Immune Supporting Properties of Milk

Part 4: Remaining members of the innate immune system in milk – The oxidase enzymes, milk fat globule membrane and osteopontin

ADPI® Center of Excellence (COE) team member Dr. David Clark shares his insight and knowledge on the immune supporting properties of milk.

Introduction

It has been pointed out in previous articles in this series that infants are not completely dependent on receiving immune support from mother's milk in the form of antibodies, the star players of the adaptive immune system (Article 1). Milk also contains multiple components of the innate immune system (Article 1 – Table 1). Several important members of milk's innate immune system have already been described including lactoferrin in Article 2 and glycomacropeptide, oligosaccharides, glycosaminoglycans, alpha-lactalbumin and lysozyme in Article 3. These molecules can be broadly classified as being proteins or carbohydrates. In this 4th article in the series, milk fat takes to the stage, or at least a subset of milk fats referred to as phospholipids. These molecules form the structure that encapsulates the energy dense triglyceride fraction of fat packaged in the milk fat globule. This envelope is referred to a milk fat globule membrane (MFGM).

In the previous articles, the analogy of an arsenal of weapons was used to describe the array of components that make up milk's immune system. Continuing with this theme, in this article MFGM would comprise a complete reserve army because not only do PLs serve as guards but also a long list of proteins embedded within the MFGM possess antimicrobial activity. Milk enzymes comprising the oxidase class including lactoperoxidase (LP) and xanthine oxidase (XO) will also be reviewed. They conduct chemical warfare against invading bacteria and viruses. Finally, an effective defense depends on good communications to recruit and mobilize forces, identify where to find the enemy and how to synchronize the attack. Alongside its built-in defensive capabilities of decoy receptor and prebiotic, the protein, osteopontin (OPN) fulfills the vital role of Signals Corps, coordinating the response of members of both innate and adaptive immune systems. These three classes of components combined with the previously described members of milk's innate immune system (Articles 2 & 3) form a formidable integrated defense that buys time for the adaptive system to get up to speed.

Whilst these components have been described individually, it is important to appreciate that it is the assembly of all the innate components present in milk that levers the power of the innate system as a whole. In addition, it is important to understand that all the immune-active components present in milk not only benefit infants receiving breastmilk or formula but likely also benefit adults through their consumption of dairy-based foods. The act of placing dairy products in your mouth delivers the immune active components to the entry point of most invading organisms – the mouth and throat. Mastication and swallowing dairy foods floods the area where the respiratory and gastrointestinal tracts merge – the back of the mouth and throat – which is exactly where most invading organisms are trying to attach.

Milk Fat Globule Membrane

A schematic diagram of the structure of the Milk Fat Globule Membrane (MFGM) is shown in Figure 4.1. In milk, MFGM comprises a tri-layer of amphipathic lipids, i.e. lipid molecules that have a water loving 'head' and an oil loving 'tail', which arrange themselves 'nose to tail' into a 3-layer laminated sheet with the oil loving tails toward the fat-filled center of the droplet and the water-loving heads towards the aqueous milk serum. Multiple proteins are embedded in this tri-layer.

MFGM-enriched fractions also referred to as Whey Protein Phospholipid Concentrate (WPPC) can be produced on a commercial scale from cream, as a byproduct of the anhydrous milk fat process, from buttermilk and from the fat component separated during WPI manufacture. Different sources and unit process combinations produce MFGM that is lipid-dominant (e.g. from cream via solvent extraction) or protein-dominant (e.g. from whey via membrane filtration).

