Milk, Mucosal Immunity and the Microbiome: Impact on the Neonate

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Foreword:
Milk, Mucosal Immunity and the Microbiome: Impact on the Neonate

There have been considerable advances in our understanding of the diverse mixture of bioactive components in human milk that influence the immune status of infants by not only facilitating development, but also by providing protection, modulating tolerance, and regulating an appropriate inflammatory response.

Human milk is the communication vehicle between the maternal immune system and the infant, actively directing and educating the immune, metabolic, and microflora systems within the infant. Data from animal models and in vitro systems have made a significant contribution towards our knowledge of the physiological and protective functions of the different components of human milk.

The 94th Nestlé Nutrition Institute (NNI) Workshop on "Milk, Mucosal Immunity and the Microbiome: Impact on the Neonate" took place in Lausanne between 23-25th September 2019. The Workshop was designed with the goal of providing a comprehensive overview on the latest human milk research and our understanding of its potential for modulating mucosal immunity, the microbiome and its impact on the neonate. The program focused on current knowledge of how both the “classical” immune and non-immune ingredients found in human milk support maturation of the immune system, including the development of tolerance and regulation of the inflammatory response in infants.

The Workshop program was brought together by the world’s experts in human milk research and nutrition: Pearay Ogra (Professor Emeritus, Jacobs School of Medicine and Biomedical Science, University of New York at Buffalo), Allan Walker (Conrad Taft Professor of Nutrition [Emeritus], Professor of Pediatrics, Harvard Medical School), and Bo Lönnerdal (Distinguished Professor Emeritus, Department of Nutrition and Internal Medicine, University of California, Davis).

The program consisted of three sessions. The first session, led by Professor Pearay Ogra, reviewed data on the immunology of milk and lactation. This session brought us from a historical perspective to the latest scientific findings that are shaping our current understanding of the complex immunobiology of mammalian milk. Chaired by Professor Allan Walker, the second session explored the microbiology of human milk and lactation, with a focus on premature infants and necrotizing enterocolitis (NEC). During the final session, Professor Bo Lönnerdal and the invited faculty shed light on the protective factors in human milk, such as human milk oligosaccharides (HMOs), bioactive milk fat components and lactoferrin, and how these influence the immune system of the neonate.

The program brought great learnings but also left us with many questions. We are just beginning to understand the complexities of milk composition, the functions of the different bioactive components and the most important roles of each in human development.

We are grateful to the three Chairpersons, Pearay Ogra, Allan Walker and Bo Lönnerdal, for putting together the scientific program. We would also like to thank all the speakers and scientific experts in the audience, who contributed to the workshop content and scientific discussions. Finally, we extend our thanks to Maria Elena Munoz and the team at the Nestlé Nutrition Institute for making this workshop possible.

The 94th Nestlé Nutrition Institute Workshop and accompanying book are dedicated to an extraordinary man: Professor Lars A. Hanson (MD, PhD), who is considered by the human milk research community as the founder of modern milk immunology. We believe that this publication and the online materials will provide a valuable scientific resource to all those who wish to learn more about human milk and its immunological properties, as well as to those for whom human milk research is already at the center of their professional lives.

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Key Note Lecture:
The Evolution of Lactation in Mammalian Species

The evolution of the mammary gland and milk has been the subject of speculation since the time of Charles Darwin. Olav T. Oftedal (Smithsonian Environmental Research Center) described the latest revelations on the paleobiology and evolution of this fascinating aspect of mammalian reproduction.

The synapsid ancestors of mammals diverged from the sauropsids (ancestral to crocodiles, lizards, and birds) around 300 million years ago during the Carboniferous period. Lactation may have first evolved to provide a source of moisture and antimicrobial compounds for parchment-shelled eggs.

