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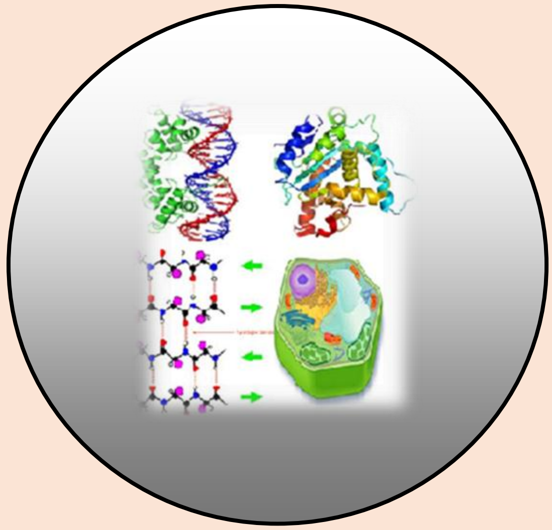
ISSN 2319-3077 Online/Electronic
ISSN 0970-4973 Print

UGC Approved Journal No. 62923
MCI Validated Journal
Index Copernicus International Value
IC Value of Journal 82.43 Poland, Europe (2016)
Journal Impact Factor: 4.275
Global Impact factor of Journal: 0.876
Scientific Journals Impact Factor: 3.285
InfoBase Impact Factor: 3.66

J. Biol. Chem. Research
Volume 36 (1) 2019 Pages No. 314-336



Journal of Biological and Chemical Research

An International Peer Reviewed / Referred Journal of Life Sciences and Chemistry

Indexed, Abstracted and Cited in various International and National Scientific Databases

Published by Society for Advancement of Sciences®

J. Biol. Chem. Research. Vol. 36, No. 1, 314-336, 2019
(An International Peer Reviewed / Refereed Journal of Life Sciences and Chemistry)
Ms 36/01/1908/2019

ISSN 2319-3077 (Online/Electronic)
ISSN 0970-4973 (Print)

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RESEARCH PAPER

Received: 10/05/2019 Revised: 10/06/2019 Accepted: 11/06/2019

Biological Importance of Milk: A Review

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ABSTRACT
Since time immemorial milk was used for development of any mammalian infant. It was thought that milk was only a food supplement responsible for growth of newly born and adult too. With the advent of science, it was proposed and authenticated that milk is not only a food supplement but also responsible for various physiological, neurological, and immunological activities in the body of any mammal. Basically, It provides protein, fats, and carbohydrate. Carbohydrates are present in the form of lactose, oligosaccharides and glycoconjugates. After the enzymatic hydrolysis the lactose provide glucose which is used as the basic food supplement and the oligosaccharides present there in are responsible for varied biological activities. Beside the essential nutrients milk also provides vitamins and minerals. It is responsible for calcium, riboflavin and protein. It also contain triglycerides, casein, and many other constituents. The milk has certain roles in physiological functions, defence mechanism, as a prebiotic and also associated with immunological properties. It also participate in secretion of antibodies, immunomodulatory effects and anti-inflammatory effects, role in brain development, mineral absorption and as tumour markers. In this review article, the authors have compiled the various biological aspect of milk and milk oligosaccharides.
Keywords: Mammalian Infant, Physiological, Neurological and immunological Activities.

INTRODUCTION
Milk is the first intake taken by any mammalian infant. It provides the first energy required for all the vital biological functions of any infant. It also gives strength to fight out any infections which try to prevail in the newly born. Milk is the most hygienic unadulterated natural gift for the babies of any mammalian. It has the properties of easy digestion and instant energy. Besides this, it has a property to develop the undeveloped pulmonary, neural and secretary systems of infants. The most important property of the milk is the development of brain which controls all the systems of the body (McWilliams, Margaret, 1979).

Basically, milk is constituted of simple and complex carbohydrates, proteins, vitamins and most of the nutritional requirements needed for the development of the body systems. Milk may be described as the most nutritionally complete food. It provides a range of essential nutrients in particular protein and a wide range of vitamins and minerals. Milk supplies calcium, Riboflavin, proteins and vitamin A requirement of a normal adult person. The proportion of various nutrients in milk also makes it an ideal diet for the new borns. It is also called the liquid complete food. It is an indispensable part of an infant's diet. The composition of milk is given below.

The human and cows milk have about 87 % of water. The major carbohydrate present in milk is lactose, which is a disaccharide composed of the monosaccharides D-glucose and D-Galactose. The chemical name of the Lactose is 4-O-β-D-Galactopyranosyl-D-Glucopyranose. Lactose is essentially unique to milk and it plays a major role in milk synthesis. It is the major osmole in milk and the process of synthesis of lactose is responsible for drawing water into milk, it is being formed in the mammalian epithelial cells. Lactose is not as sweet as other Disaccharides such as sucrose and monosaccharides such as fructose and glucose. Lactose is cleaved in the intestines to glucose and galactose. By an enzyme called lactase. Some other carbohydrates which are found free in milk are amino-sugars, sugar phosphates, neutral/acidic oligosaccharides and neucleotide sugars. Some complex oligosaccharides are important in establishing the microflora of neonatal intestine such as Bifidus factor identified in milk (Boehm et al., 2005). The fat component of the milk is composed of a complex mixture of lipids (Welsh and May, 1979, Kabara, 1980, Ogra and Losonsky, 1984). Triglycerides which are the major types of lipids in milk are composed of these fatty acids covalently bound to a glycerol molecule by ester linkage. Milk fat is the major source of lipids used by neonate mammal for accumulating body adipose tissues in the initial days after birth. Milk fat is easily digested by the new born and the composition is best suited to them. The total protein component of milk is composed of many specific proteins. The primary groups of milk proteins are the caseins (Orlando et al). Caseins have an amino-acid composition which is important for the growth and development of the nursing young. Caseins are easily digestible in the intestine compared to many other food proteins available. Caseins reacts with the acid found in the stomach and also with an enzyme called rennin and form a soft curd. The protein caseins can be separated from the whole milk with the action of acids. The reaction is similar to what happens in the stomach when milk is consumed. Once caseins are removed from the milk, all other proteins left are known as whey proteins. There are many whey proteins in the milk and the specific set of whey proteins found in mammary secretions varies with the species, stage of lactation, the presence of any intra-mammary infections and other factors. The major whey proteins in the milk are α-Lactalbumin (Pelligine et al., 1999) and β-Lactglobulin. α-Lactalbumin is an important protein in the synthesis of lactose and it plays a key role in the process of milk synthesis. Other whey proteins are important immunoglobulins which have anti-infective factors especially high in colostrums (Tadasu Urashima, 2001, Tadasu Urashima et al 2003, Nakamura and Kowese, 2003). Whey proteins also include various enzymes, hormones, growth factors, disease resistant factors, iron lactoferrin and various nutrient transporters. The major minerals found in milk are calcium and phosphorous. These minerals are of prime importance for growing neonates and children (Chonan and Watanuki, 1996, Brink and Van Berestejn 1993, Greger et al., 1989, Debongnie et al., 1979, Kobayashi et al., 1972). They are specially required for bone and teeth growth and development of soft tissues. Milk also has all the vitamins (Proudfit and Robinson, 1978) which are required by the human body. The fat soluble vitamin A, D, E, K are found in the milk fat while the B-complex vitamins are found in the aqueous phase of the milk. Milk also contains some bioactive factors such as hormones, enzymes and cellular proteins (Smith, et. al 1971) etc. Milk becomes important food stuff in every stage of life cycle and even in various clinical conditions due to variety of nutrients, it supplies and even larger variety of products one gets from it.

