Immune Supporting Properties of Milk
Part 5: The Immunoglobulins – Milk's Guided Missiles

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

Introduction

There is significant evidence suggesting that milk evolved to provide the newborn with defense against infections. From an evolutionary perspective, the provision of nutrients to the suckling infant by milk was a secondary function. The immune supporting system contained within milk has a complicated architecture but the components can be assigned as a part or members of either the adaptive or the innate branches. The multiplicity of components in milk that contribute to these two branches of the immune system were introduced in the opening article in this series. The subsequent 3 articles addressed several key contributors to the innate system. The second article exclusively discussed lactoferrin, a multifunctional protein which could be described as the 'Swiss Army Knife' of the innate system in milk. Parts 3 and 4 described a further 8 distinct members of the arsenal of milk-borne innate system components. This final 5th article wraps up the series and components of the adaptive system take center stage. As in the previous articles, recent findings and reports of potential efficacy against Sars-Cov-2, the causative agent of COVID-19 will be highlighted.

Immunoglobulins - The sharp tip of the adaptive immune system

The adaptive immune system is what most people associate with immunity. The adaptive system is comprised of the 'smartest weapons' in the immune arsenal and the antibodies that make up immunoglobulin fraction in milk are the tip of the spear. Note that 'antibody' is a descriptive term that reflects the functionality of the group of proteins that comprise the immunoglobulins which are found in the whey fraction of milk[1].

As well as being a strength, the complexity of the 'weaponry' associated with the adaptive system can also be a weakness. In simple terms, it takes time to either identify existing or develop a new antibody that is specific to an antigen on the surface of an invading pathogen and then to synthesize it on a sufficient scale. Once the immune system is 'mature' the B-cells of the infected host, the guardians of the 'antibody libraries', search them to identify whether an effective antibody exists in the cell archives. Meanwhile, new antibody

[1] In many cases the term antibody and immunoglobulin are used interchangeably. Immunoglobulin is a more precise term as it usually allows identification of the sub-class of immunoglobulin molecule. For example, IgG refers to immunoglobulin type G etc.

variants are synthesized directly as a 'backup plan'. The latter process involves segments of the antibody (immunoglobulin) genes undergoing recombination, generating an enormous repertoire of antigen-binding sites segments (the variable region) of the immunoglobulin molecule. This phenomenon is called "gene rearrangement". This is a time and energy consuming process that is a considerable challenge to the vulnerable newborn. In addition, the adaptive immune system is un(der)developed in many newborns. So the newborn has a very limited, if any 'antibody archive' to search, limiting options to 'new' antibody synthesis alone.

As a stopgap, the suckling infant's adaptive immune system is 'jump started' by receiving ready-made antibodies from the mother through her milk. As the infant plays no part in the synthesis of these antibodies, their receipt by the infant from the mother is described as passively-acquired adaptive immunity. The importance of this transfer varies between species. For example, foals are particularly vulnerable to life threatening infection during the first weeks of life, if they do not consume the mare's colostrum shortly after birth. Indeed, in most mammals, the concentration of immunoglobulins is highest in colostrum, the first milk, and gradually declines as the composition transitions to mature milk over a period of months. The 'targeting' profile of the antibodies in milk also changes with time, as it reflects the current 'palette' of antibodies synthesized by the mother, which in turn mirrors the infectious challenges she, rather than the infant has encountered at any given point in time.

The mothers' antibodies are secreted into the whey fraction of colostrum and milk in the mammary gland. The most abundant and familiar antibody type is the 'Y' shaped immunoglobulin G (IgG) as illustrated in **Figure 5.1**. The stem of the 'Y' is conserved and the outer tips of the 'Y' contain the variable parts, which house the antigen binding sites of the molecule. Other immunoglobulin types such as secretory IgA and secretory IgM are also present in milk. These are complex molecules and require adaptation to efficiently transfer into the milk – hence the prefix 'secretory' IgA and IgM (**Article 1, Table 1**). It is notable that the prevalence of immunoglobulin type in milk varies between species. The most abundant Ig type in breast milk is secretory IgA, whereas IgG is the predominant type in cow's milk.

The sequence of events that precede and follow the binding of an antibody to an antigenic sequence on the surface of a pathogen is beyond the scope of this article. Only a summary of key steps for IgG is presented as an illustration in **Figure 5.1**.