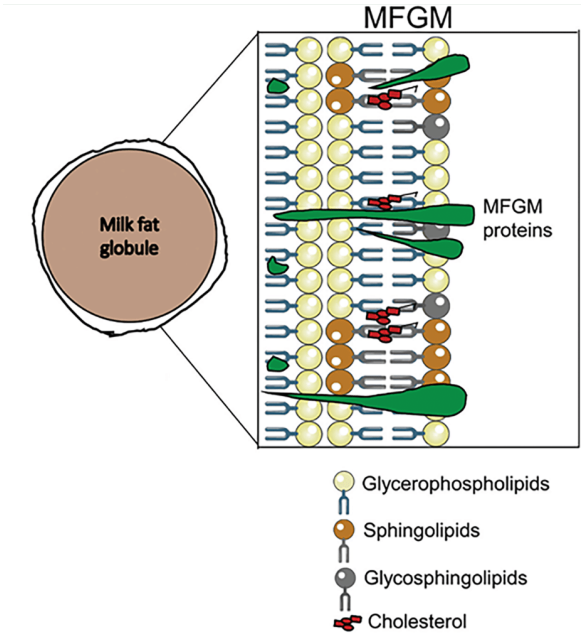


Figure 4.1: Schematic representation of MFGM structure showing the triple layer membrane comprised of different classes of phospholipids encapsulating the fat globule. Multiple different types of protein are embedded in the membrane, some traversing it (modified from Raza et al., 2021).

This variation in composition complicates interpretation of clinical studies. The protein fraction alone comprises 244 different identified proteins in the whey-derived MFGM preparation and 133 in the buttermilk-derived preparation. The major proteins present include xanthine oxidase, mucins, butyrophilin, lactoferrin, and lactadherin (Timby et al., 2017).

Milk Fat Globule Membrane (MFGM) initially gained attention as an ingredient in infant formula. However, benefits have been reported across a broad range of age groups and should not be neglected. Scientific evidence in support of this broader bio-functional activity was reviewed recently by Raza et al., (2021). These authors evaluated 15 separate clinical trials published between 2010 and 2019 involving adults exhibiting a wide range of health states described as healthy, overweight, post-menopausal or older.

Reported benefits range from improved growth and cognitive development in infants to decreases in risk factors for cardiovascular disease, such as LDL, cholesterol and triglycerides and improvements in insulin sensitivity in adults and even improvements in walking speed, leg muscle mass, and muscle fiber velocity in frail elderly subjects.

However, interpretation of data on the immune supportive effects of MFGM remains a challenge. Clinical trials with MFGM supplementation of infants or children showed beneficial effects with respect to gastrointestinal and respiratory infections (Zavaleta et al., 2011; Timby et al., 2015). In these studies, different forms of MFGM exerted different responses, which is likely a reflection of their different composition. For example, protein-rich MFGM containing ganglioside lipids prevented diarrhea associated with rotavirus infection in infants, whereas phospholipid-enriched fractions did not (Raza et al., 2021). Turning to different age groups, Ten Bruggencate et al., (2016) showed that adults supplemented with protein-rich MFGM exhibited reduced diarrhea episodes, following infection with an attenuated enterotoxigenic *E.coli* compared to controls receiving an amino acid balanced control protein. Summarizing findings relating to bacterial infections, it appears that supplementation with different MFGM formulations reduces fever, respiratory and diarrhea symptoms and length of the disease across a wide range of age groups. However, definitive conclusions are complicated due to the differences in MFGM composition.

The antiviral activity of MFGM has tended to focus on viruses that cause problems in infants. Monaco et al., (2021) assessed the infectivity of rotavirus in an *in vitro* study. They showed that MFGM decreased the infectivity of two different rotavirus types that had different dependency on sialic acid for binding to both monkey (the most widely used model for rotavirus) and human cell lines. This would suggest that MFGM is more than merely a decoy receptor. In a previous study, Fuller et al., 2013 identified anti-rotavirus components present in MFGM were associated with its organic soluble phase, strongly suggesting they were lipid in nature.

One of the proteins that forms part of the MFGM is the enzyme, Xanthine Oxidase, which is a convenient segue into the next section of this article.