Milk contains a species-specific mixture of lactose and a variety of neutral and acidic oligosaccharides. Lactose is dominant in most eutherian milks, but oligosaccharides dominate in monotremes, marsupials and caniform carnivores. The distribution of oligosaccharides across the mammalian taxa suggests that oligosaccharides may be ancestral. Compared to the milk of other mammals, human milk has the greatest oligosaccharide diversity.

The milk constituents of particular evolutionary interest include the casein micelles, milk fat globule membrane, components of the milk sugar synthetic pathways and whey proteins. Many of these are unique to the mammary gland and can be found across all three existing mammalian taxa. All of the mammalian milks studied so far contain the four primary types of caseins (αs1-, αs2-, β- and κ-caseins), suggesting that these originated from a pre-mammalian ancestor. Caseins are members of the secretory calcium-binding phosphoproteins (SCPP). Based on related SCPP genes, caseins are thought to have originated from a proto-lacteal secretion that delivered calcium to eggs.

The milk fat globule is another mammary-specific component. Interestingly, some of the key proteins in the milk fat globule membrane participate in immune function in other tissues. These observations suggest that the ability to defend against microbial invasion was of great functional importance during the evolution of milk components.

“From an evolutionary perspective, if a milk protein occurs across the three major mammalian taxa (monotremes, marsupials and eutherians), this is evidence that it evolved before these groups diverged, and was inherited from ancestral taxa.”
The period after birth is marked by rapid changes in the infant’s tissues. Not only does the infant undergo rapid tissue growth, but it is exposed to multiple antigens from the external environment. Spanning the many decades of milk research from its earliest days to the latest advances, the first session kicked off with Pearay L. Ogra’s historical overview of the study of lactation and milk immunology. This body of work underscores the concept that milk not only nourishes the neonate, but also plays a crucial role in driving immunological development of the infant. Next, Jiri Mestecky gave an in-depth look at the antibody-producing cells of the mammary gland and how they function during lactation. Breastfeeding confers numerous benefits, not only to the infant, but also to the mother. Helena Tlaskalová-Hogenová reviewed how these advantages reflect a continuation of the tight relationship between mother and child after birth. Many factors in breastmilk are known to affect mucosal immunity in early life and to modulate an individual’s susceptibility to allergic diseases in later life. Which components of human milk could play such a role? To conclude the session, Valerie Verhasselt examined TGF-β, vitamin A, and the immunoglobulins.

**Immunology of Milk and Lactation: Historical Overview**

Pearay L. Ogra (State University of New York) opened the session by presenting a historical overview of the study of milk immunology and lactation.

During the past few decades, significant new information has become available about the evolutionary biology of mammalian lactation, the functional characterization of antibody and cellular immunologic products, the role of oligosaccharides and other proteins and peptides, and about the distribution and biological function of the microbiome associated with lactation.

Development of the mammary glands and the process of lactation is an integral component of mammalian evolution. For most mammalian species, suckling is essential for the survival of the neonates. Newborns are immunologically immature; thus, colostrum and milk contain a wealth of biologically active products derived from the maternal immunologic and microbiological circulation as well as from maternal mucosal surfaces. These include major immunoglobulin isotypes that can be found in the maternal circulation, secretory IgA immunoglobulin, a variety of soluble proteins, casein, nutritional components, hormones, a large number of cellular elements and their secreted functional products (cytokines, chemokines), several peptides, lipids, polysaccharides and oligosaccharides, and a diverse spectrum of microorganisms. These indicate that milk not only nourishes the neonate, but it also plays a key role in establishing immunological development in the offspring.

“Colostrum and milk, the major products of lactation, contain a wealth of biologically active products.”
Common Mucosal Immune System

The mammary gland consists of distinct cell types that differ in phenotype and function. Jiri Mestecky (University of Alabama at Birmingham) focused on the antibody-producing cells and their role during lactation.

Studies on the structure and biological activities of IgA antibodies in human milk, particularly those specific for environmental antigens of microbial and food origin, have revealed an important finding: the mammary gland is a component of the common mucosal immune system.