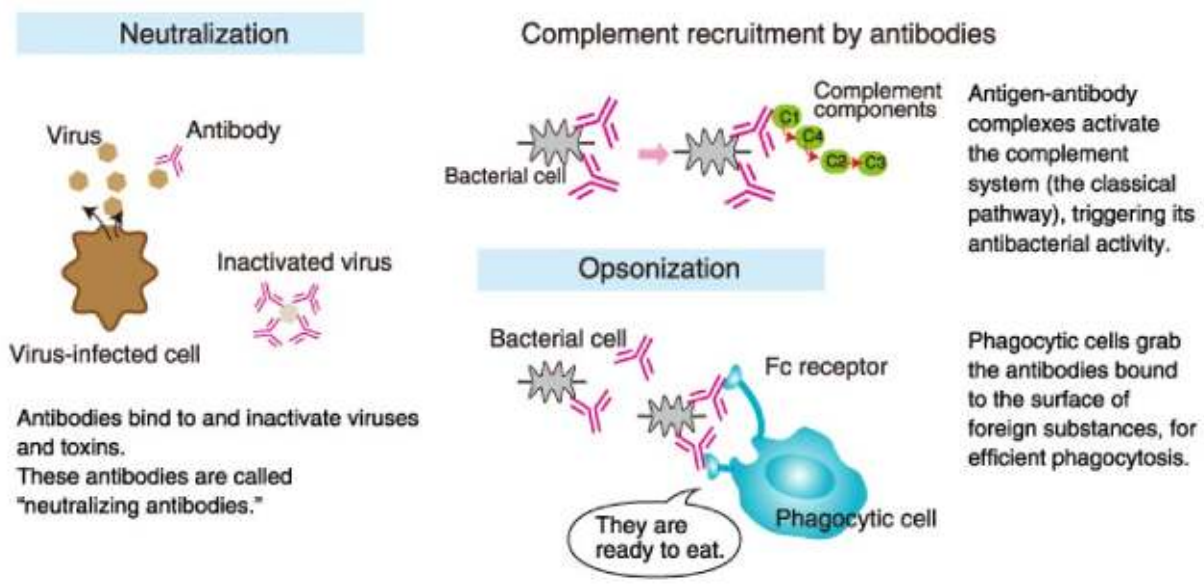

Figure 5.1: The roles of antibodies (immunoglobulin). Adapted from https://www.mblbio.com/bio/g/support/method/antibody-role.html

IgG contributes to immune defense in three distinct pathways. Firstly, in the case of viruses, IgG can attach to the cell binding site on the surface of freely circulating target viruses, interfering with the virus's ability to bind to and infect another host cell – the neutralization mechanism. Alternatively, IgG can interact with antigenic sites of pathogen and act as an anchor for other components of the immune system – a mechanism called complement recruitment. This initiates a cascade of events resulting in destruction of the invading component. Finally, IgG can 'mark' a target infected host cell or infecting pathogen cell for disposal by phagocytic cells of the immune system – so-called opsonization.

The adaptive system once set in process destroys and eliminates these 'marked' cells. This lethal capability requires multiple checks and balances not only to stimulate a quick reaction to a threat but also a damping of response to ensure it does not accelerate out of control and cause extensive damage to 'self'. Milk also delivers multiple components that contribute to these vital processes via up- and down-regulation of various cells and components of the immune system. The roles of several of these immune modulating components of milk including osteopontin and xanthine oxidase (Article 4), α-lactalbumin (Article 3) and lactoferrin (Article 2) were discussed in previous articles.

Are the immunoglobulins in cow's milk only effective against infectious pathogens found in the barn?

The spectrum of immunoglobulins in a given sample of milk is a reflection of infections that the lactating mother most recently encountered, perhaps during pregnancy and certainly since giving birth. Consequently, the immunoglobulins in cow's milk reflect challenges that the cow recently encountered in the barn or during grazing. That does not mean that cow's milk immunoglobulins have no value. For example, cow's are vulnerable to respiratory infections such as pneumonia that can also have serious effects in humans. In the U.S., pneumonia is the most common reason for hospitalization of children under 5 and is the recorded cause of death of 50,000 adults each year (American Thoracic Society, 2019).