Oxidase enzymes – Xanthine Oxidase

Xanthine oxidase (XO) accounts for up to 20% of the total protein fraction of MFGM. Confusingly, this enzyme is also called hypoxanthine oxidase or xanthine:oxygen oxidoreductase. XO is widely distributed and is indigenous to many tissues in humans. The main function of XO is the safe disposal of breakdown products of ingested and unwanted endogenously synthesized DNA and RNA. This involves the conversion of the purine bases found in these nucleic acids into uric acid, which is excreted via the kidneys. During this conversion superoxide and hydrogen peroxide is generated. (Farkye, 2002; Kostic et al., 2015).

All this appears perfectly logical until we consider milk. Milk contains significant quantities of XO but its main substrates, xanthine and hypoxanthine are essentially absent! This remained an enigma for many years but now possible explanations are coming to light. Recently, Al-Shehri et al., (2015) showed that XO in milk becomes activated once in contact with saliva. It turns out that saliva naturally contains sufficient levels of xanthine and hypoxanthine to allow generation of hydrogen peroxide by XO. The latter highly reactive oxidizing chemical will be familiar to dairy processors as a sanitizing agent. This finding becomes even more intriguing, as hydrogen peroxide is required to activate lactoperoxidase (LP), which will be discussed below. The reactive products generated by LP from hydrogen peroxide effectively multiplies anti-bacterial potency by 30-80 times! More on LP in a moment. Turning back to XO, a recent study which exposed cow's milk XO to simulated conditions within the infant mouth – temperature, pH and levels of xanthine and hypoxanthine found in infant saliva – resulted in growth inhibition of several Gram-negative and -positive bacterial pathogens of *S.aureus, E.coli,* and *S.endocarditis* in a dose-dependent manner. In these cases even very high levels of inoculum (10^6 cfu/mL) were inhibited (Ozturk et al., 2020).

Studies of the direct effects of XO activity on viral infections are rather sparse. Several of the catalytic products of XO cause an inflammatory response via stimulation of cytokines, including various interleukins. There has been speculation that over stimulation of synthesis and activity of indigenous XO in the lung could be a contributing factor to the so-called cytokine storm phenomenon. This morbidity is observed in cases of acute respiratory distress syndrome (ARDS), which can occur in severe COVID-19 infections. This is currently only a hypothesis and requires validation (Pratomo et al., 2021). However, as you will read below, this could amean that XO could serve as a useful biomarker for susceptibility of at risk patients to severe COVID-19.

Oxidase enzymes – Lactoperoxidase

The best know oxidase enzyme in milk is lactoperoxidase (LP), a basic protein that carries a net positive charge at milk pH and as a result is easily extracted and isolated by ion exchange

processes. As with XO, under suitable conditions, LP can generate highly reactive chemicals but in this case with even higher potency than the peroxide from XO. These reactive chemicals destroy invading bacteria, viruses and damaged cells in a rather indiscriminate manner, so need to be closely regulated. LP is secreted not only by the mammary gland but also mucosal glands in other secretory tissues including salivary, lung, bronchial and nose. LP is folded around an iron molecule contained within a heme group (protoheme IX). This structure confers a greenish color to the protein in a comparable manner to that of iron in hemoglobin making it appear red. The greenish color of LP at least in part contributes to the color of the mucus expelled from the nose following a common cold!