Milk is the only naturally occurring food stuff that contains lactose which is made up of glucose and galactose. Glucose is the form in which sugar is absorbed by the body and galactose is utilized for the synthesis of myelin sheath, the insulating cover of the nerve fibers. Lactose also favours calcium absorption, firstly by increasing the permeability of the small intestine for influx of calcium ions and secondly, by lowering the pH of the intestine consequently favoring calcium absorption. Milk also supplies all the essential amino-acids. Milk also contains essential fatty acids that are good for the body system. The calcium phosphorous ratio of milk is most suited for bone development. Iron content of milk is rather low but the absorption of iron from milk is high and so it is highly utilized by the body. Milk is rich in riboflavin, thyamine and also has high amounts of vitamin A and D. It also has high moisture content and to some extent it releases the constipation tendency in newborns and immobilized patients. Water content of milk is dependent upon the synthesis of lactose, without some water in it milk would be a viscous solution of lipids and proteins and would be extremely difficult to remove from the gland. Upon birth the mammalian neonate is not able to meet its own water supply and would dehydrate rapidly without the water component of milk. In pregnant ladies, a balanced diet rich in proteins, minerals and vitamins is essential for the proper growth and health of mother and foetus. Milk can provide all these nutrients in adequate amounts. Milk also helps the pregnant ladies for the following:
(a). Rapid growth of the foetus (Hytten and Leitch)
(b). Enlargement of uterus, mammary glands and placenta
(c). Increase in maternal circulating blood volume
(d). Formation of amniotic fluid
(e). Storage reserve for delivery and lactation
(f). Transfer of amino acids from the mother to foetus.

Apart from supplying the essential nutrients, it also helps to overcome some common problems of the pregnant ladies-
(a). Helps relieve heartburn,
(b). Lack of apatite,
(c). Constipation,
(d). Gastric ulcers,
(e). Anemia.

In the next stage lactating women require a high protein, high calorie balanced diet (Aebi and Whitehead, 1980). The newborn depends entirely on milk for first few months of his life and the quality and quantity of milk is dependent on mother's food intake. Milk can help the lactating women to a great extent in getting proper nutrition for herself and her infant. In infancy the infant doubles its weight in six month and his weight becomes three times by the time he is one year old. The requirement for high protein and various other nutrients is easily met by milk.
During adulthood nutrients are required for the purposes of energy, for replacement of worn out tissues and maintenance of body functions. To meet these requirements an adult needs to take at least 250 mls of milk every day. In old age, milk is not only required for its nutrients but is also taken because of its buffering action and is best suited to many physiological changes. This includes alterations in taste and smell sensations, decrease in saliva secretion, lowered gastrointestinal functioning, loss of teeth and decreased metabolic rate. It is also important to improve the cognitive abilities of the elderly (Carlson, 1972) supplying them with important essential fatty acids. Milk acquires an important and irreplaceable place in the balanced diet and is also equally important in diseased conditions.

The nutrient configuration, bland flavour and taste and neutral action of milk makes it therapeutically very important. In gastrointestinal diseases (Arvanitakis, 1979) milk products like curd, butter milk and butter are highly preferred. Milk itself is a bland food and it does not irritate the intestinal mucosa. Milk and milk products are indispensable especially in a vegetarian diet and they can be given to the patients of peptic ulcer, gastritis, colitis and diarrhoea. In post-surgical and burn conditions (Bistrian et al., 1974) where patients require a high protein and high calorie diet for formation of new tissues and replacement of various body fluids milk is very important. In various hepatic diseases like jaundice and infective hepatitis, milk is given in skimmed form to provide the patient with the required high protein, high carbohydrate and moderate fat (Gabuzda and Shear, 1970) diet. Patients of Coronary Heart Disease are advised low calorie, low saturated fat, low carbohydrate and normal protein, mineral and vitamin diet. Skimmed milk and its products can help in providing these required nutrients (Wenger, 1979). In some kidney diseases like Glomarulonephritis and nephrotic syndrome, proteins are easily passed in urine and there is low serum albumin. High protein and normal balanced diet is advised in these conditions (Burton, 1974). In diabetes mellitus (Nutall and Brunzell, 1979), a balance diet high in protein (20% of the daily calorie intake) is good for health because it supplies the essential amino acids needed for tissue repair. In patients of NIDDM consumption of protein along with carbohydrate lowers the blood glucose concentrations due to amino acid stimulation of insulin secretion. Milk has a low glyceamic index and is therefore important. In Cancer patients (Aker, 1979), milk is given as an important part of diet to meet the increased metabolic demand of the diseased, to prevent catabolism as much as possible and to prevent excessive weight loss. The primary cause of protein-calorie malnutrition in children between ages 1 to 6 years is inadequate and faulty diet. Malnutrition weakens the child immunity and regularly occurring infections make him more malnourished. In disease of Kwashiorkar and Marasmus milk is important to provide good quality protein and calories (Neumann, 1977).