The specificity of the antibodies in milk can also be adjusted by vaccination. This was the basis of the CDC advice to pregnant mothers to receive the COVID-19 vaccination. This ensures passive transfer of immunity to the developing fetus during pregnancy and subsequently to the infant via the mother's milk should she breastfeed. In similar fashion, it has been shown that the specificity of the immunoglobulins present in cow's milk can also be adjusted through vaccination. Over the years, multiple companies have produced hyperimmunized cow's milk to use as a basis for the formulation of products with targeted immunoglobulin specificity. One of the most longstanding products is NuVim® (https://nuvim.com/), which is a whey protein-containing beverage powder still sold as a supplement in the U.S. The original technology on which this product is based was developed by Stolle Milk Biologics (https://smbiologics.com/) in 1958 (Mehra et al., 2006).

Figure 5.2: NuVim® MUNEFLEX™ powdered nutritional supplement containing cow's whey immunoglobulin. (Source: https://nuvim.com/about/).

Vaccination of cow's has been considered as a strategy to slow the escalation of the avian flu and SARS pandemics (Alisky, 2009) and more recently COVID-19 (Arenas et al., 2021; https://www.science.org/news/2020/06/cow-s-antibodies-could-be-newest-weapon-against-covid-19). This may sound like science fiction but variations of this technology have been used for over a century! One of the first applications was in the development of antivenoms for snake bites (Pucca et al., 2019). Whilst the mechanism of action was not understood at the time, antivenoms were developed by injecting attenuated snake venom into horses, donkeys or sheep (the immunization step), extraction of serum from the blood of these animals, followed by its injection into the victim of the snake bite. In this example, the antibodies are isolated from blood plasma rather than milk but they will also be present in milk too. Exploiting antibodies in plasma is at the root of convalescent plasma therapy, which was used successfully in the dark, early days of the COVID-19 pandemic to treat seriously ill patients. The results from early studies were inconclusive but this was attributed to the limited availability of human donors that had recovered from COVID-19. The resulting short supply of therapeutic plasma limited it to the treatment of patients with the most advanced and serious COVID-19 infections. Some of these shortcomings may be addressed in an upcoming Stage 3 trial of SAB-185, a fully-human polyclonal antibody therapeutic isolated from transchromosomic (Tc) Bovine™ herds. These transgenic animals produce fully-human antibodies targeted at specific diseases, including COVID-19 and influenza (https://www.sab.bio/2021/09/24/sab-biotherapeutics-announces-sab-185-receives-positive-dsmb-review-and-advances-to-phase-3-in-nih-sponsored-activ-2-trial-for-treatment-of-covid-19/).

Can cow's milk immunoglobulins survive digestion in the human GI tract?

During feeding, milk floods the oral cavity where the gastrointestinal and respiratory tracts converge. This delivers milk immunoglobulins to precisely the entry point to the host that is take by most infectious agents. This helps to explain the significant reduction in risk of respiratory, ear and gastrointestinal infections observed in breastfed infants up to 6 months of age compared to formula fed subjects. The benefits appear to persist much longer too. In a recent study, the reduced incidence of gastrointestinal and ear infections continued through each 3 month period of extension of breastfeeding up to 18 months (Frank et al., 2019).

For many years, it was assumed that benefits deeper into the gastrointestinal tract rapidly diminished due to digestion. The higher pH of the underdeveloped stomach of the infant was considered essential to reduce the extent of digestion of immunoglobulin in the stomach. However, this benefit does not appear to be limited to the upper GI tract as evidence shows that significant amounts of immunoglobulin survive digestion and retain functionality through to infant feces (Ulfman et al., 2018). Surprisingly, this is not limited to infants. Other studies have reported that significant amounts of milk immunoglobulins survive intact at least through the small intestine also in adult subjects (Jasion & Burnett, 2015).

Ulfman et al. (2018) provide an excellent review of not only the limited digestion of milk immunoglobulins but also the results from clinical studies in infants, children and adults. Their publication includes studies on colostrum and isolated Ig fractions from both normal and hyper-immunized animals. The majority of clinical data involves infant subjects. The presence of IgG in normal cow's milk that was active against rotavirus was first reported in the 1970s (Ellens et al., 1978). Since then, multiple studies have delivered similar

findings with milk from non-immunized cows, demonstrating clearly that diarrhea resulting from rotavirus infections in infants can be prevented by consumption of colostrum or immunoglobulin.

The strongest evidence supporting the protective effects of bovine immunoglobulins from non-immunized cows in adults comes from studies in HIV patients afflicted with recurrent diarrhea. As HIV patients are strongly immunosuppressed, they have diminished capacity to resist infections and are highly susceptible to diarrhea, especially induced by *Cryptosporidium, Amoeba* and *Campylobacter*. Multiple studies (6) reported reduced stool frequency, decreased fatigue scores, increased subject weight and T cell counts (Ulfman et al., 2018) in HIV patients receiving bovine colostrum..