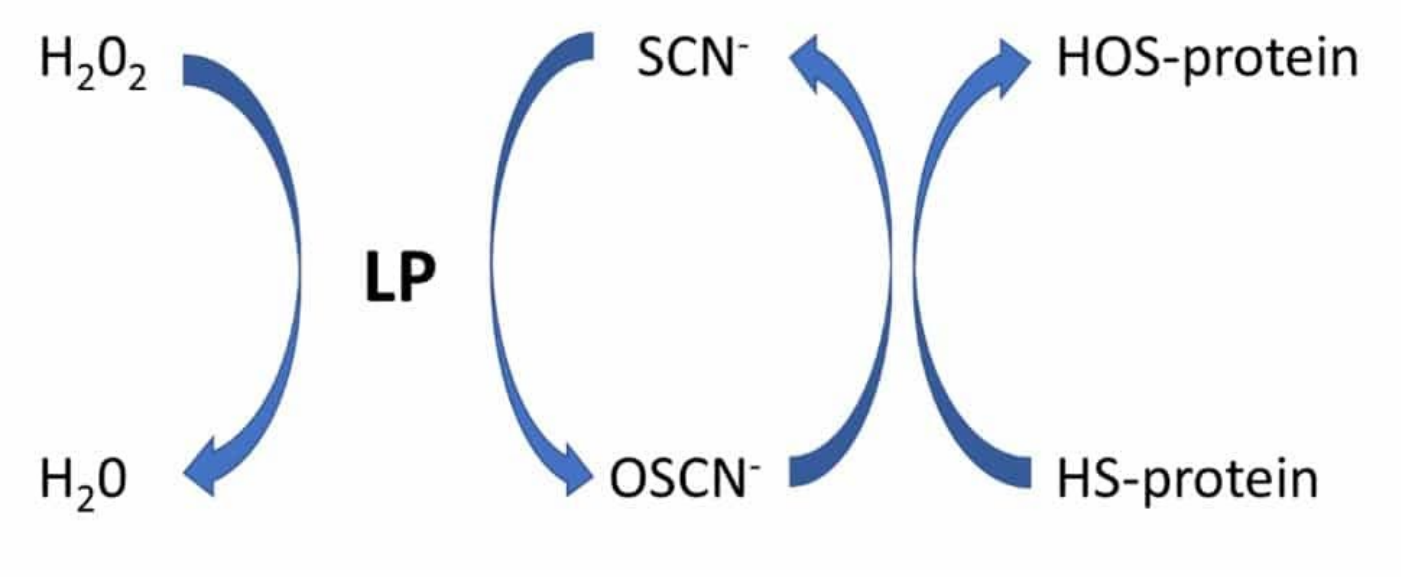
In isolation, LP has no anti-microbial activity. However, in the presence of hydrogen peroxide and thiocyanate, it becomes the LP-system, which is a potent bacteriocide. The LP-system destroys bacteria and viruses outright rather than just preventing their multiplication. This differentiates LP-system activity from that of lactoferrin. The LP-system was first identified in raw milk but is active only while it is supplied with hydrogen peroxide. Only low levels of peroxide (10-15ppm) are required to power the LP-system compared to the much higher levels of peroxide alone (300-800ppm) needed to elicit a comparable antibacterial effect to that of the LP-system. Hydrogen peroxide can be generated naturally *in situ* in milk by probiotic bacteria including catalase-negative lactic acid bacteria. However, in many technological applications, activation of the LP-system requires a more predictable level of control that can turn it on only when needed. This can be achieved by chemical means e.g. solubilizing sodium perchlorate or by quite elegant enzymatic reactions using glucose oxidase, for example.

Table 4.1 Concentration of thiocyanate in various human body fluids compared to cow's milk

Human source	Levels of thiocyanate (ppm)
Blood Serum	1.9-8.4
Saliva (Adult	37-198
Lachrymal Fluid (Tears)	10
Gastric Juice	23-64
Cow's Milk	2.9

LP in the LP-system uses hydrogen peroxide to oxidize thiocyanate (SCN^-) anions to the short-lived and highly reactive hypothiocyanate anions ($OSCN^-$). Thiocyanate anions are widely distributed through human tissues and secretions such as saliva, tears, gastric juice and blood serum (Table 4.1).

Figure 4.2: Mechanism of action of the LP-system



The LP-system requires thiocyanate concentrations in the 10-25 ppm range for efficient activation. Thus, there is sufficient present in saliva and gastric juices to power the LP-system (Table 4.1). In technological applications of the LP-system, thiocyanate is generally added in the form of the sodium or potassium salt. The hypothiocyanite anion is an extremely potent oxidizer of sulfhydryl groups (-SH) in proteins. Many critical membrane-bound proteins in bacteria contain -SH groups, which are central to their functionality e.g. cross membrane transport etc. Destruction of these functionalities along with enzymes that are involved in bacterial metabolic pathways such as glycolysis by the LP-system has been demonstrated in multiple studies. Mammalian cells do not seem to share this same level of sensitivity to damage by the LP-system.