Physiological functions and nutritional implications of milk oligosaccharides

Free oligosaccharides are natural constituents of all placental mammals' milk and also found in bacteria, fungi, plants etc. The composition of milk progressively changes post parturition to meet the changing and specific requirements of the suckling neonate. During the first few days post parturition, the "early milk" (colostrums) has a composition quite different from that of "mature milk". Colostrums contain many other biologically active constituents; these include growth factors, antimicrobial compounds and immune enhancing components. The role of milk in these first few days in the life of a newborn is not only to provide nutrition but also to provide protection against infection while the immune system is still developing (Kunz and Rudloff, 1993, Renner Band Sawatzi, 1993, Newburg, 2000, Sumiyoshi et al., 2003, Tadasu Urashima et al., 2003). Variation of oligosaccharide structure in Human Milk indicates that oligosaccharides are involved in many functional effects related to the gastrointestinal tracts as well as to systematic processes (Nakamura et al., 2003). There is considerable evidence that oligosaccharide affect intestinal flora and bowel habit (Giovanni et al., 1990). The oligosaccharide might affect mineral absorption (Giovanni et al., 1990) and lipid metabolism and end-products of bacterial metabolism can play a role in colon-cancer prevention. Further prebiotic oligosaccharide directly or via, modulating the intestinal flora can influence the immune system (Boehm et al., 2005). Additionally dietary oligosaccharide influence on the brain development. Oligosaccharide (Boehm and Stahl, 2003) and glycoconjugates in milk have a direct inhibitory effect on certain virulence related abilities of pathogenic microorganisms (Newburg et al., 2005). Human milk oligosaccharide inhibits monocyte, lymphocyte and neutrophill adhesion to endothelia cells and acts as anti-inflammatory agents (Lars Bode et al., 2004). Human milk derived oligosaccharides stimulates cytokine production of cord blood T-cells and exert anti-inflammatory properties and also increase immunomodulatory effect (Thomas et al., 2004).

Recently Human milk oligosaccharide has shown that some oligosaccharide on cell surfaces such as sialyl Lewisx play important role in cell-cell interaction (Poirier and Kimber 1997). Artificial mimics of these oligosaccharides are potentially useful for the treatment of inflammation, cancer metastasis, autostasis and autoimmune diseases (Kevin et al., 1998, Reiji Kannagi, 1997). So now Human milk oligosaccharides are used for· studying the biosynthesis of antigen I, antigens i. Oligosaccharide present in individual sample of milk can vary with ABO or Lewis blood type of donor as the enzymes involved in their synthesis are also responsible for the formation of structural determinants of these blood types. Immunological study showed that blood group' determinants are expressed in sugar chains and the expression is stronger in mucin type sugar chains than in the asparagines linked sugar chains (Koscielak 2001, Jacques Le Pendu 2004, Thurl et al., 1997). Application of a finger printing method to the analysis of milk oligosaccharide led to elucidate the basis of blood types in human (Danielle et al., 2003, Johanna Hofmann et al., 2017, Erney et al., 2001).

Milk oligosaccharides as non-specific defense mechanism

Milk oligosaccharides and glycans are made by the same type of glycosyl transferases that are responsible for the synthesis of human cell surface glycans, so they have common structural moieties (Rudloff et al., 2002). Most enteric pathogens (Moran and Gupta, L. Joshi (2011) use cell surface glycans to identify and bind to their target cell as the critical first step in pathogenesis therefore soluble glycans from milk act as competitive inhibitors against important pathogens at the intestinal surface and thereby protect the breastfed infant from diarrhea. Since human milk contains more than 80 oligosaccharides and these milk oligosaccharides are not digested in the small intestine, so a large range of oligosaccharides are available to inhibit pathogen adhesion to the intestinal epithelium. Human milk oligosaccharides inhibit adhesion of Pneumococci and influenza virus to, pharyngeal or bucca epithelial cells and also inhibit binding of streptococcus pneumonia and Haemophilus influenza virus. Similarly, sialylated oligosaccharides inhibit binding of pathogenic strains of E. coli. In neonates' neutral oligosaccharide from human milk protect the intestinal tract of neonates from Vibrio cholera and fucosylated oligosaccharides inhibit the interaction of an enterotoxin of several virus with cells of intestine. Sialylated oligosaccharides also inhibit adhesion of ulcer causing human pathogen Helicobacter pylori to epithelial cells (Ora Burger et al., 2000, Siiri Hirmo et al., 1996, Martin et al., 2002, Sudarmo et al., 2003, Newburg et al., 2004, Uauy and Araya 2004, Martin et al., 2002, Autar et al., 2003). Campylobacter is a bacterium causing bacteria induced diarrhea, the main intestinal ligand for this bacterium is the HH2 histo blood group antigens and milk fucosyl oligosaccharide containing α-I-2-linked fucose (Morrow et al., 2004, Guillermo et al., 2003). Thus α-1, 2-linked fucosylated glycoconjugates in milk strongly protect against campylobacter infection (Cervantes et al., 1995). Noroviruses (Huang et al., 2003) are a major cause of diarrhea in humans and especially in infants. Most milk oligosaccharides contain glycans that specifically block Noroviruses binding to histo blood group antigen receptors which provide protection to infants from noroviruses infection (Huang et al., 2003). Human milk oligosaccharides also inhibit adhesion of Neisseria meningitides, a human specific pathogen causing meningitis and septicemia (Hakkarainen et al., 2005). Thus naturally occurring human milk oligosaccharides are the major components of an innate immune system of human milk that plays a significant role in the ability of the mother to confer protection to her infant against disease (Johansson et al., 2005). Human milk oligosaccharide have similar function in non- human species as- the Gal α (1-3) Gal β (1-4) GlcNAC sequence in rabbit glycolipid acts as receptor for the binding of Clostridium difficile toxin A and a trisaccharide Gal α (1-3) Gal β (1-4) Glc found in Bovine, ovine caprine colostrums, bear and elephant milk are inhibitor of this toxin in intestinal mucosa. Glycolipid containing N-glycosyl neuraminic acid in the form of trisaccharide sequence, Neu 5-Glc α-(2-3) Gal β (1-4) Glc, acts as receptor for binding of *E. coli* which cause life threatening diarrhea in piglet (Martin-Sosa et al., 2002).

