THE ROLE OF THE BIOACTIVE FACTORS IN BREAST MILK ON THE IMMUNE SYSTEM OF THE INFANT

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Abstract: *Bioactive factors in breast milk represent a "true immune system". It compensates for the adaptive immune system immaturity of the newborn, as well as for the insufficient development of many aspects of the innate immune system. The immunological action and the protective benefits of breastfeeding against the infections acquired in the infant and young child made up the subject of numerous recent studies. They come, on the one hand, to bring strong support, with clinical records, to the concept related to the importance of breastfeeding in the normal development of the immune system in infants, and on the other hand, they support the constant concern of the manufacturing companies to include additives in formulas milk, in order to bring them as closer as possible to the breast milk. This paper presents the bioactive factors in breast milk with their functions and mechanisms of action in the adaptive and innate immune response. It also illustrates the aspects related to the natural dynamics of the immune benefits of breast milk. In conclusion, the role of bioactive factors in immunity has emerged along with the evolution of the concept of "immune system in breast milk", being shown clear correlations between this one and the immune status of the breast-fed infant and his subsequent immunological development.*

Cuvinte cheie: *sistem imun, factori bioactivi, alăptarea, imunomodulator*

Rezumat: *Factorii bioactivi din laptele matern constituie un „adevărat sistem imun". Acesta compensează imaturitatea sistemul imun adaptativ al nou-născutului, precum şi insuficienta dezvoltare a multora dintre componentele sistemului imun înnăscut. Acţiunea imunologică şi beneficiile protective ale laptelui matern contra infecţiilor dobândite la sugar şi copilul mic constituie subiectul a numeroase studii recente. Acestea vin, pe de o parte, să aducă o puternică susţinere, cu evidenţe clinice, a conceptului legat de importanţa alăptării în dezvoltarea normală a sistemului imunitar la sugar, iar pe de altă parte sprijină constanta preocupare a companiilor producătoare de a include aditivi în formulele de lapte, care să le aducă cât mai aproape de laptele matern. Această lucrare prezintă factorii bioactivi din laptele matern cu funcţiile lor şi mecanismele de acţiune în cadrul răspunsului imun adaptativ şi înnăscut. De asemenea ilustrează aspecte legate de dinamica naturală a beneficiilor imune ale laptelui matern. În concluzie, rolul factorilor bioactivi în imunitate s-a conturat o dată cu evoluţia fundamentării conceptului de „sistem imun în laptele matern ", fiind evidenţiate corelaţii clare între acesta şi statusul imun al sugarului alăptat, precum şi al dezvoltării sale imunologice ulterioare.*

The immunological action of breast milk is a topical issue and represents the starting point for many studies that support the concept of extending breastfeeding as much as possible. These studies document the protective effect of the bioactive factors in breast milk, including respiratory infections, otitis media, infections of preterm infants and provide data to support the current recommendations of the American Academy of Pediatrics for exclusive breastfeeding up to six months. Thus, we are witnessing strong support with clinical records, of the concept related to the importance of breastfeeding in the normal development of the infant immune system and its defence against infectious diseases during childhood, when the immune system is still immature.(1)

The concept of immune system in breast milk

The idea of the concept of the existence of an "immune system in breast milk" started in 1970. This concept is strengthened towards the end of the XVIIIth century and mid-twentieth century with clinical observations and laboratory data. The last link in the chain of evidence that underpinned the concept of "immune system in breast milk" was the discovery of the presence of leukocytes in the breast milk. The concept was later extended to include the immunomodulatory agents among the antimicrobial and anti-inflammatory agents (table no. 1).(2,3)

Table no. 1. Factors in breast milk produced late by the infant's immune system

Antimicrobial agents	Anti-inflammatory agents	Immunomodulatory agents
Secretory IgA, lactoferrin,, lysozyme etc.	IL-10, lactoferrin,, lysozyme, PAF-Acetyl Hydrolase etc.	IL–4, IL–10, IL–12, IFNγ, TNFα, G–CSF, RANTES, T cells with memory etc.