The LP-system found in a number of commercial products ranging from oral care through crop protection to aquaculture (Figure 4.3).

Figure 4.3: Examples of commercial products containing Lactoperoxidase as an antimicrobial ingredient. Clockwise from top left: Koppert Enzicur natural antifungal treatment for white mildew (GHS Asia), the LP system for milk preservation (https://www.youtube.com/watch?v=JI0esrNAsks), Zendium toothpaste (https://zendium.shop/products/complete-protection-total-care-kit).

In 1991, the Codex Alimentarius recognized the use of a LP-system (Figure 4.3) in countries where technical, economic or practical situations did not allow for the cooling of raw milk (IDF Fact Sheet, Jan 2013). In practice, this involved addition of thiocyanate (14 mg/L) and sodium percarbonate (30 mg/L) to raw milk either in bulk by trained farm personnel or from pre-weighed sachets for small scale use. In 2005, a further assessment of safety and application of the LP-system for the preservation of raw milk was published by the World Health Organization, which extended called for extension of the guideline to 4-7 hours storage/transport at 31-35°C for of raw milk in which the LP system had been activated (WHO, 2005).

Several oral care products are formulated to include LP and part of the LP-system (Figure 4.3). One way of ensuring LP-system activation at time of use in oral care products is to formulate with thiocyanate, glucose oxidase and starch. The glucose oxidase can only generate hydrogen peroxide once hydrolysis of the starch releases its substrate, glucose. Hydrolysis of the starch is initiated by the action of brushing teeth due to contact with salivary amylase in the mouth.

The LP-system has been shown to be active against influenza A and B viruses including H1N1 (Sugita et al., 2018). In their study, hypothiocyanite generated by the LP-system showed antiviral activity when the virus was exposed both before and after adsorption to model mammalian cells. The cells themselves were not affected by exposure to hypothiocyanite.

The LP-system can be assembled with components from other sources. Indigenous production of hydrogen peroxide is comparatively widespread in the mucosal tissues of mammals by the duox oxidase system (Geiszt et al., 2003). In the presence of hydrogen peroxide, LP will not only oxidise thiocyanate but also iodide (I^-) in this case to hypoiodite (IO^-) and hypoiodous acid (HOI). It has been suggested that the observed inverse relationship between iodine intake and COVID-19 infection rate could be more causal than just a correlation. In regions with diets rich in iodide such as Japan, where intake is boosted by the significant consumption of seafood and particularly

seaweed, COVID-19 infection rates were found to be lower, despite its high population density (Smith et al., 2022). It is not inconceivable that combining consumption of LP in dairy products with iodine supplementation could provide a dietary means to reduce SARS-Cov-2 infections and certainly merits further review.

Osteopontin

To round off the discussion of innate immune system components in milk we turn attention to osteopontin (OPN). OPN sometimes referred to as lactopontin is a multifunctional protein found throughout the body but in highest concentrations in milk. OPN is a highly phosphorylated and glycosylated acidic protein. While it is present in bovine milk at much lower concentrations than breast milk, (18mg/L compared to 140-160 mg/L), its acidic nature allows it to be readily extracted by ion exchange chromatography. Initially, much focus was put on the role of OPN in bone formation and mineralization, hence the reference to 'osteo' in its name. However, it is now clear that it plays important roles in developing the immune, intestinal and nervous systems in early life. The importance of OPN's contribution to the immune system is important because through signalling, it coordinates the activity of elements of both the innate and adaptive systems in children and adults. Ingredients enriched in OPN derived from bovine milk are now being included in infant formula, particularly in Asia.