Milk oligosaccharides as prebiotic

The human intestine lacks enzymes able to hydrolyze β-glycosidic linkage with exception of lactose. Thus human milk oligosaccharide and most animal milk oligosaccharide as well as non-milk oligosaccharides are considered to be indigestible (Mark et al., 2000, Buddington et al., 2002). These dietary non-digestible human milk oligosaccharides reach the colon and are utilized by health promoting colonic bacteria and are known as prebiotic. So prebiotic is non-digestible food ingredients that beneficially affecting the host by selectively affect the growth and activity of bacteria in colon that can improve the host health. Milk oligosaccharides are neither digested nor absorbed in the upper intestinal tract of humans but are delivered intact into the colon where they act as nutrients for colonic microflora. The neutral oligosaccharides present in human milk act as growth enhancers for bacteria of the genus *bifid bacterium* in infants. So the population of bifid bacterium increased in breast fed infants (Newburg, David S. (2000, Cherie et al., 1998, Agostoni et al., 2004, Amy Sie-Yik Lau et al., Euler et al., 2005, Fanaro et al., 2005, Ben et al., 2004, Boehm et al., 2002, Janette et al., 1996). This increased metabolic activity of larger population of bifidobacteria in the lumen and will decrease the intestinal pH and this in turn inhibit proliferation of pathogenic Gram-negative bacteria such as-*Shigella flexneri* and *E. coli*. Milk oligosaccharides also act as growth promoting factor for *Lactobacillus bifidus*, the predominant intestinal flora of breast fed infants (Martin et al., 1998). So the incidence of infection in breast-fed infants is reduced in comparison to bottle-fed infants.

Immunological Activities Associated with Milk

Milk is a complex biological fluid which has been viewed primarily as a food that provides energy and essential nutrients for optimal health, growth and development of the young mammal. However it is now realized that milk also contains a variety of bioactive components, usually occurring in small quantities, that includes a mix of cells, bioactive proteins, hormones, growth factors, and other immunological factors. Levels of immunoactive compounds parallel the development of the immune system of the newborn animal, which is functionally immature at birth and undergoes extensive differentiation and recognition during the early postnatal period (Xanthou, 1993). For example, levels of complement components, neutrophil activity macrophage activation by interferon-γ, production of secretary Ig-A (sIgA), numbers of T cells displaying the CD45RO effector/memory phenotype and production of IgG antibody to T cell-dependent immunogens all are low during early infancy (Chheda et al., 1996, Goldman et al., 1998). In addition, relative to adults, newborn infants produce lower quantities of cytokines, e.g., granulocyte/macrophage colony-stimulating factor, interferon-γ, interleukin(IL)-3, IL-4, IL-6 and tumor necrosis factor (TNF)- α. Consequently, the capacity of infants to respond to an infectious challenge is low, and provision of immune and immunomodulating factors by way of milk may be critical to the health and survival of the newborn offspring (Bill Woodward). This provides functional evidence that infants receiving diets rich in immunomodulating compounds are better suited to protect themselves from infectious agents. An additional consideration is the role that milk-associated immunological factors play in the protection of the mammary gland and ultimately in the mother-infant dyad, itself. Without a protective "food source", i.e., the mother and her milk supply, the young mammal would certainly perish. This multifunctional role of milk-borne immunological factors certainly play a role in the evolution of mammals. Therefore, not only are immunologically active milk components essential for the protection of the suckled newborn, but they are also critical for the protection of the mammary gland and, therefore, for the survival of the species. Breast-feeding protects human infants against respiratory tract infections, otitis media, botulism, urinary tract infections (Marild et al., 1990, Pisacana et al., 1992) and necrotizing enterocolitis (Saarinen, 1982, Amon, 1984, Wright et al., 1989, Lucas and Cole, 1990, Duncan et al., 1993).

Moreover, while environmental exposure of newborns to bacteria, viruses and other microorganisms is an important concern, it has recently become evident that there is also risk of transmission of human immunodeficiency virus (HIV, cytomegalovirus (CMV) and other viruses through the milk of infected mothers. Hence, the immunologically active components in milk are involved in protecting the young animal against both external pathogens and pathogens of maternal origin.

Milk oligosaccharides as Secretary Antibodies

Human milk contains significant amount of secretory antibodies. When significant amounts of secretory antibodies (sIgA) were found in human milk in 1961, they were assumed to be the major agents whereby milk protected nursing infants. The very high concentrations of sIgA in colostrums seemed consistent with human milk being a medium through which the maternal adaptive immune system could transmit mucosal protection to the infant gut, augmenting the gift of maternal antibody through prenatal transfer of serum antibodies across the human placenta. This seemed an effective mechanism to protect infants from pathogens to which the mother had prior exposure (Natal'ya et al., 2006, Malandain, 2005, Johnson et al., 2003, Debre et al., 1930).

Immunomodulatory effects of milk oligosaccharide

Human Milk oligosaccharide structures like lacto-N-fucopentaose 111 (LNFPIII) and lacto-N-neotetraose (LNneoT) showed an effect on murine IL-IO production. The human milk derived acidic oligosaccharide fraction 198 is also found to enhance the production of certain cytokines after long-term exposure (20 d) in vitro in the CD4+ as well as in the CD8 + T-cell subfraction (Thomas Eiwegger et al., 2004).