However, unlike other components of the mucosal immune system that are directly stimulated by such antigens, the mammary gland is populated by precursors of antibody-producing cells during lactation. These cells acquire their commitment to the production of antigen-specific antibodies of the IgA isotype in mucosal inductive sites in the gastrointestinal and respiratory tracts. After selective homing to the mammary gland, these cells proliferate, locally differentiate and produce secretory IgA, which is then selectively transported into the milk.

Immunomodulatory Components of Human Colostrum and Milk

Breastfeeding represents a continuation of the tight relationship between mother and child after birth. Helena Tlaskalová-Hogenová (Czech Academy of Sciences) reviewed the numerous benefits of breastfeeding for the mother and the infant.

Both colostrum and milk contain immunologically active factors that increase the resistance of the newborn against infection. Not only does breastfeeding protect against diarrhea, respiratory and urinary tract infections, but there is evidence to show that milk components accelerate the development of the immune system and reduce the risk of inflammation in the infant. The wide variety of cytokines, oligosaccharides, and hormones in human milk modulate long-term immunity by decreasing the risk of allergy and immune diseases.

A large quantity of secretory IgA is delivered from the mother to the infant via colostrum and breastmilk. This reacts to a broad spectrum of antigens, and its resistance to enzymatic digestion guarantees its activity even in the distal parts of the infant’s gut. These maternal antibodies originate in maternal gut lymphoid tissue and protect the gut mucosa of the immunologically immature infant, as the antibodies are directed primarily against microbes which may colonize the newborn gut mucosa. Indeed, most milk components substantially affect the microbial colonization of the infant’s gut, which in turn influences the development of other components of the infant immune system.

Breastfeeding also confers many benefits to the mother. Breastfeeding stimulates the production of oxytocin, thereby increasing the feeling of well-being in the mother. The other maternal benefits of breastfeeding are a reduced risk of breast and ovarian cancer.

“Due to the importance of breastfeeding, further investigations on the induction of antibodies of relevant specificity in human milk are encouraged.”

Although the effectiveness of different routes of mucosal or systemic immunization has been extensively evaluated in various animal models, there are only limited studies on the induction of antibody responses related to human milk. Information is particularly lacking with respect to the route of immunization and suitable antigen–delivery systems. The majority of studies have explored the oral route of immunization with microbial antigens. Further insight on the immune modulatory effects of human milk will add to our understanding of how early life nutrition may impact immune development.

“The components of breastmilk act primarily locally on the mucosal membranes, preventing the penetration of microbes and other antigenic components into the circulation and thus ensuring effective defense without the damaging inflammation.”
Influence of Breastfeeding on Immune Trajectory and Long-Term Immune Health

The composition of breastmilk evolves continuously alongside the developing infant and the challenges within its environment. Valerie Verhasselt (University of Western Australia) focused on the factors in breastmilk that have been shown to influence immunity and long-term health.

Compared to infants who are not breastfed, breastfed infants are exposed to a wide variety of allergens and bioactive factors in breastmilk that are found in low concentrations. Evidence from animal studies has shown that the neonatal immune system requires very low doses of antigen exposure to stimulate the appropriate immune reactivity. Indeed, breastmilk intake is necessary for the development of oral tolerance. Several factors in breastmilk are known to affect mucosal immunity in early life and to modulate an individual’s susceptibility to allergic diseases in later life. Among these are TGF-β, vitamin A, and immunoglobulins.

How does breastmilk affect infant immunity? Infants show a relative lack of TGF-β in their mucosal tissues, as well as a physiological deficiency in vitamin A and low mucosal and systemic immunoglobulin secretion. Breastmilk provides the infant with these important factors, which affect the integrity of the gut epithelial barrier, antigen transfer and presentation for successful induction of the regulatory immune response. Ultimately, this results in a reduced risk for allergic diseases over the long term.