The bridging role of OPN between the innate and adaptive systems is complicated not only by the wide range of activating and deactivating functions but also by the dependence of those functions on the precise degree of post-translational modification of the individual molecules of OPN. These modifications can include combinations of O-linked glycosylation, sialylation, phosphorylation, and tyrosine sulfation (Clemente et al., 2016). This is beyond the scope of this article, not least because here we focus on OPN-delivered from milk. Most likely OPN from milk exerts its influence primarily in the environment of the gut but bear in mind the gut is considered the largest organ of the immune system (Chassaing et al., 2014). To complicate matters further, OPN is protected from digestion to a certain extent by complexation with other proteins, including lactoferrin in milk (Jia et al., 2021). However, it is reported even peptides release by digestion of OPN retain or even increase some signaling activities!

Table 4.2: Some key signaling roles of Osteopontin	
Upregulates expression of cytokine interleukin 12	Schack et al., 2009
Acts as an opsonin that enhance bacterial phagocytosis	Schack et al., 2009
Cell adhesion	Giacelli and Steitz, 2000
Cell migration	Giacelli and Steitz, 2000
Regulates inflammation	Giacelli and Steitz, 2000
Inhibits expression of interleukin-10	Ashkar et al., 2000

Several of the critical signaling functions of OPN are highlighted in Table 4.2. This ability to regulate levels of synthesis of a number of cytokines, especially interleukins is at the heart of the bridging role of OPN between the innate and adaptive systems. Cytokines are the chemical messages that stimulate the various cells of the immune system in different ways. There is an army of immune cell types operating behind both the innate and adaptive immune systems and OPN through cytokine regulation sends them amongst other things, 'instructions to engage' invaders. For example, stimulation of the expression of the cytokine interleukin-12 (IL12) by OPN activates T-helper 1 cells, which are important in clearance of pathogens such as viruses and certain bacteria (Schack et al., 2009). OPN also decreases the expression of interleukin-10 (IL10) the presence of which reduces inhibition or 'takes the brakes off' of T-helper 2 cells. In addition, OPN has opsonin-like behavior which stimulates the 'consumption' of invading bacteria by macrophages (Schack et al., 2009). More details about these immune cells and their roles will be provided in the next and final article in this series.

As with XO and LP, OPN is not only found in milk but is also produced by many other tissues in the body and plays part in the immune response throughout life. OPN is actually produced by most cell types of the immune system, including B and T cells, natural killer (NK) cells, NKT cells, macrophages, neutrophils, dendritic cells (DC) *etcetera*. OPN is involved in inflammation by inducing cell adhesion and migration, regulating the differentiation of proinflammatory lymphocytes, and inhibiting the apoptosis (death) of inflammatory cells (Clemente et al., 2016).

The focus on unravelling the general signaling functions of OPN described above and their inherent complexity have limited direct studies on effects of OPN on viral infections. Nevertheless, there are some which are worthy of note. For example, infection of intestinal epithelial cells and mouse intestine with rotavirus – a diarrhea-causing virus in infants – caused marked increases in OPN mRNA levels (an indicator of upregulation of indigenous OPN synthesis) and secreted OPN protein. OPN-deficient mice suffered prolonged disease (Rollo et al. 2005). Interest in OPN in relation to COVID-19 has grown recently due to its potential use as a biomarker for prediction of the likely

severity of COVID-19 infection. Studies in both adults (Varim et al.,2021) and children (Reisner et al., 2021) have been very promising. Unlike many other biomarkers that have been investigated, OPN levels in those who contract severe COVID are significantly higher. Of course, this is 'off subject' as it relates to indigenous OPN that is synthesized within other tissues in the body, such as lung as opposed to OPN from milk. Nevertheless, it demonstrates that the details of OPN functionality are still being unraveled.

Closing Remarks

This article rounds off the discussion of the remaining members of the innate immune system found in milk. Here three components – MFGM, the oxidase enzymes and osteopontin are added to the previous list of lactoferrin (Article 2) along with milk oligosaccharides, glycomacropeptide, glycosaminoglycans, alpha-lactalbumin and lysozyme (Article 3) described previously. The innate immune system described constitutes a formidable array of defenses that have been evolving in milk since the first mammals appeared an estimated 178 million years ago. Some members of the innate complement joined comparatively recently on the evolutionary time scale – for example alpha-lactalbumin appears to have originated from a mutated lysozyme gene almost 300-400,000 years ago. It is a sobering fact that despite the ingenuity of scientists invested in the discovery and development of antibiotic and antiviral drugs over the last century, bacteria and viruses constantly find ways to outwit and circumvent them. In contrast, nature through evolution has provided mammals with a rich source of defenses that are still able to fight long established and new invaders alike!