Anti-inflammatory effects of milk oligosaccharide

Human milk oligosaccharide show anti-inflammatory property. Excessive leukocyte infiltration causes severe tissue damage in a variety of inflammatory diseases. The initial step in leukocyte extravasations is mediated by selectins and oligosaccharide on their glycoconjugates ligands (Milo et al., 2004, Gnoth et al., 2000,). The human milk oligosaccharides contain binding determinants for the selectins (David R. Bundle, 1989). They are also able to affect leukocyte rolling and adhesion to endothelial cells under dynamic conditions. 3’-sialyl- lactose and 3’-sialyll3-fucosyl-lactose present in human milk serves as anti-inflammatory components by inhibition of monocyte, lymphocyte and neutrophill adhesion to endothelial cells. Lacto-N- Fucopentaose III (LNFPIII) is a Human milk sugar containing the biologically active Lewis- X (LeX) trisaccharide (Kunz and Rudloff, 2008, Wieruszeski et al., 1985). LNFPIII LeX is also expressed by immunosuppressive helminthes parasites, by bacteria and on a number of tumor / cancer cells. LNFPIII activates macrophages in vitro' as indicated by up regulation of Gr-l expression on F 4\80 (+) cells and F 4/80 (+) cells is able to activate natural killer cells, inducing up regulation of CD69 expression and gamma interferon production. LNFPIIII stimulated macrophages secrete prostaglandin Einterleukin-10 (IL-10) and tumor necrosis factor alpha. An oligosaccharide fraction isolated from goat milk reduces intestinal inflammation in a rat model of dextran sodium sulfate- induced colitis and contribute to the recovery of damaged colonic mucosa (Lara-Villoslada et al., 2006).

Milk oligosaccharides as Tumor Marker

Monoclonal antibodies of several tumor cell lines or carbohydrate antigens have provided evidence that membrane glycoprotein or glycolipid which may function as differentiation antigens or tumor- associate antigens occur as free oligosaccharide in milk. The sialyl- Le structure in glycolipid or glycoprotein has been defined as gastrointestinal tumor associated antigen. Two newly isolated oligosaccharides B-1 and B-2 have both the sialyl Le^a and Le^x or Le-1 structure respectively. These structures have been found in mucin type glycoprotein and glycolipid. In a variety of human cancer.

Oligosaccharides having the Sialyl Le^a and blood group H structure or with both sialyl Le and difucosyl Le-Le structure also occur in milk and Le^a-Le^x structure exhibits high affinity to an antibody directed to a human squamous lung carcinoma (Kitagawa et al., 1989, Martensson et al., 1988, Yamashita et al., 1982, Hiroshi Kitagawa et al., 1993).

Milk Oligosaccharides and Brain development

In addition to the biological significance of the unique oligosaccharide composition of human milk, their possible functions are in neonatal host defense and inflammatory events. Oligosaccharides, along with lactose, may play a role in postnatal brain development. Many newborn mammals undergo a period of rapid postnatal brain development that requires large amounts of glycolipid, which are components of the cell membranes of neurons and myelin.

Galactocerebroside, with Gal as its polar head group, is the predominant glycolipid in myelin. The liver may not be capable of providing the entire Gal needed by the young mammal during this period of myelination and brain development. Thus, a possible role of milk oligosaccharides in which Gal is a main component is ensuring that Gal levels in the infant is not become limiting during this time. A prerequisite for this mechanism is that oligosaccharides are not completely excreted via faeces, but are to some extent absorbed in the digestive tract. The conclusion that can be drawn from these studies is that some oligosaccharides were absorbed without being digested. Therefore, one cannot exclude the possibility that beside their local action, oligosaccharides may have systemic effects as well, e.g., on brain glycoconjugate composition. This suggestion was confirmed by comparing the effects of intraperitoneal and intragastric applications of NeuAc on rat brain composition. It was found that both the oral and intraperitoneal routes resulted in significantly more cerebral and cerebellar glycolipid and glycoprotein NeuAc than did glucose injection. Furthermore, the advantage of oral dose of NeuAc-lactose, the major acidic fraction in human milk, over application of free NeuAc for brain composition in rats has been shown by Witt et al. A further indication that dietary carbohydrates may be important for normal brain composition is the observation that in patients with classic galactosemia, exogenous Gal may be important for the maintenance of a correct ratio' of UDP-Glc to UDP-Gal in some cells. The impairment of UDP-Gal concentration in affected subjects could be, in parts, responsible for the altered biosynthesis of brain glycolipids in these subjects. Because the oligosaccharide pattern in the milk of elephants is even more complex than that of human milk, it is fascinating that the two species show similar patterns of postnatal ontogeny; they grow slowly, have relatively large and highly developed central nervous systems developing mainly after birth, are highly intelligent, and exhibit a high degree of learned behavior. The degree of encephalization is considerably higher in humans and elephants than in nonhuman primates (e.g. rhesus monkeys), reflecting the differences in milk oligosaccharide concentrations. Thus, it is speculate that lactose-derived oligosaccharides and in particular their Gal moieties, may play a role in the development of the infant brain. Sialic acid present in human milk also contribute to the increased concentration of NeuAc, present in cerebral and cerebral glycoconjugates of breast fed and thus playa important role in development of the infant brain(Tom Gardiner, 2000 Wang and Brand-Miller, 2003, Nakano et al., 2001, Nakamura et al., 2003).

Effect of milk oligosaccharides on mineral absorption

Human milk oligosaccharides contribute to the high efficiency of Ca absorption of breast fed infants. In adults dietary oligosaccharides also improve Ca absorption. Fructooligosaccharides are also able to stimulate Ca absorption. There are also some reports that Magnesium, Iodine and iron metabolism can be improved by dietary oligosaccharide (Sabater-Molina et al., 2009, Ellen et al., 2009).

Possible Application of Milk Oligosaccharides for Drug Development

By applying a finger-printing method to the analysis of human milk oligosaccharides, several oligosaccharides were found to be deleted in the milk of non-secretor or Lewis negative individual (Kobata, 2003). 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 end. 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 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. 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 and milk oligosaccharides as future anti-adhesion drugs for bacterial diseases (Kunz et al., 2000, Kobata, 2010, Thurl et al., 1997, Sharon, Ofek, 2000, Kobata, 2003).