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Session II: Microbiology of Milk and Lactation: Influence on Gut Colonization

The past decades have seen a transformation in our view of human milk, from the earliest consideration of human milk as a vehicle for pathogens to our current understanding of its unique microbial, biochemical, and cellular composition. The immature digestive and immune systems of the human infant require a highly regulated microbial colonization process to facilitate optimal development. Samuli Rautava began the session with a description of the milk microbiome and its role as an early colonizer of the infant gut. Although there is strong evidence to support the vertical mother-to-infant transmission of the bacteria in human milk, we have little knowledge of the function of the microbial communities found in milk. Juan Rodriguez followed with an overview of the origin and potential function of milk bacteria. Bacteria, however, are not the only players in human milk. Pia Pannaraj moved one step further in order to explore the identity and function of the viruses in human milk. Josef Neu shifted the focus to other fascinating players within this bioactive cocktail, including the carbohydrates, enzymes, and immune cells. Finally, Allan Walker discussed how our current knowledge of breastmilk and its microbiome could be used in the prevention and treatment of necrotizing enterocolitis.

Chairperson: Allan Walker
Massachusetts General Hospital for Children
Milk Microbiome and Neonatal Colonization: Overview

Samuli Rautava (University of Turku & Turku University Hospital) discussed the milk microbiome and how it could contribute towards the colonization of the infant gut.

The composition of the milk microbiome includes bacteria that are considered to be characteristic of the intestinal microbiome, such as the bifidobacteria. Indeed, data from preclinical and clinical studies suggest that the bacteria in milk may originate in the maternal gut.

How would gut bacteria travel towards the mammary gland? In mice during the perinatal period, increased bacterial translocation has been observed from the intestinal lumen to the mesenteric lymph nodes. Along these lines, the human milk microbiota of mothers who have delivered by caesarian section is significantly different from those who have undergone labor. Furthermore, the milk microbiome can be affected by maternal health and metabolic state, as well as by antibiotic use.

“Human milk has relatively recently been discovered to harbor a distinct microbiome.”

There is a growing body of data indicating that human milk bacteria are a source of colonizing bacteria for the infant gut. First, specific bacterial taxa are detectable in both human milk and infant feces. Second, the microbiome of the infant gut more closely resembles that of its own mother’s milk than the microbiome of unrelated women’s milk. Finally, based on source tracking analyses of samples from 107 mother-infant pairs, approximately 15% of the fecal microbiome in predominantly breastfed infants originate from the bacteria in milk during the first 30 days of life. An understanding of the biological significance of the human milk microbiome will open new therapeutic avenues to modulate neonatal gut colonization and reduce the risk of diseases associated with aberrant gut colonization or suboptimal breastfeeding.

Human Milk Microbiota: Origin and Potential Uses

The origin of the microbiome in human milk is currently a topic of intense research. Juan Rodriguez (University of Madrid) elaborated on the origin and possible applications of the milk microbiome.

Colostrum and milk bacteria are among the first colonizers of the infant gut and thus may play a key role in driving the development of neonatal microbiota. Although there is strong evidence to support the vertical mother-to-infant transmission of the bacteria in human milk, we have little knowledge of the function of this microbiota. In fact, the complex composition of human milk (including the nutrients, bioactive molecules and cells) makes it difficult to isolate the specific functions of different milk bacteria.

Nevertheless, several studies have demonstrated that bacteria isolated from human milk have an inhibitory effect on a wide range of pathogenic strains, thereby reducing the incidence of gastrointestinal and upper respiratory tract infections in infants. Furthermore, human milk bacteria may participate in the maturation of the infant immune system. Some strains are more metabolically active in the infant gut. A well-balanced milk microbiota is also important for maternal breast health. Indeed, mastitis is associated with various bacterial strains that show antibiotic resistance and the ability to form biofilms.

The origin of the bacteria present in human milk remains a topic of debate. It is thought that the skin surface close to the exit point may provide some bacteria to milk. In addition, the existence of oral and entero-mammary bacterial transport pathways opens up exciting new avenues for research and therapy for ameliorating the effects of preterm birth and infant diseases.