These agents intervene to compensate for the delay of the immune system during childhood. This explains the role of breast milk in fighting against common childhood infectious diseases or immune-mediated disorders such as Crohn's disease. It seems that the immunomodulatory action of the bioactive factors manifests itself at the level of other mucosa than at the gastrointestinal mucosa level. These diverse results emphasize

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the superiority of breast milk in infant feeding. The current concerns for extending breastfeeding and the concerns of the companies producing milk powder regarding the introduction of some bioactive factors as close to those present in breast milk emphasized lately more and more components of the breast milk, as well as their functions in the further development immune system of the child.(4)

Correlations between the immune system of breast milk and breast-fed infant immune status

In the evolution towards substantiating the concept of *"immune system in breast milk"*, there have been revealed some correlations between this one and the immune status of breast-fed infant, as well as the subsequent immunological development. These correlations can be structured as follows:(3,5)

1. There is some delay in postnatal development of the immune system of children, which are compensated by the agents present in breast milk:
   - direct compensation: examples: secretory IgA, lactoferrin, lysozyme, cytokines;
   - indirect compensation: examples: nucleotides, oligosaccharides
2. Defence anti-infective agents in breast milk are resistant to digestive enzymes and are thus able to fulfil their functions in the digestive tract of the infant and beyond the gastrointestinal mucosa. Examples: secretory IgA, lysozyme, lactoferrin, oligosaccharides.
3. Also, in certain components of breast milk, there are also released other defence agents, due to their partial digestion in the gastrointestinal tract of the child. Examples: lactofericina, βcasomorfina, fatty acids, monoglycerides.
4. Cells that produce antibodies in breast milk have intestinal and bronchial origin.
5. Defence factors in breast milk protect against pathogens without causing inflammation. Moreover, some of the bioactive factors in breast milk inhibit inflammation, while others have immunomodulatory activity.
6. Certain bioactive factors in breast milk can initiate and enlarge the less expressed functions of the immune system of the child. Examples: cytokines, anti-idiotypic antibodies.
7. Some agents in breast milk can alter the physiological state of the digestive tract to adapt to the extra uterine life. Example: The influence of the permeability of the intestinal tract.
8. The interaction between the defence agents of breast milk and certain pathogens cause an immune response from the part of the child's body without the occurrence of symptomatic infections.
   Examples: immunity acquired for the infection with cytomegalovirus.
9. Certain bioactive factors in breast milk bring about the logarithmic increase of the enteric commensal bacteria in infants' gut. Thus, Bifidus factor and some oligosaccharides accentuate the increase of bifidobacteria and lactobacilli.

Classification of bioactive e factors in breast milk

Bioactive factors can be classified according to several criteria: the functions they accomplish (figure no. 2), their chemical nature, their mechanism of action (figure no. 3) within the adaptive immune response and the innate immune response. Also, the various bioactive components are present in breast milk in varying amounts, depending on the stage of lactation, the mother's health, psycho-social condition, and last but not least by the mother's immune status.

The benefits of breastfeeding on the development of the immune system and the anti-infectious defence in infants and toddlers.

There are several studies showing that shortening the breastfeeding period increases the risk of developing lower respiratory tract infections and digestive infections, and therefore, an increase in admissions caused by these diseases in infants and small children. The main findings of these studies were that breastfeeding up to 6 months seems to have protective effects on the respiratory and gastrointestinal development of the child during the first six months. Also, breastfeeding up to four months or more proved to protect the infant against lower respiratory tract infections in ages between 7 months and 12 months.

Table no. 2. Classification of the bioactive factors in breast milk according to their functions

Categories	Types
Antimicrobial factors	Secretory IgA, lactoferrin, Lactaderina, Lysozyme, α-lactalbumin, Fibronectin, defensin, MIF (macrophage migration inhibitory factor), MUC1, C3, CCL-28
Anti-inflammatory factors	Prostaglandins E2, F2α, lactoferrin, lysozyme, EGF and polyamines, Cortisol, PAF acetyl hydrolase, α1-antichemotripsin, uric acid, ascorbate, βcaroten, IL-10, TGFβ1
Immunomodulatory factors	IL-6, MIF, M-CSF, G-CSF, GM-CSF, erythropoietin, IL-10, TGFβ1, IL-8, RANTES, MIP-1, CCL-28, IL-7, IL-4, IL-10, TGFβ2, MIG, IL-10, IL-12, TNFα, IL-18, IFNγ

