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## Immunologic components of human milk

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The ontogeny of immune system starts early in the embryo continues during fetal life and is completed only several years after birth.<sup>1</sup> In addition, the fetus, who develops in a highly protective germ-free environment, lacks antigenic experience. These factors cause problems of immunological adaptation during the transitional period from the intra- to the extra-uterine life, and are responsible for the "physiological" immaturity of the immune function in newborn infants.<sup>2</sup>

The unfavourable effects of neonatal immunodeficiency are limited by some naturally occurring compensatory mechanisms, such as the transplacental passage of IgG antibodies from mother to fetus during pregnancy, and the introduction of protective and immunologic components of human milk in the infant. Breast feeding maintains the maternal-fetal immunological link after birth, may favour the transmission of immunocompetence from the mother to her infant, and is considered an important contributory factor to the neonatal immune defence system during a delicate and crucial period for immune development.

### **Antifective and immunomodulatory factors in human milk**

There is convincing evidence that breast feeding may reduce the incidence of gastrointestinal and respiratory infections in infants, and sepsis in VLBW newborns.<sup>3,4</sup> A host of factors with immunologic, hormonal, enzymatic, trophic, and bioactive activity present in breast milk can provide passive protection (Table 1).

In addition, breast milk is rich in maternal cells,<sup>5</sup> that may produce cytokines and exert a modulatory effect on the neonatal immune system. Macrophages and leukocytes, that are mostly concentrated at the beginning of the lactation are mainly included among the cellular components.

Breast milk feeding, therefore, may represent a mean of transmission of immunocompetence from the mother to her infant.

Indeed, some aspects of cellular or humoral specific immune function, NK cell activity, or the response to vaccination have been reported to be improved in breast fed infants.<sup>6</sup>

Most of the immunologic components of human milk may interact synergistically each other or with factors related to the mucosal or systemic immune response. Other factors may develop or may be activated only after they reach the intestinal tract, or may behave differently depending on the context.<sup>7</sup>

The non digestible oligosaccharides, for instance, may directly inhibit the adhesion of diarrheal pathogens<sup>8</sup> or indirectly produce a protective and immunomodulatory result through a prebiotic effect on the infant intestinal microflora.<sup>9</sup>

Particularly well known is the protective role of secretory IgA, which are lacking in newborn infants, but are present at very high concentration in the colostrum (about 1 g/dL), and in mature milk (about 0,1 g/dL); the percentage of IgA2, more resistant to the peptic acidity in the stomach and to digestion by enteric enzymes and bacterial proteases, is much higher in mother milk than plasma; indeed, IgA1/IgA2 ratio is about 6/4 in milk, as opposed to 9/1 in plasma. Neonatal IgA intake with milk is about 0.5-1 g/day. IgG and IgM are also present at lower concentration, and provide the infant with about 10-100 mg/day.<sup>10</sup> The distribution of specific antibodies within IgG subclasses in human milk may compensate for the reduced transplacental transfer of some antibodies, as those against pneumococci.<sup>11</sup>

The entero-broncho-mammary link of IgA+ B lymphocytes and mucosal immune system is considered a mean of transfer of highly specific protection from mother to her infant. When the nursing mother is exposed to antigenic material from environmental pathogens, M cells of Peyer patches in the GALT (gut associated lymphoid tissue) or BALT (tracheobronchial tree mucosa) acquire and present the anti-

**Table 1. Immunologic and protective components in human milk.****SOLUBLE**

- Adaptive immunity compounds: Immunoglobulins sIgA (11S), 7S IgA, IgG, IgM, IgE, IgD, Free secretory component, Anti-idiotypes.
- Cytokines, Chemokines and Receptors: IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-16, IL-18, IFN- $\gamma$ , TNF- $\alpha$ , G-CSF, M-CSF, GM-CSF, GRO- $\alpha$ , MCP-1, TGF- $\beta$  1 e 2, sCD14, Toll Like receptor, sFas, sFasL.
- Histocompatibility antigens.
- Innate immunity factors: Complement, Chemotactic factors, Properdin factors, Interferon, Alphafetoprotein, Anti-staphylococci factors, MBL (mannose binding lectin),  $\beta$ -defensin-1, Anti-adherence substances (Oligosaccharide, Mucins, Lactadherin, Glycolipids and Glycosaminoglycans, k-casein), Milk fat globule, Hormones and Growth factors (Prolactin, Cortisol, Insulin, Thyroxin, Prostaglandins, EGF, VEGF, NGF, TGF, Erythropoietin), Antiviral factors (Fatty Acids and Monoglycerides), Migration inhibition factor (MIF),  $\alpha$ -lactalbumin.
- Carrier proteins: Lactoferrin, Transferrin, Vitamin B12 binding protein, Steroid binding protein.
- Enzymes: Lysozyme, Lipoproteinlipase, Leukocytes enzymes, Anti-proteases, PAF-acetyl-hydrolase.
- Others: Nucleotides, Carrier proteins, LCPUFA.
- Prebiotics, Bifidus factor, Oligosaccharide.

