

EDITORIAL

Breast milk as an alternative source of cytokines for offspring

Newborn mammalian offspring are relatively immature in their capacity to produce cytokines and other growth factors. The provision of alternative sources of cytokines via the placenta and amniotic fluid antenatally and breast milk postnatally counterbalances the potentially detrimental effects of this. In this respect, the mammary gland predates the placenta in evolution, as the former is well developed in nonplacental mammals such as monotremes (platypus and echidna) and marsupials (koalas and kangaroos). Thus breast milk may be of particular importance in the development of the mammalian newborn [1].

The importance of exogenous sources of cytokines for the health and well-being of the newborn is best demonstrated in $TGF\beta$ knockout mice who do not exhibit a phenotype associated with a deficiency in this cytokine until weaning has occurred. The knockout offspring of wild-type mothers survive until weaning because there is an alternative source of $TGF\beta$ during intrauterine development and suckling as $TGF\beta$ is detectable in both the amniotic fluid and breast milk of the wild-type mice [2]. Mice are not the only animals in which cytokines and growth factors are demonstrable in the pregnancy-associated biological fluids. This is common to all species examined and for a wide array of biological factors.

The biological role of breast milk

Breast milk provides numerous defence factors to the developing gut. Human milk contains antimicrobial agents, such as lactoferrin, which contributes to the protection against infection without provoking inflammation. The passive transfer of immunoglobulins (Ig), predominantly IgA, imparts additional protection particularly at the mucosal surface in the gut. Additionally, breast milk is a source of factors that promote the growth of favourable commensal organisms, such as lactobacilli and bifidobacteria [3].

The major population of leucocytes in breast milk is neutrophils that comprise approximately 80%, with macrophages comprising 15% of the total white cell population [1,4]. Breast milk macrophages have an activated phenotype [5]. The remaining leucocytes are lymphocytes which are predominantly memory (CD45RO+) T cells. There is a relative increase of CD8+ cells in breast milk vs blood such that the CD4:CD8 ratio is 0.88 which is much lower than that in peripheral blood. This may reflect a requirement for CD8+ cells by the gut of the developing newborn [4]. Breast milk leucocytes would only be of value to the newborn if

they survive in the gastrointestinal tract. Breast milk macrophages are resistant to trypsinization enabling survival in the gut [6]. Furthermore, human milk leucocytes enterically administered to baboons adhere to the mucosa and remain there for up to 60 h [7].

A large number of immunomodulatory molecules, including cytokines and growth factors, have been identified in human milk. The cytokines are dominated by those considered anti-inflammatory, such as Interleukin (IL)-10 [8], and are thought to have an important role in regulating responses to very high doses of antigens to which the neonatal gut is exposed. As for the breast milk leucocytes the environment in the neonatal gut, as well as factors present in the milk itself, ensure the biological activity of these molecules is maintained.

Breast milk contains a number of inhibitors of protease activity, including α_1 -antichymotrypsin and α_1 -antitrypsin [9]. Also gastric pH in the neonate is higher than in the adult thereby further limiting proteolysis in the gut. Indeed, there is no evidence of intragastric digestion of proteins over the first few days of life and the stimulation of HCl production in the stomach is very low for the first month of life. Thus conditions in the gut favour the survival and absorption of intact polypeptides, which may therefore transmit immunomodulatory signals. This does appear to be the case as enterically-administered bovine serum albumin is transported intact from the gut of rabbits and can be detected in the plasma [10]. This occurs for several weeks after birth. Also, insulin is absorbed across the intestinal wall of infant rats [11].

The cytokines identified in breast milk include many with well-described roles in immune responses, including, IL-6, $TNF\alpha$, $TGF\beta_1$, $TGF\beta_2$, M-CSF, IL-8 and IL-10. Interestingly, many of these cytokines exist as higher molecular weight forms in breast milk suggesting that they are bound to a carrier molecule. For example, the aqueous fraction of milk contains IL-10 at the expected size of 37 kDa for a cytokine dimer whereas in the lipid fraction it has a molecular weight of 80 kDa [8]. $TNF\alpha$ has long been thought to be bound to a carrier protein with recent evidence suggesting that this carrier protein is soluble TNF receptor as both soluble TNFRI and soluble TNFRII can be detected in breast milk [12]. To what extent the biological effect of the cytokines is modulated by the presence of soluble receptors and other cytokine antagonists is open to speculation. Both IL-1 receptor antagonists and soluble IL-6 receptors have been measured in human breast milk [13]. Thus the proposed effect of a cytokine present in breast milk must be

considered in the context of the levels of possible antagonists in the same sample.