“Although Lactobacillus and Bifidobacterium have attracted much attention from scientists and the industry, some staphylococcal and streptococcal strains which are dominant in human milk may also play important empirical probiotic roles in the breastfed infant.”
Human Milk Virome

Pia Pannaraj (University of Southern California) moved beyond the human milk microbiome to explore the virome of human milk and its possible effects on the developing infant.

Emerging evidence shows that human milk viruses are also transmitted from mother to infant via breastfeeding. These viruses include eukaryotic viruses, the bacteria-infecting viruses called bacteriophages, and other viral particles. Like the bacteria found in human milk, these viruses are also instrumental for shaping the virome and microbiome of the infant gut.

The early infant virome is dominated by bacteriophages. Bacteriophages are viruses which are specific for particular bacterial strains and can function as natural regulators of bacterial populations. The bacteriophages have the ability to kill bacteria or to supply them with potentially beneficial gene functions, contributing towards the evolution of the infant gut flora over time.

Gut Microbiota, Host Gene Expression and Cell Traffic via Milk

Human milk represents a bioactive cargo of diverse components that can regulate gene expression and cell function. Josef Neu (University of Florida) gave insight on the identity and function of some fascinating milk constituents.

In addition to microbes, human milk is also a source of carbohydrates, enzymes, immune molecules, and cells. Carbohydrates such as lactose are partly absorbed by the host; however, the undigested portion may be used by microbes which further metabolize the carbohydrate into short chain fatty acids, acetate, propionate and butyrate, all of which are bioactive. Some of the enzymes found in milk include lipase and alkaline phosphatase. The bioactive proteins lactoferrin and lysozyme are also present. Immune molecules such as IgA are found in high concentrations. In addition to these molecules, immune cells, stem cells and small interfering RNAs (siRNAs) are also present in human milk, likely as modulators of immunity and transcriptional regulation.

Based on the data available so far, it appears that the first 2-3 years of life represents a critical window for the establishment of gut microbial diversity. This period of early childhood is marked by rapid growth alongside maturation of the metabolic, endocrine, neural and immune pathways. The colonization of microbes throughout the infant’s body during this time plays an important role in the establishment and maturation of these processes. It is likely that the virome transmitted via breastfeeding will also be important in shaping the microbiome and maturation of immunity during this critical period of development.

“Eukaryotic viruses, bacteriophages and bacteria co-exist in the infant gut in an interdependent and dynamic relationship.”

“A major challenge is to obtain mechanistic evidence on the function of the diverse milk components. Much of the current information we have is associational. Although we know that the human neonate is born with a non-sterile gastrointestinal tract, we lack concrete evidence on the mechanisms and consequences of gut colonization in utero and after birth. A better understanding not only of the composition of human milk, but also of how these components affect the infant, should help us optimize milk for the greatest number of infants.”
Breastmilk and Microbiota in Protection from NEC in Prematures

Allan Walker (Harvard Medical School) explored the use of breastmilk components for the prevention of necrotizing enterocolitis in newborn infants.

Necrotizing enterocolitis (NEC) is an inflammatory condition of the gastrointestinal tract that occurs in newborn infants, primarily in those weighing less than 1500 grams. It is partly due to the interaction between colonizing bacteria and an immature intestine. This evokes an innate immune response in the immature intestine, resulting in increased expression of TLR-4 on the intestinal surface as well as increased signaling molecules and NFκB in the enterocytes alongside a decrease in regulatory molecules. Altogether, this results in a condition of inflammation rather than immune homeostasis.