Table no. 3. Classification of the bioactive factors in breast milk according to their mechanism of action within the immune response

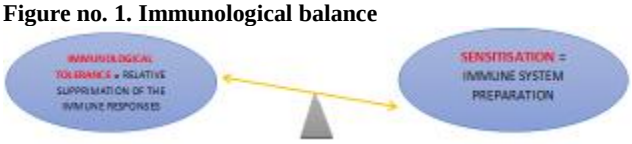
Components of the adaptive immunity	Components of innate immunity
Humoral Immunoglobulins: secretory IgA, IgG, IgM, IgE, IgD Anti-idiotip antibodies Cells -Monocytes -Neutrophils -Lymphocytes	Antimicrobial factors: complement chemotactic factor, interferon, α-fetoprotein, α-lactalbumin, Lactaderina, mucins, defensins, cytokines, chemokines and receptors: proinflammatory and immunomodulatory: IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-16, IL-18, TNFα, IFNγ, M-CSF, G-CSF, GM-CSF Anti-inflammatory: IL-10, TGFβ1 Prebiotic factors: Bifidus Factor, oligosaccharides Proteins with transportation role: lactoferrin, transferrin, vitamin B12-binding protein Proteins with enzymatic role: lysozyme, lipoprotein, antiproteases (α1-antitrypsin and α1-antichemotripsina). Other immunomodulatory proteins: Nucleotide

The constituents of breast milk that promote the immune system sensitivity and the immunological tolerance of cause a significant immunological development of the naturally fed infant. Regarding responses to antigens, during childhood there is a fine balance between tolerance and sensitization (figure no. 1).

It was hypothesized that food intolerance during childhood would be the result of failure to adequately develop tolerance and the successful development of the immunological tolerance contributes to reducing the incidence of food allergies in the children who were breastfed for a longer period of time.

Tolerance is an active process and there are in vitro studies suggesting that food antigens present in breast milk stimulates food tolerance and intestinal microflora of the infant through the immunosuppressive cytokines IL-10 and TGFβ.(6,7)

Figure no. 1. Immunological balance



One of the benefits of breastfeeding in the development of the infant immune system is related to *maternal Major Histocompatibility Complex (MHC)*. Thus, there are clinical data supporting that breastfeeding can cause the child to develop a tolerance to MHC antigens of the mother. Thus, in case of a kidney transplant from mother to child, it has been shown statistically, that the survival rate is higher in the children who had been breast fed. Also, the development of a "tolerance to MHC antigens of the mother" is important for the contact between the immune cells contained in breast milk (macrophages, neutrophils, lymphocytes, endothelial cells) and the cells of the immune system of the infant. Recent data support the intervention of long chain polyunsaturated fatty acids (LC-PUFA) in the development of the immunological tolerance.(8,9)

Scientific evidence advocating for the *sensitisation* of the immune system of the child through breast milk immunogenic factors has led to increased interest in using this capacity to the transfer of maternal vaccines. Therefore, it has been attempted to use breast milk as a vehicle for various vaccines, being influenced by the exposure to anti-idiotypic antibodies n the milk.(10)

Natural dynamics of the immune benefits of the breast milk

The intestinal immune system of the child is growing rapidly in the early postnatal period of time due to the contact with food and microbial antigens. In addition, in order to eliminate the infectious agents and to minimize the damage caused by them, the immune system must develop the ability to discriminate between antigens that are harmless (as against it shows tolerance) and those that are potentially dangerous. It is believed that the induction of tolerance occurs first in the intestine and it is facilitated by the specialized B and T cells, by the production of secretory IgA and by the distortion of the Th2 lymphocytes response. Failure to regulate immune tolerance and active immune responses contributed to the issuance of the assumption that there is a close relation between food and allergy, as well as between autoimmune system and the inflammatory bowel disorders. Walker and Wagner and many other researchers have investigated the dynamic changes, the interactions and the evolution of breast milk, as well as the immune benefits offered to the child. The dynamic nature of breast milk, and all the bioactive factors, the interaction of the child with the mother's immune system through breast milk, makes this one truly "unique", incomparable, the "ideal nutrition for infants".(8,11)