FACTORS RESPONSIBLE FOR BIOLOGICAL ACTIVITY OF OLIGOSACCHARIDES

- Oligosaccharide bear structure homology to cell surface glycoconjugates used as receptors by pathogens, thus protecting nursing infants. Human milk oligosaccharide containing α 1,2- linked fucose inhibits the stable toxin-producing Escherichia coli in vitro (Newburg et al., 2004, David S. Newburg, 1999, David et al., 2004) and its toxin induced secretory diarrhea in vitro and in vivo. Glycoconjugate found in human milk also inhibit binding by Campylobacter jejuni in vitro and in vivo and also inhibit binding by calciviruses in vitro. Thus specific fucosyl oligosaccharides of human milk have been observed to inhibit specific pathogens. Some important enteric pathogens, for example- rotavirus, are inhibited by human milk oligosaccharide or other glycoconjugates that are not fucosylated. Thus the association described here addresses only one possible set of enteric pathogens that may be inhibited by one family of milk oligosaccharide; other oligosaccharides that inhibit other pathogens are probable. Finally it can be concluded that the family of α l,2-linked fucosylated oligosaccharide, probably in conjugation with other families of oligosaccharide, constitute a powerful innate immune system of milk (Mehra, Kelly, 2006, Trine et al., 2005).
- Infection by rotavirus is responsible for much of the diarrhea in infants around the world. The ability of rotavirus to infect MA- 104 cells in culture is inhibited by human milk, and this inhibition is due to a mucin-associated 46 kDa milk glycoprotein named lactadherin. Furthermore, after sialic acid is removed from lactadherin, its ability to inhibit rotavirus is essentially lost, which suggests that the glycan portion of the molecule is responsible for inhibition and that specific terminal sialic acid is required for inhibition. Lactadherin from human milk also inhibits rotavirus (EDIM strain) gastroenteritis in mice (Jos et al., 2005).

- Due to presence of sialic acid in milk, they serve as anti-inflammatory components and reduce platelet-neutrophill complex formation leading to a decrease in neutrophill B2 integrin expression. While neutral human milk oligosaccharide fraction had no effect. Sialylated human milk oligosaccharide also inhibit binding of pathogenic strains of Escherichia coli and ulcer-causing human pathogen H. pylori on the other hand neutral human milk oligosaccharide may protect the intestinal tract of neonates from Vibrio cholera (Mehra R. Kelly, 2006, Trine et al., 2005, Jos et al., 2005, Trine et al., 2005).
- Prebiotic is non-digestible food ingredients that beneficially affect the host by selectively affecting the growth and activity of bacteria in colon that can improve the host health. Milk oligosaccharides are non-digested due to the presence of β-glycosidic linkage. So this β-glycosidic linkage plays an important role for its prebiotic activity (Ayyappan Appukuttan Aachary and Siddalingaiya Gurudutt Prapulla, 2010, Euler, 2005).
- N-and O-linked oligosaccharide causes the release of histamine and other mediators of the allergenic, response which then lead to the development of allergeic symptoms (Fotisch and Vieths, 2001).
- Oligosaccharide mimics containing galactose and fucose specifically label tumour cell surfaces and- inhibit cell adhesion to fibronectin (Evelyn et al., 2005).
- Supplementation of milk formula with galacto-oligosaccharides improves intestinal micro flora and fermentation in term infants (Boehm, 2002).
- Galactose and sialic acid present in milk oligosaccharide are required for optimal development of the infant's brain (Wang, 2009).

Other contents of Milk having Biological Activities

Antimicrobial Protein and Peptides

Lactoferrin, an iron-binding protein present in human milk at remarkably high concentrations (1-2 g/L or 10-20% of total protein content) and in bovine milk at lower concentrations, has been reported to exert a bacteriostatic effect against *E. coli* (Bullen et al., 1972), and to be bactericidal against several pathogens to which infants may be exposed, e.g., *Vibro cholerae, Salmonella* and *Staphylococcus* (Arnold and Brewer, 1980. Recently, a peptide called lactoferrin has been isolated from human and bovine lactoferrins. These peptides contained 47 and 27 amino acids, respectively, and have strong bactericidal effects in *vitro* and in animal models (Teraguchi et al., 1995). Lactoferrin is located at the N-terminal end of lactoferrin and does not contain an iron-binding region (Yamauchi et al., 1993). Lysozyme is present in breast milk in unusually high concentrations (0.1-0.3 g/L) and is found in bovine milk at lower levels. This enzyme can degrade the cell walls of Gram-position bacteria and has strong antimicrobial activity in *vitro* (Ellison and Giehi, 1991). Ellison and Giehl recently have demonstrated that lactoferrin can bind to, and remove, lipopolysaccharide (LPS) from the outer membrane of Gram-negative bacteria, thereby allowing lysozyme access to the underlying proteoglycan matrix to cause lysis. Whether this potent synergism occurs *in vivo* remains to be explored.

Many other proteins and peptides in milk have been reported to exert antimicrobial and/or immunostimulatory actions, although it should be recognized that these activities have been demonstrated only *in vitro,* or in animal models.

Nucleotides

Nucleotides are present in human milk in significant concentrations but, until recently, these compounds have not been added to infant formulas (Janas and Picciano, 1982). In animal models, nucleotides have been reported to prevent malnutrition-and starvation-induced immunosuppression

(Janas and Picciano, 1982, Kulkarni et al., 1987), to aid resistance to *Staphylococcus aureus* and *Candida albicans* (Kulkarni et al., 1986, Fanslow et al., 1988), to enhance T-cell maturation and function (Van Buren et al., 1985) and to stimulate gastrointestinal growth and maturation (Uauy et al., 1990). Recently, Tanaka (Tanaka et al., 1996) have shown that individual nucleotides, particularly AMP, can induce apoptosis in human fetal intestine in culture. These authors suggest that AMP, and possibly other nucleotides, may reinforce intestinal mucosal integrity by increasing cellular proliferation and differentiation via enhanced apoptosis.