One interesting observation now made by many investigators is that the levels of cytokines in milk collected over the first few days of lactation (colostrum) are in higher concentrations than mature milk collected as lactation progresses. This may compensate for the diminished volume of breast milk ingested during the first few days of life. As the ingested volumes of milk increases the cytokine concentrations decline, thus, the infant receives a relatively constant dose of the cytokine until weaning.

Cellular sources of breast milk cytokines

A number of investigators have addressed the issue of the source of cytokines detectable in breast milk. Studies such as that by Hara *et al.* [14] indicate that they are not derived from serum, M-CSF levels were 10–100 times higher in the breast milk than matched maternal serum and, more importantly, there was no correlation between serum and milk levels. Observations such as this indicate local production by milk leucocytes or breast tissue. Breast milk leucocytes have been identified as containing mRNA for both TGF β 1 and TGF β 2 [15] as well as TNF α [16]. In this issue, Järvinen *et al.* [17] confirm that breast milk macrophages are at least one source of TNF α protein. Epithelial cells are also a source of cytokines. Primary mammary epithelial cells produce both protein and mRNA for M-CSF [14] and IL-10 mRNA [8] and protein for IL-6 and IL-8 have been detected from immortalized mammary epithelial cell lines [18].

Breast milk cytokines and allergic disease

The apparent importance of breast milk as an alternative source of biological mediators in the face of the infant's endogenous shortcomings raises the possibility that an over or under abundance of such factors may have dramatic consequences for the offspring. Allergic disease is one of the best studied outcomes in this respect. Thus prolonged breast feeding has been recommended to prevent or delay the development of allergic disease by infants, however, not all studies confirm the putative advantages of breast-feeding [19–21]. One of the most recently published studies suggests there is a protective effect of breast milk, with exclusive breast feeding for the first 4 months of life associated with a significant reduction in the risk of asthma at 6 years of age [22].

There are a number of studies that have examined the composition of breast milk from mothers whose children develop allergic disease. These studies have concentrated on comparisons in the breast milk from mothers of babies who develop cow's milk allergy (CMA) vs those who do not. In one study the HLA-DR expression on breast milk

macrophages from mothers whose babies had challenge proven CMA was significantly decreased compared to that on cells from mothers who had healthy infants [23]. There was no difference in the number of macrophages recovered from the milk of the mothers in either group.

The authors of this study postulated that decreased expression of HLA-DR may result in decreased antigen presentation by these macrophages which could lead to an adverse reaction in the infant's gut upon subsequent exposure to antigen. These authors have further examined the association of the function of breast milk macrophages with CMA by comparing TNF α production by these cells from mothers of children with or without CMA [17]. Breast milk macrophages from mothers whose infants had CMA produced significantly less TNF α *in vitro* than those from mothers with healthy infants. As in the previous study the authors postulated that this would modify the antigen presenting functions of these macrophages in the gut. However, the authors did not consider the levels of TNF α in the breast milk itself. A negative feedback system may be in operation whereby TNF α levels, possibly derived from nonmacrophage sources such as the epithelial cells result in reduced production of this cytokine by breast milk leucocytes such as macrophages. Thus even in the absence of an abundance of macrophage-derived cytokine the infant may be exposed to appropriate levels. Still, the possibility that altered levels of cytokines in the breast milk modify immune function in the infant's gut is a very attractive hypothesis.

The potentially altered cytokines profiles of breast milk (and placenta and amniotic fluid prior to birth) associated with subsequent atopic disease may explain the peculiar association between maternal atopy and atopic disease in the offspring [24]. A few studies have considered that there may be differences in the cytokines provided in the breast milk of atopic vs nonatopic mothers. Rudloff *et al.* found no differences in cytokine levels, including IFN γ , IL-4, IL-10 and MIP-1 α , in colostrum from allergic vs nonallergic mothers [25]. However in a study using a more sensitive IL-4 ELISA there were significantly elevated levels of IL-4 and also a trend to elevated IL-13 in breast milk of allergic mothers compared to nonallergic mothers [26]. Prospective studies are required to thoroughly assess the differences in the composition of breast milk from mothers with different allergic histories and the consequences of this for the offspring.

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