In a first instance, breast milk evolves during the lactation progresses in terms of volume, biochemical composition and content of the bioactive factors. The ontogeny of child development is dependent on the evolution of breast milk to provide not only the necessary nutrients, but also the immune protection, immune stimulation and modulation of the development through some important components supplied in suitable quantities during different periods of development and continued adaptation to the extra uterine life. There are many factors that influence the volume and composition of breast milk: lactation stage, parity, milk production volumes, infant feeding, maternal diet, maternal energy status, health status, family stress.(12)

The composition of various bioactive factors in breast milk also varies during lactation. The development of the mucosal immune system of the child, *as well as the systemic immune system show different needs for different factors, according to their role and effect on the child.* Therefore: secretory IgA presents the highest values in colostrum and transitional milk; lactoferrin levels decrease during the first 12 weeks of lactation; lysozyme levels increase during the first 12 weeks of lactation; Both proteins, lactoferrin and lysozyme, remain relatively constant in breast milk between the 6th month and the 24th month of lactation; Relative percentages of individual nucleotides and the total potentially available nucleotides in breast milk changes over time (from colostrum to mature milk).(8,13)

In the human host, there is a dynamic and dramatic change in nutritional requirements due to the metabolic response to infections. In an acute infection, there are several changes in the metabolic response of the host. There are described many variables that contribute to this response. Growth and nutritional status prior to infection, immune function, infection severity, duration and progression, nutritional intake of the individual are as important as the location of the infection in certain organs: *gastrointestinal infections limit the availability and absorption of nutrients; liver infection alters the metabolism of carbohydrates and amino acids, the shock causes metabolic disorders (hypoxia, acidosis and uncoupling of oxidative phosphorylation).*

A simple example of the benefits of breastfeeding during infection is improving the condition of infants with diarrhea, when resorting to continued breastfeeding or when this is reintroduced early (fluid supplementation is no longer necessary). There is evidence of the presence in milk of several factors (*IGAS, glycans)* that act against some *specific infectious agents* that cause diarrhea in children. The metabolic response in an *acute infection* requires increased amounts of *carbohydrates and amino acids* for *energy* production, and *nucleotides* to activate the *cellular immune response*.(3,14,15)

Another aspect of the *dynamic nature* in terms of immune protection of children through *bioactive factors* in human milk is that they act *additively and synergically.* Isaacs describes the synergistic effect of certain antiviral specific lipids and of some peptides against HSV (herpes simplex virus). Inactivation of HSV is synergic, the components attack the pathogens in different points of replication and requires lower concentrations of factors and less time to effectively inactivate the virus.

The anti-microbial activity of breast milk is not measurable only in terms of quantity of a specific factor or of its apparent activity in vitro. As an example, one of the antiviral activities of lactoferrin is its proteolysis dependence in vivo, releasing anti-HSV peptides, which were not emphasized in vitro.(16,17,18)

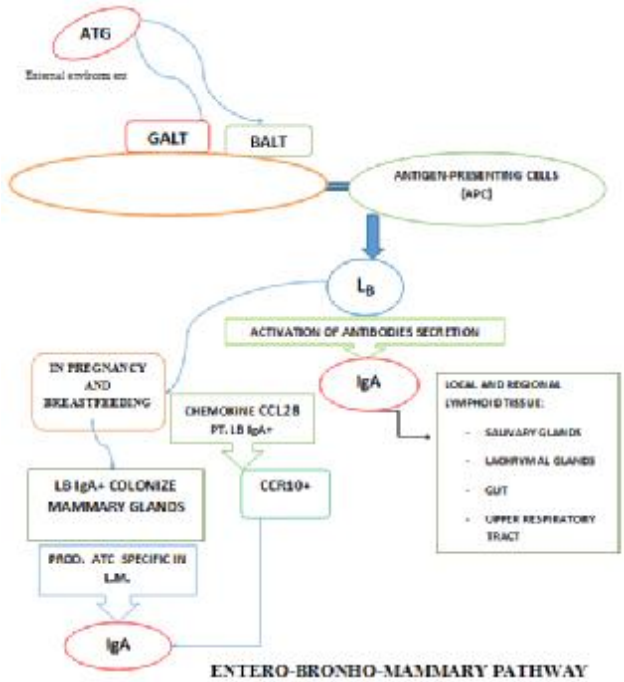
The most important contribution to the dynamic nature of breast milk is the *MALT system (mucosa-associated lymphoid tissue).* When a child and mother are exposed to a potential pathogen in the environment, the mature immune system of the mother can react more quickly and efficiently than the child's. The contact of the pathogen agent with mother's mucosa (respiratory, intestinal or vaginal) leads to an immune response that can increase the number of cells, including the secretory IgA specific cells and cytokines, in breast milk for the baby.