In the next and final article in this series, we will take a look at the immunoglobulin fraction in milk. A lactating mammal secretes these antibodies into her milk. Through her milk, the mother provides the infant with the 'tip of the spear' of her adaptive immune system. Certainly, antibodies comprise the 'smartest weapon' in her immune armory!

References

Al-Shehri SS, Knox CL, Liley HG, Cowley DM, Wright JR, Henman MG, et al., 2015. Breast milk saliva interactions boost innate immunity by regulating the oral microbiome in early infancy. *PLoS ONE*, 10, Article e0135047.

Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, Rittling SR, Denhardt DT, Glimcher MJ, Cantor H., 2000 Eta-1 (Osteopontin): An Early Component of Type-1 (Cell-Mediated) Immunity. *Science* 287, 860-864.

Chassaing B, Kumar M, Baker MT, Singh V, Vijay-Kumar M, 2014. Mammalian gut immunity. *Biomed J*, 37, 246-258. doi: 10.4103/2319-4170.130922.

Clemente N, Raineri D, Cappellano G, Boggio E, Favero F, Soluri MF, Dianzani C, Comi C, Dianzani U, Chiocchetti A. 2016. Osteopontin Bridging Innate and Adaptive Immunity in Autoimmune Diseases. *J Immunol Res*, Article ID 7675437, 15 pages. http://dx.doi.org/10.1155/2016/7675437

Farkye NY, 2002. Enzymes indigenous to milk. Xanthine Oxidase. *Encyclopedia of Dairy Sciences*, 941-942.

Fuller KL, Kuhlenschmidt TB, Kuhlenschmidt MS, Jiménez-Flores R, and Donovan SM, 2013. Milk fat globule membrane isolated from buttermilk or whey cream and their lipid components inhibit infectivity of rotavirus in vitro. *Journal of Dairy Science*, 96(6), 3488-3497.

Geiszt M, Witta J, Baffi J, Lekstrom K, Leto TL, 2003. Dual oxidases represent novel hydrogen peroxide sources supporting mucosal surface host defense. *The FASEB Journal* express article10.1096/fj.02-1104fje.

Giachelli CM, Steitz S., 2000. Osteopontin: a versatile regulator of inflammation and Biomineralization. *Matrix Biology* 19, 615-622.

IDF Factsheet –January 2013. The Lactoperoxidase system.

Jia Q, Wang Y, Zhu J, Yu H, Tong X, 2021. A literature review on lactopontin and its roles in early life. *Transl Pediatr.* 10(7), 1924-1931 | https://dx.doi.org/10.21037/tp-21-293.

Kostic DA, Dimitrijevic DS, Stojanovic GS, Palic IR, Dordevic, AS, Ickovski JD, 2015. Xanthine Oxidase: Isolation, Assays of Activity, and Inhibition. *J Chem.* Article ID 294858 http://dx.doi.org/10.1155/2015/294858

Monaco MH, Gross G and Donovan SM, 2021. Whey Protein Lipid Concentrate High in Milk Fat Globule Membrane Components Inhibit Porcine and Human Rotavirus in vitro. *Front. Pediatr*. 9:731005. doi: 10.3389/fped.2021.731005

Ozturk G, Shah IM, Mills DA, German JB, de Moura Bell JMLN, 2020. The antimicrobial activity of bovine milk xanthine oxidase. *Int Dairy J.* 102: . doi:10.1016/j.idairyj.2019.104581.