Breastmilk has been widely used to counteract NEC. The best results have been obtained using a combination of breastmilk and probiotics. When combined with breastmilk, the bacterial strain *Bifidobacteria infantis* has been shown to produce a molecule that modulates the inflammatory response in the immature infant gut by inhibiting the production of interleukin-8, a pro-inflammatory cytokine. Complex carbohydrates in breastmilk have been shown to interact with colonizing bacteria such as *Bifidobacterium infantis* to produce short-chain fatty acids which trigger anti-inflammatory signaling pathways to modulate the inflammatory response in immature enterocytes. Clinical trials that include large numbers of infants are needed in order to test potential strategies for the treatment and prevention of NEC.

“NEC is an extensive intestinal inflammatory disease that is caused, in part, by an inflammatory response to initial bacterial colonization of the immature infant gut.”

Session III: Protective Factors in Human Milk

Breastmilk contains many components capable of modifying the intestinal microbiota and modulating immunity in the infant. Some of these components include antimicrobial peptides and bioactive proteins like lactoferrin and lysozyme, oligosaccharides that nourish commensal bacteria and provide pathogen decoys, and a high concentration of secretory IgA, the primary mucosal antibody responsible for pathogen exclusion on mucosal surfaces. Lars Bode began the session by reviewing one of the most abundant components of human milk, the oligosaccharides. This diverse family of molecules shows incredible variation between individuals, as well as throughout the course of lactation. Building upon the concepts of the first talk, Franz Hanisch discussed how to apply the key learnings from our understanding of milk oligosaccharide structure and function in designing new anti-viral treatments. Nicholas Embleton discussed the conflicting findings from the ELFIN trial of lactoferrin supplementation on the incidence of late onset sepsis in preterm infants. The difficulties in obtaining conclusive evidence from clinical trials is largely due to the challenge of pinpointing the actions of individual nutrients that function in a complex mixture. The true complexities of human milk were showcased in the final two talks by Bo Lönnerdal and Vanessa Dunne-Castagna, who described the far-reaching effects of osteopontin and secretory IgA on the infant gut and in the circulation beyond.
Milk Oligosaccharides – Structure and Function

Human milk is unique for the high concentration and structural diversity of its oligosaccharides. Lars Bode (University of California, San Diego) gave an overview of this diverse group of molecules.

Human milk oligosaccharides (HMOs) are the third most abundant component of human milk, often exceeding the total amount of milk proteins. Over 150 different HMOs have been identified so far. Interestingly, the HMO composition varies between individual women and throughout the course of lactation. Once ingested, many HMOs resist degradation in the digestive tract. A small percentage is absorbed and reaches the systemic circulation. The remainder is either metabolized or excreted intact in the feces.

What are some of the functions of HMOs? Not only are these molecules known to act as prebiotics, but some also have antimicrobial, anti-adhesive, and immune-modulating properties both locally in the gut and systemically once they have reached the circulation. Individual HMOs can have potent and specific effects. For example, the HMO disialyllacto-N-tetraose (DSLNT) improves survival and reduces pathology scores in an animal model of necrotizing enterocolitis (NEC). Along these lines, pre-term infants that receive human milk with low levels of DSLNT are at higher risk of developing NEC. In addition, the ratio and relative abundance of HMOs also play an important role in their actions on downstream targets.

The complex nature of HMOs calls for the combination of suitable preclinical models, mother-infant cohort association studies, as well as randomized clinical trials in order to establish true structure-function relationships and provide the evidence required to improve infant health and development.

Oligosaccharides and Viral Infections: Milk Oligosaccharides versus Algal Fucan-Polysaccharides

Can we harness the properties of human milk oligosaccharides as anti-viral food additives? Franz Hanisch (University of Cologne) discussed the possibilities.

Norovirus infections are a common cause of gastroenteritis worldwide, with epidemic outbreaks resulting in hundreds of thousands of deaths each year. In humans, noroviruses bind to the gastrointestinal epithelia via recognition of the blood-group active mucin-type O-glycans. Human milk oligosaccharides (HMOs) mimic the structures of blood-group active mucin-type O-glycans, and therefore represent an ideal source of potential competitors of viral glycan receptors. The trisaccharide 2-fucosyllactose (2’FL) is able to block norovirus binding quite efficiently, and has been approved as a safe food additive. Other high molecular-weight HMOs have been shown to exert even more potent effects.