Immunoglobulins, Anti-Idiotypic Antibodies and Hyperimmunization

Milk contains high concentrations of antibodies, IgG being the predominant class in cow's milk and sIgA the predominant class in human milk (Goldman et al., 1982). The sIgA antibodies in milk are known to recognize a wide variety of microorganism that are found in the respiratory tract and intestine, e.g., bacterial pathogens such as *E. coli, V. cholerae, H. influenzae, S. pneumoniae, C. difficile* and *Salmonella*, viruses including rotavirus, CMV, HIV, influenza virus and RSV, and yeasts such as *Candida* albicans (Goldman, 1993).

Immunomodulating Agents including Anti-Inflammatory Components: Cytokine and Non-Cytokine Factors

Milk contains a wide variety of cytokines, some of which are found in concentrations that suggest physiological activity. The discovery of cytokines in milk opened new possibilities for effects of breast milk components on diverse cellular targets. Cytokines are polypeptides that act in an autocrine, paracrine or endocrine fashion by binding to specific cellular receptors, thereby affecting cell protein synthesis and/or other cellular activities. Human milk was early found to be anti-inflammatory as it protected against infection in the absence of clinical evidence of inflammation. One possible explanation for this is that human milk contains soluble cytokine/chemokine receptors and receptor antagonists. For example, breast milk at all stages of lactation contain soluble TNF-α receptors I and II, as well as IL-I receptor antagonist (Buescher and Malinowska, 1996).

The Role of Milk Secretory IgA in Protecting Infants from Bacterial Enteritis

In most of the world diarrhea is the leading cause of death in infancy. The risk of bacterial enteritis includes acute life-threatening fluid losses, renal failure, and impaired growth. Exposure to bacterial enteropathogens varies geographically. In areas where sanitation is poor variety of pathogens occurs at high frequency. In more developed countries a limited range of pathogens is found with only organisms easily spread direct contact or through contaminated food being common. Milk is generally believed to provide the suckling infant with protection from bacterial enteritis (Feachem, R.G. and Koblinksy, M.A., (1984, Victora et al., 1989, Hanson et al., 1991). The basis for this defense mechanism is complex. Breast-feeding serves as an uncontaminated source of food so that there is decreased exposure to enteropathogens. It contains pathogen-specific Secretory IgA (sIgA) and non-specific protective factors including cell receptor analogs (glycolipids, glycoproteins, oligosaccharides), and anti-infectives such as lactoferrin and lysozyme (Pickering and Kohl, 1986). Breast-feeding lowers stool pH so that acid-intolerant enteropathogens fail to thrive in the intestinal milieu. The best studied of the milk factors is sIgA.

Vibrio cholera

Cholera is a clinical syndrome caused by intestinal infection with specific serotypes of Vibrio cholerae in which severe, often life-threatening, water and electrolyte losses occur in stool. In much of the world cholera is a major cause a childhood death. The organism has the ability to cause watery diarrhea because it adheres to intestinal epithelial cells and delivers a toxin, cholera toxin that causes ADP ribosylation of an adenylate cyclase regulatory protein (Sears and Kaper, 1996).

Milk has been shown to contain sIgA to choleratoxin (Simhon et al., 1979) and to *V. cholerae* antigen (Carlsson et al., 1976). The O antigen also called somatic antigen or lipopolysaccharide (LPS) has repeating carbohydrate units linked that from part of the outer membrane lipopolysaccharide of Gram negative bacteria.

Shigella spp

Shigella spp. infections are among the most serious that occur in infants and toddlers. These infections are characterized by high fever, watery or bloody diarrhea, and toxicity. The clinical features reflect the fact that the fundamental virulence trait of these organisms is their ability to invade the intestinal epithelium. There is evidence that breast-feeding may decrease the risk and severity of illness due to *Shigella.* Clemens related breast-feeding to illness severity and showed that breast-feeding was protective (Clemens et al., 1990).

Escherichia coli

Breast-feeding has been correlated with protective against diarrhea caused by ETEC. A community-based prospective study by Long et. al. 1994 in urban Mexican children revealed that strictly formula-fed infants colonized by ETEC-LT were symptomatic with diarrhea nearly three times as often as strictly breast-fed infants and twice as often as infants receiving mixed diet.

Salmonella and Non-Typhoidal Salmonella

Infections with the various *Salmonella spp.* is associated with a variety of intestinal and extraintestinal manifestations including typhoid fever, gastroenteritis, sepsis, and focal infections. Several studies have suggested that breast-feeding protects infants from *Salmonella* infections. Retrospectively, France et. al. 1980 found that of 253 infants less than 12 months of age who were infected with *Salmonella* (based on microbiology records of three institutions) only one was breast-fed at the time of study and only twelve had ever been breast-fed (France et al., 1980).

Campylobacter jejuni* and *C. coli

Campylobacter spp. are important intestinal pathogens particularly in the first four years of life. They cause both watery diarrhea and bloody diarrhea. IN many places they are among the most common causes of bacterial diarrhea. Some strains are invasive and others produce a heat labile toxin related to cholera toxin. Breast feeding can be protective against these pathogens.

Antimicrobial and antibacterial Actions of Lactoferrin

Human milk provides infants with nutrients, harmones, enzymes and growth factors. These substances contribute to the child's health and offer protection against infectious diseases. Passively transferred components such as IgA antibodies, lysozyme and lactoferrin exert protective role against a wide variety of microorganisms (Lonnerdal and Atkinson, 1995). Lactoferrin (Lf) is an iron-binding glycoprotein, formerly called red milk protein, first isolated from bovine and human milk (Groves, 1960, Johansson, 1960). The protein is found in many other secretions such as tears, saliva, pancreatic juice and secondary granules of neutrophils (Mason et al., 1966). Lactoferrin is present in the milk of most species. Human milk is particularly rich in Lf, which represents about 20 % of total milk protein. A large number of studies has indicated that Lf plays an important role in host defense. In particular, it inhibits antimicrobial activities (including bacteriostatic and in some cases, bactericidal effect) against a wide range of microorganisms (Sanchez et al., 1992, Brock, 1997).