Pratomo IP et al., 2021. Xanthine Oxidase-Induced Inflammatory Responses in Respiratory Epithelial Cells: A Review in Immunopathology of COVID-19. *Intl. J Inflammation* |Article ID 1653392 | https://doi.org/10.1155/2021/1653392.

Raza GS, Herzig K-H, Leppäluoto J, 2021. Invited review: Milk fat globule membrane—A possible panaceafor neurodevelopment, infections, cardiometabolic diseases, and frailty. *J. Dairy Sci*. 104, 7345–7363. https://doi.org/10.3168/jds.2020-19649

Reisner A, Blackwell LS, Sayeed I, Myers HE, Wali B, Heilman S, Figueroa J, Lu A, Hussaini L, Anderson EJ, Shane AL, Rostad CA, 2022. Osteopontin as a biomarker for COVID-19 severity and multisystem inflammatory syndrome in children: A pilot study. *Exp Biol Med (Maywood)* 247(2), 145-151. doi: 10.1177/15353702211046835

Rollo EE, Hempson SJ, Bansal A, Tsao E, Habib I, Rittling SR, Denhardt DT, Mackow ER, and Shaw RD, 2005. The Cytokine Osteopontin Modulates the Severity of Rotavirus Diarrhea. *J Virology*, 79, 3509-3516. https://doi.org/10.1128/JVI.79.6.3509-3516.2005

Schack L, Lange A, Kelsen J, Agnholt J, Christensen B, Petersen TE, Sorensen ES, 2009. Considerable variation in the concentration of osteopontin in human milk, bovine milk and infant formulas. *J Dairy Sci*., 92, 5378-5385.

Smith ML, Sharma S, Singh TP, 2022. Iodide supplementation of the anti-viral duox-lactoperoxidase activity may prevent some SARS-CoV-2 infections. *Eur J Clin Nutr* 76:629–630; https://doi.org/10.1038/s41430-021-00995-2

Sugita C, Shin K, Wakabayashi H, Tsuhako R, Yoshida H, Watanabe W, Kurokawa M, 2018. Antiviral activity of hypothiocyanite produced by lactoperoxidase against influenza A and B viruses and mode of its antiviral action. *Acta Virologica* 62: 401 – 408.

Ten Bruggencate SJ, Frederiksen PD, Pedersen SM, Floris-Vollenbroek EG, Lucas-van de Bos E, van Hoffen E, and Wejse PL, 2016. Dietary Milk-Fat-Globule Membrane Affects Resistance to Diarrheagenic Escherichia coli in Healthy Adults in a Randomized, Placebo-Controlled, Double-Blind Study. *J Nutr* 146, 249–55.

Timby N, Domellöf M, Lönnerdal B, Hernell O, 2017. Supplementation of Infant Formula with Bovine Milk Fat Globule Membranes. *Adv Nutr*, 8, 351–5; doi:10.3945/an.116.014142.

Timby N, Hernell O, Vaarala O, Melin M, Lönnerdal B, Domellöf M, 2015. Infections in infants fed formula supplemented with bovine milk fat globule membranes. *J Pediatr Gastroenterol Nutr* 60, 384-9. doi: 10.1097/MPG.0000000000000624.

Varım C, Demirci T, Cengiz H, Hacıbekiroglu I, Tuncer FB, Çokluk E, Toptan H, Karabay O, Yıldırım I, 2021. Relationship between serum osteopontin levels and the severity of COVID-19 infection. *Wien Klin Wochenschr* 133:298–302. https://doi.org/10.1007/s00508-020-01789-5

WHO, 2005. Benefits and Potential Risks of the Lactoperoxidase system of Raw Milk Preservation. *Report of an FAO/WHO technical meeting FAO Headquarters*, Rome, Italy, 28 November – 2 December, 2005.

Zavaleta N, Kvistgaard AS, Graverholt G, Respicio G, Guija H, Valencia N, Lonnerdal B, 2011. Efficacy of an MFGM-enriched Complementary Food in Diarrhea, Anemia, and Micronutrient Status in Infants. *J Pediatr Gartoenterol Nutr.* 53, 561-568.