Gal beta 1-4GlcNAc sequences. Arch Biochem Biophys 1987;257:217-29.
69. Newburg DS, Pickering LK, McCluer RH, Cleary TG. Fucosylated oligosaccharides of human milk protect suckling mice from heat-stable enterotoxin of *Escherichia coli*. J Infect Dis 1990;162:1075-80.
70. Ashkenazi S, Newburg DS, Cleary TG. The effect of human milk on the adherence of enterohemorrhagic *E. coli* to rabbit intestinal cells. Adv Exp Med Biol 1991; 310:173-7.
71. Schroten H, Hanisch FG, Plogmann R, Hacker J, Uhlenbruck G, Nobis-Bosch R, Wahn V. Inhibition of adhesion of S-fimriated *Escherichia coli* to buccal epithelial cells by human milk fat globule membrane components:a novel aspect of the protective function of mucins in the nonimmunoglobulin fraction. Infect Immun 1992; 60:2893-9.
72. Yolken RH, Peterson JA, Vonderfecht SL, Fouts ET, Midthun K, Newburg DS. Human milk mucin inhibits rotavirus replication and prevents experimantal gastroenteritis. J Clin Invest 1992;90:1984-91.
73. Newburg DS, Ashkenazi S, Cleary TG. Human milk contains the Shiga toxin and Shiga-like toxin receptor glycolipid, Gb_3. J Infect Dis 1992;166:823-36.
74. Cervantes LE, Soto LE, Newburg DS, Ruiz-Palacios GM. α1-2 Fucosylated chains (H-2 and Lewis^b) are the main human milk receptor analogs for *Campylobacter*. Pediatr Res 1995;37:171A.
75. Lambert DM. Role of oligosaccharides in the structure and function of respiratory syncytial virus glycoprotein. Virology 1988;164:458-66.
76. Crane JK, Azar SS, Stam A, Newburg DS. Oligosaccharides from human milk block binding and activity of the *Escherichia coli* heat-stable enterotoxin (Sta) in T84 intestinal cells. J Nutr 1994;124:2358-64.
77. Kitagawa H, Nakada H, Numata Y, Kurosaka A, Fukui S, Funakoshi I, Kawasaki T, Shimada I, Inagaki F, Yamashina I. Occurrence of tetra- and pentasaccharides with the sialyl-Le^a structure in human milk. J Biol Chem 1990;265:4859-62.

乳汁中的寡醣成爲藥物的可行性

木幡 陽

使用指印法 (finger-printing method) 去分析人乳中的寡醣，發現在非分泌型 (non-secretor) 及路易士陰性 (Lewis negative) 個體的乳汁中缺乏一些寡醣。這個發現提供了一些線索來解釋人類血型與酵素的關係。此外，從一些含量較多寡醣的消失導致發現五種新的且含量較少的寡醣，而這些含量較少的寡醣是隱藏在含量較多的寡醣之中。後來，有超過七十種不同構造的寡醣因而被發現。這些寡醣是以十一種核心寡醣 (core oligosaccharide) 爲基礎，經過唾液醣化 (sialylation) 及／或岩藻醣化 (fucosylation) 的衍生而成。其共同特點是在它們的還原端皆含有乳醣 (lactose)。這個證據加上在人類兩種血型的乳汁中所見到的刪除 (deletion) 或缺乏現象，顯示出這些寡醣是醣類移轉酶群 (glycosyltransferases) 藉由同時作用 (concerted action) 而形成，而這些酵素也負責上皮細胞黏膜上醣蛋白中醣鏈的形成。寡醣的加長是藉由一種 iGnT (酵素) 的作用而開始。這個酵素是負責將β-*N*-乙醯葡萄醣胺 (β-*N*-acetylglucosamine, β-GlcNAc) 接到半乳醣 (galactose, Gal) 第三個碳的位置成爲 GlcNAcβ1→3Gal的結構，使其可以繼續構成醣共軛上醣鏈的*N*-乙醯乳醣胺 (*N*-acetyllactosamine, Galβ1→4GlcNAc)。因此，人乳寡醣包含的許多構造，都是經由*N*-乙醯乳醣胺 (*N*-acetyllactosamine, Galβ1→4GlcNAc) 連接到不同醣蛋白的醣鏈而開始的。

許多實驗證實，致病腸內菌與濾過性病毒上所含的凝集素 (lectin, adhesion or agglutinin) 與目標細胞表面上特定醣共軛的醣鏈相結合開始進行感染。而人乳寡醣可用以阻撓並抑制這些細菌與濾過性病毒的附著而避免感染。因此，乳汁中的寡醣具有研發成藥物的可行性。(長庚醫誌 2003;26:620-36)

關鍵字：乳汁，人類，寡醣，腸內菌，血型，β3-N-乙醯葡萄醣胺移轉酶

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