Antimicrobial Functions of Milk Lipids

Milk is not only a source of nutrients for the newborn but also a source of immunoglobulins and non-immunoglobulins secretary products similar to those of other parts of the secretary immune system, which play an important role in protecting mucosal surfaces from infection. Infants who are breast-fed have been found to have a lowered incidence of gastrointestinal infections then infants fed formula or cow's milk (Larsen Jr and Homer, 1978, Cunningham, 1979, Myers et al., 1984).

The incidence of any infection in very low birth weight infants is significantly lower in infants fed human milk than in infants fed formula (Hylander et al., 1988).

Antimicrobial and Immunomodulating Actions of Milk Leukocytes

Leukocytes, the main cellular component of mammary secretions, include mononuclear phagocytes (macrophages), polymorphonuclear neutrophils (PMN) and lymphocytes. These cell types normally make up more than 90 % of the somatic cells in colostrums and milk. The remaining fraction may contain a varying number of epithelial cells. Leukocytes and epithelial cells in milk are termed body, or somatic, cells to differentiate them from contaminant microbial cells. Milk somatic cells counts (MSCC) range from several hundred to several million cells per ml, depending on the species, the stage of lactation and, above all, the level of inflammation.

MILK OLIGOSACCHARIDES AND THEIR BIOLOGICAL ACTIVITIES

In recent years milk has emerged as prodigious source of new and structurally complex carbohydrates which are promising therapeutic agents evaluating against various disease like AIDS and tumour. Milk of various origins is prescribed for different ailment in old and traditional system of medicine like ayurveda and Unani system of medicine. A number of oligosaccharides having biological importance have been isolated from milk of different origin like human, goat Buffalo, Donkey etc that have shown antitumour, anticancer, antigenic and immunostimulant activities. There are numerous other beneficial biological effects of glycoconjugates found in milk e.g. lactoferrin, the major iron-binding protein in human milk, appears to function in the process of iron absorption in infants through interaction with a small intestine receptor, fucosylated glycans on the carbohydrate chain of lactoferrin are necessary for receptor recognition. A histidine rich glycoprotein has been identified in human milk that binds copper and zinc with high capacity (Hutchens, 1992). The glycoprotein in human and pig milk containing fucose galactose, mannose, galactosamine, glucosamine and sialic acid that bind vitamin B12, and ensures its bioavailablity. HMFG membrane glycoproteins in breast milk express blood group related determinants primarily on mucin like epithelial membrane antigens. Breast milk and its glycoconjugates are also important for normal development and function of the biliary system, e.g. human IgA and breast milk stimulate bile duct growth in an animal model, suggesting a possible role in the developing neonate. Milk is a rich source of glycoconjugates such as oligosaccharides and many have been extensively characterized. The oligosaccharides typically contain residues of most of the necessary glycoconjugate sugars, including fucose, sialic acid (N-acetylneuraminic acid. NANA) galactose, manose, N-acetyglucosamine and N-acetylgalactosamine. Human milk oligosaccharides bind to a wide range of lectins on the surface of epithelial cells lining the mouth, oesophagus and stomach and throughout the gastrointestinal system in the new borne baby. This is turn prevents opportunistic infection while the baby’s immune system is developing. Oligosaccharides lectin binding has also been used to target therapeutic agents to diseased cells which express high densities of specific lectin on their surface e.g. GalNAc clusters have been used to target antisensenucleotides to hepatocytes to potentially allows treatment of hepatitis A. A trivalent clusters have been utilized in approach because they are able to able to bind to the required lectins as strongly as galactose terminated multiantenary oligosaccharides isolated from milk has also been used as lead compound for various anti-infective and antiadhesive drugs e.g. bacterial infection caused by the MS fimbrial such as *E.coli* 015H7 and certain salmonella strains oligosaccharide receptors in vivo that contain mannose units. Hence anti-infective oligosaccharides containing mannose could potentially inhibit the infective process. A large number of heterogeneous N containing oligosaccharides of various sizes have been isolated and characterized from milk. All the milk oligosaccharides terminate with lactose or N-actety1 lactosamine at the reducing end and can be classified as acidic or neutral based upon the presence or absence of NANA.

Many milk oligosaccharides contain the basic lacto N tetraose sequence (Gal-GlcNac-Gal-Glc) or one of its derivatives, which has been shown to be a particularly potent bifidus factor. In addition to this NANA may play an important development role in early infancy. Studies in rats have demonstrated that significant amount of brain ganglioside synthesis occurs during the suckling period and that the absolute amount of NANA in brsin gangliosides can be modified by delivery availability of NANA.

Human Milk N-Acetyl Neuraminic acid content at different lactational intervals

Lactational Stage (weeks)	NANA (mg/dl)	NANA-nitrogen (mg/dl)	%NPN
0-2	113+8.6	5.16	10.9
2-4	70+7.9	3.20	7.3
4-6	34.4+6.3	1.58	4.0
6-8	25.8+3.4	1.17	3.0
10-28	13.5+1.6	0.61	1.5

Supply of NANA from human milk, which can be hydrolyzed by neuraminidase in the intestinal epithelium, may contribute significantly to glycoprotein and glycolipid synthesis in young mammals especially if NANA synthesis is not yet fully developed. Brain ganglioside development in humans occurs predominantly in third trimester and during the initial postnatal period suggesting that the nutritional availability of high levels of NANA, as found in early breast milk may impart a developmental advantage of infants in the first few days of life. Nitrogen present in GlcNAc may serve as a precursor for in vivo synthesis of nonessential amino acids and hence play an important nutritional role in protein synthesis, particularly for the infant for whom total dietary nitrogen supply for Human Milk may be limited. Alternatively, GlcNAc and GalNAc may be taken up at the mucosa and directly incorporated into intestinal glycoprotein such as the mucoproteins.

ACKNOWLEDGEMENTS

Authors are thankful to the Chemistry Department, Lucknow University for providing lab facilities.

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