What feature of HMOs confers the viral receptor binding ability? It is the oligo-valency of the fucose moiety in hepta- to deca-saccharides that contributes towards viral binding. Interestingly, high valency α-L-fucose is also a feature of the natural polysaccharides derived from some algae. The fucoidans derived from brown algae have been shown to display anti-viral properties against Herpes simplex, cytomegalovirus, and human immunodeficiency virus. Hanisch’s group have reported promising results on the anti-norovirus effects exerted by α-L-fucose from fucoidans of different sources. These findings demonstrate how an understanding of the structure and function of HMOs can be applied towards the design of new anti-viral strategies for currently untreatable norovirus infections.

“The mixture and relative abundance of different HMOs to each other is what makes them most effective, suggesting mediation through complex interactions of multiple different HMOs on different molecular targets.”
Clinical Trials of Lactoferrin in the Newborn: Effects on Infection and the Gut Microbiome

Lactoferrin is a potent anti-microbial factor present in human milk. Nicholas Embleton (Newcastle Hospitals NHS Trust) highlighted the findings from the ELFIN trial of lactoferrin supplementation on the incidence of late onset sepsis in preterm infants.

Human breastmilk is known for its bacteriostatic activity against strains such as *E. coli*. Some of this activity is derived from lactoferrin, an iron-binding glycoprotein present in high amounts especially in early colostrum.

The ELFIN trial is the largest trial of lactoferrin supplementation. It enrolled 2203 very preterm infants that were recruited before 72 hours of age and were allocated to lactoferrin supplementation or placebo. The primary outcome was the occurrence of late onset sepsis (LOS). Results showed no significant difference in the rate of LOS between the lactoferrin (28.9%) and placebo (30.7%) arms. The incidence of necrotising enterocolitis (NEC) stage II/III and all-cause mortality also did not differ.

The findings from the ELFIN trial are contradictory to those from previous studies. A large multicenter trial conducted in Italy had previously shown that overall rates of LOS were significantly lower in infants receiving lactoferrin, and there was also a reduction in NEC. While large-scale clinical trials are essential, mechanistic studies are also needed in order to better understand the treatment effect on disease pathology. The results from the ELFIN trial highlight the fact that nutrients do not function in isolation, and that the optimal source of immune-nutrients remains fresh human milk.

Effects of Milk Osteopontin on Intestine, Neurodevelopment and Immunity

Bo Lönnerdal (University of California, Davis) focused on the multifunctional protein osteopontin, which is involved in cell proliferation and differentiation, biomineralization, immunomodulatory activities, and myelination.

Osteopontin is expressed in various cell types, such as epithelial cells and immune cells, and is found in most human bodily fluids including milk and blood. Human milk contains high concentrations of osteopontin, which is relatively resistant to gastrointestinal digestion and may contribute towards intestinal development in the infant. Furthermore, orally ingested osteopontin appears in the circulation, suggesting that this molecule exerts multiple systemic effects. There are established methods to isolate osteopontin from cow’s milk, and milk osteopontin is commercially available.

What are the key functions of this pleiotropic molecule? The results from preclinical and clinical studies highlight three major functions of osteopontin in early life. First, milk OPN contributes to intestinal development, by stimulating proliferation and differentiation and modulating immunity in human intestinal cells. Second, milk osteopontin promotes neurodevelopment in early infancy. *In vivo* data from mouse models indicate that brain myelination and cognitive development were enhanced in mouse pups receiving milk from wild-type dams compared to those receiving milk from osteopontin knock-out dams. Finally, milk osteopontin stimulates immune development. Infants fed osteopontin-supplemented formula had significantly lower levels of serum TNF-α and fewer days of illness compared to infants fed regular formula. Those receiving osteopontin-supplemented formula had an immune cell profile more similar to that of breastfed infants.