These studies clearly demonstrate that the administration of passive immunity in the form of bovine immunoglobulins can be protective against a range of pathogens across different age groups. In broad terms, the results of the multiple clinical studies suggest that normal milk and colostrum is effective against rotavirus but that defense against bacterial infection is frequently more effective with milk or colostrum from hyperimmunized cows. Further research is required to establish is this is a significant observation and that it reflects the different levels of complexity between these two classes of pathogen.

Concluding Remarks

This closing article draws to a close this whirlwind tour of how milk delivers a complex package of components to support immunity. It was necessary to break down the exquisitely complex immune support system that is found in milk into manageable groups of components across the five articles to explain how the different elements function. As a result, it is tempting to consider the milk system as a group of individual components and lose sight of the fact that it has evolved as an interacting, complementary mixture that coexists in the complex (nutrient) matrix that is milk.

It remains unclear just how important it is to maintain this natural balance of these immune active components as found in milk. However, it should be remembered that this complex mixture has persisted through millions of years of evolution. Evolution usually does not allow unnecessary, non-functional or redundant components to survive. If it did it would place a drain on nutrient and energy resources. It seems likely that synergism exists between individual immune modulating components in milk that delivers a greater benefit than the sum of the individual parts. Indeed, the balance of the mix of immune modulators in cow's milk may already have been adjusted inadvertently through breeding and diet by the dairy farmer in the quest for increased milk productivity per cow. Over multiple decades the target has been increased volume and protein and fat content. It is unclear whether hidden behind these advances, the potency of the immune supporting mix has been diminished. Nevertheless, cow's milk retains an impressive array of weapons in its arsenal to fight infection.

In many respects, developments in infant formula are in the vanguard of the quest to address how immune supportive functionality can be re-instated in a dairy-based product. Many new product launches are shifting focus from nutrition alone and to boosting levels of immune supportive ingredients such as lactoferrin, milk fat globule membrane and oligosaccharides, to reflect levels found in breastmilk. (Incidentally, this pathway is the reverse of that followed by evolution, i.e. immune support first, then nutrition).

Drinking or consuming dairy products can potentially deliver components of the innate and passive adaptive immune system to the primary point of entry of an infectious agent to the host –

While milk evolved to provide immune and nutritional support to vulnerable infants, these benefits translate to consumers of all ages. Infants, children and adults share similar anatomy at the junction between the oral/gastrointestinal and respiratory passages. The moist mucosal surface that coats these passages provides an abundance of attachment points for pathogenic bacteria and viruses. Flooding this area with a sticky, mouthcoating fluid rich in anti-microbial components – milk - would appear to be a logical defensive strategy to counter infection. In my opinion the dairy industry does not capitalize enough on the opportunity space that this presents. Milk is an amazing bio-functional system, the complexity of which will likely never be challenged or replaced by cultured dairy protein expressed by a GMO in a fermentation tank! We have this complex system ready assembled by the cow. Why not focus on developing dairy products based on maintenance of the component balance of the natural milk immune support system through mild processing. For example, the system could be kept intact and native by concentration using mild modern processes, such as cold filtration. The objective would be to minimize denaturation of proteins, preserving them in their bio-functional native state. This would provide a liquid raw material – or by using a mild drying process – a powder, which could be the starting point for development of a range of dairy products based on preservation of the natural balance of immune supporting ingredients designed and built by nature.

Making structure-function claims for milk and immune support may be some way off. Currently, most authorities recommend consumption of dairy products as a good source of vitamins A, D and protein to support a healthy immune system. However, some countries have gone further. For example, in March 2020, the Chinese Centre for Disease Control and Prevention's (CCDC), National Institute for Nutrition and Health and the Chinese Medical Doctor Association joined with the Chinese Dairy Industry Association and issued revised guidelines for dairy consumption during the COVID-19 pandemic. The document included an interesting reference to lactoferrin and α-lactalbumin as follows:

"Research [has] shown that lactoferrin inhibits virus invasion by blocking HSPG (heparan sulfate proteoglycan), the anchor point where a virus binds to and invades into a cell) on the cell membrane, thus playing an important role when [a virus such as] SARS-CoV infects the human body,"

and

"[α-lactalbumin may potentially also] affect the immune response, by regulating the intestinal flora or [stimulating] glutathione synthesis, thereby improving immune function."

It seems that others are already thinking in this way. Let's see how things develop.

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