**CELLULAR**

Total counts: Colostrum, 1-3x10<sup>6</sup>/mL; mature milk, ~1x10<sup>6</sup>/mL.

Cell types: Macrophages, ~60%; Neutrophils, ~25%; Lymphocytes, ~ 10%; Epithelial cells.

gen to B cells that become active to secrete IgA and migrate to local and regional lymphs. Then they reach salivary and lacrimal glands, intestine, upper airways, and urogenital tract. During pregnancy and lactation, due to hormonal stimuli, IgA+ B lymphocytes colonize mammary gland, and produce specific secretory IgA that may bind to pathogen and prevent infection.<sup>12</sup>

Several reports seem to confirm that the immunologic components of human milk can influence the infant immune response:<sup>13-22</sup>

- the short term effects on immunologic and inflammatory response;<sup>23-29</sup>
- the long term protection against the development of allergy,<sup>30</sup> insulin-dependent diabetes, Crohn disease, ulcerative colitis and tumors in infancy.<sup>31</sup> The latter effect may also be favoured by the HAMLET, human  $\alpha$ -lactalbumin made lethal to tumor cells, a novel type of protein produced after the modification of  $\alpha$ -lactalbumin, under the conditions that exist in the stomach of nursing infant, that induces apoptosis of tumor cells.<sup>32,33</sup>
- The differences of response to vaccination between breast and formula fed infants.<sup>34-36</sup>

**Cytokines in human milk**

Of the several factors with immunologic, hormonal, enzymatic and trophic activity, cytokines are believed to play a significant role in the immunomodulation and immune-protection of breast milk. Most of the cytokines that are known to be deficient in the neonate, particularly in preterm infants, have been found in significant amounts in breast milk: IL-1 $\beta$ , IL-6, IL-8, IL-10, IL-12, IL-18, IFN- $\gamma$ , TNF- $\alpha$ , TGF- $\beta$ , G-CSF, M-CSF, GM-CSF.<sup>37-45</sup> Breast milk is rich in cytokines producing cells, mainly macrophages and

activated T-lymphocytes; most milk T cells display the CD45RO marker of activation,<sup>46</sup> at variance with the neonatal T cells that are predominantly expressing the *naïve* CD45RA phenotype.

We recently evaluated cytokines production in breast milk by CD4 and CD8 lymphocytes, expressing either the CD45RA or the CD45RO antigen. Intracellular cytokines synthesis of fluorescent stained cells was measured by three-color flow cytometry. Our data showed a substantially higher percentage of cytokines producing cells in breast milk than in cord blood. IFN- $\gamma$  was the most relevant cytokine found in breast milk, as opposed to the very low levels found in cord blood.<sup>47</sup> The high percentages of Th1-type cytokines (IL-2 and, particularly, IFN- $\gamma$ ) may play an important role in the differentiation of neonatal T cells toward a preferential Th1, rather than Th2, pathway. Such hypothesis seems to be in accordance with a report on the differential response to measles vaccination, showing a preferential Th1 activation in breast fed infants.<sup>48</sup>

Immunologic characteristics of maternal milk may vary according to the race. We recently measured the levels of breast milk IL-4, IL-8, IL-10, TNF- $\alpha$  and IFN- $\gamma$  to evaluate their race related variations. These cytokines were chosen due to the possible role in modulation of neonatal immune development.<sup>49</sup> IL-8 levels were significantly higher in milk samples from Asian mothers as compared to African ones; in addition, IL-10 concentration resulted significantly higher in Italian than in African women.

Our data seem to confirm that the immunological factors of breast milk may contribute to one of the most important and peculiar characteristic of human milk, that is its variability of nutritive, bioactive and functional components.

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