“Whilst we search for immune-nutrients that improve health and reduce disease, we must continue to better understand how to support mothers to meet their infants’ intake needs using their own milk.”

“Milk osteopontin plays important roles in development of the intestine, brain, and immunity.”
Effects of Milk Antibodies and other Proteins on the Commensal Flora

Secretory IgA (SlgA) is one of the key immunoglobulins among all the isotypes that are present in human milk. Vanessa Dunne-Castagna (University of California, Davis) reviewed the latest data on how milk SlgA affects the intestinal microbiota of the infant.

The SlgA in milk is derived from a diverse pool of intestinally-induced plasma cells homing in to the lactating mammary gland. Milk SlgA is heavily glycosylated, containing O- and N-linked glycan moieties that can that bind to select commensals to promote mucosal localization and increase their colonization potential in the infant gut. There is also evidence that SlgA undergoes non-specific binding in the gut, further promoting colonization of commensal bacterial strains and conferring a benefit to the host.

What are some of the effects of SlgA binding in the intestine? Experiments in mouse models have shown that the non-specific association of SlgA with commensal bacteria reduced dextran sulfate sodium (DSS)-induced colitis. This protective effect was absent when the SlgA binding was experimentally inhibited. In vitro, non-specific SlgA association with commensals reduced the induction of pro-inflammatory cytokines. The binding of SlgA to commensal bacteria also safeguards them against intestinal proteolysis, thereby facilitating the survival of beneficial strains.

Taken together the available data shows that milk SlgA has multifaceted abilities, retaining high-affinity anti-pathogenic protective functions while simultaneously promoting the colonization of the infant gut with commensal bacteria. The use of cutting-edge biochemical and molecular techniques continues to reveal new functions of this important component of human milk.

“These studies demonstrate the complex utility of milk SlgA in shaping the intestinal microbiota in the infant, both in protection from pathogens and in promotion of commensal colonization.”
Conclusions

Human milk is the product of millions of years of evolution, resulting in a finely-tuned system that supports infant growth and development. Breastmilk is a dynamic entity whose composition and function alter throughout lactation to accommodate the changing needs of the infant. Early post-natal life is characterized not only by profound growth, but also by the need to rapidly adapt to the challenges of the external environment. Indeed, neonatal tissues are constantly exposed to an onslaught of new antigens. Beyond supporting linear growth, therefore, breastmilk also performs a range of important functions such as modulating the infant gut microbiome and the regulation of immunity.

We now understand that breastmilk harbors its own microbiome and virome, as well as many immunologically active components that guide the infant’s developing mucosal immune system. The origin of the bacteria found in human milk is an area of active research. There is evidence to suggest the presence of a maternal enteromammary system for bacterial translocation between the gut and mammary gland. Whatever the mechanism, the transfer of the microbial communities from breastmilk to the infant is a key step in establishing a healthy infant intestinal microflora. This in turn protects the infant against many respiratory and diarrheal diseases. Understanding how the human milk microbiome contributes to the beneficial effects of breastfeeding will open exciting new therapeutic opportunities. Modulating or mimicking the milk microbiome may present a means to affect early gut colonization and reduce the risk of those disorders associated with aberrant gut colonization or suboptimal breastfeeding.

In today’s era of cutting-edge technologies that enable us to harvest data from multiple avenues, we have just begun to scratch the surface of the landscape of human milk. We are beginning to uncover new information on well-known components of human milk, such as the oligosaccharides, osteopontin, and lactoferrin, but more work is needed in order to obtain a better picture of how these factors interact within their biological milieu. The bioactive complexity of the human milk cargo presents a real challenge for the design and interpretation of clinical trials. In addition, more mechanistic studies are needed in order to clarify how these factors interact to modulate the infant immune system. Answering these questions will pave the way for preventive and curative approaches that are tailored to this critical window of life and will ensure long-term immune health.
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