Also, additional nutrients (carbohydrates, amino acids, fats and nucleoside) and micro-nutrients (vitamins, zinc and selenium) are immediately made available for the child, in order to accelerate the metabolic response to infections. These occur before the mother is aware of the exposure to infection, or to the potential risk of the infant through their mutual exposure. Most of the ***protective components*** of breast milk may interact

synergistically with each other or with factors related to the immune response of mucosa and by the systemic immune response.

The entero-broncho-mammary pathway of the secretory IgA B lymphocytes (LB IgA +) and the mucosal immune system are considered a means of transferring high specific protection from mother to child through breastfeeding.(19)

Figure no. 2. Entero-broncho-mammary pathway



ENTERO-BRONHO-MAMMARY PATHWAY

Therefore, when the nursing mother is exposed to an antigenic stimulus (pathogens from the environment), the cells of the mucosal immune system become antigen-presenting cells (APC); these are: M cells at the level of the Payer plaques in the gut-associated lymphoid tissue (GALT) and the cells at the level of mucosa of the bronchus-associated lymphoid tissue (BALT). CPA presents environmental antigens B lymphocytes, so they are activated to secrete IgA that will migrate to the local and regional lymphoid tissues. Migration of IgA is typically to the salivary glands, lachrymal glands, intestine, upper respiratory tract, urogenital tract. During *pregnancy and lactation*, due to the *hormonal secretion*, there occurs the colonization of the mammary glands with antibody-producing B lymphocytes (LB IgA +) that secrete in the milk specific IgA, which can bind pathogens and prevent infections.(19)

CONCLUSIONS

1. The immaturity of the adaptive immune system of the newborn and the insufficient development of many aspects of the innate immune system are two reasons, strong enough, to advocate the infant feeding.
2. The bioactive factors in breast milk bring benefits to the immune system of the child through a variety of mechanisms, including the direct or indirect anti-microbial activity, the stimulation of the development of the immune function, the modulation of the immune function, the anti-inflammatory effects, the improvement of tissue growth and development of the child.
3. All these activities of the bioactive factors in breast milk led to the emergence and consolidation of the concept of *"immune system in breast milk"*.
4. The agents present in breast milk intervene in the postnatal development of the immune system of the child, directly speaking, exercising their biological functions, or indirectly, by triggering some immunological mechanisms.
5. The bioactive factors in breast milk can interfere with the immune system development in nursing infants due to protection through the digestive enzymes by protease inhibitors, particularly α1- antitrypsin and α1-antichymotrypsin.
6. Protease inhibitors are added two other mechanisms that promote survival and absorption of polypeptides immunologically active under an undamaged form, namely reduced gastric digestion of proteins in the first 3 months of life due to limited secretion of pepsin and H+ ions, as well as the immaturity of the digestive capacities of the newborn.
7. Because the infant gut mucosal cells that release secretory IgA are rare or absent in the infant intestine, it is important that the percentage of IgA2, more resistant to gastric acidity, digestive enzymes and bacterial proteases, is much higher in breast milk than in plasma.
8. The entero-broncho-mammary pathway of secretory IgA B lymphocyte (LB IgA +) and the mucosal immune system are considered means of transferring high specific protection from mother to child through breastfeeding with the involvement of chemokine (CCL28) and CCR10 + receptors.
9. Breast milk also contains bioactive factors that once arrived in the infant's gut bring about the logarithmic increase of the enteric commensal bacteria, prebiotic factors such the bifidogenic factor, oligosaccharides.

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