Do Milk-Borne Cytokines and Hormones Influence Neonatal Immune Cell Function?¹

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ABSTRACT Cytokines, growth factors and various hormones collectively control the proliferation, survival, differentiation and function of immune cells. A wide array of these compounds is present in maternal milk and ingested by neonates during a period of rapid maturation of gut-associated and peripheral lymphoid tissues. The functional consequences of most milk immunomodulatory constituents in neonates are unknown. However, there is evidence that milk prolactin acts as a developmental regulator of the neonatal immune system, supporting the premise that milk constituents with immunomodulatory activity may serve as neonatal immunodevelopment agents. J. Nutr. 127: 985S–988S, 1997.

KEY WORDS: cytokines prolactin milk immune system development

Maternal milk is a complex biological fluid principally viewed as a food providing energy and essential nutrients for growth and development of newborns. However, milk also contains a multitude of bioactive enzymes, hormones, growth factors and immunomological agents with diverse biochemical specificities (Ellis and Picciano 1992, Goldman et al. 1996). Many of these agents were originally viewed as insignificant byproducts of milk secretion, because they are present in milk at less than picogram quantities. These components are typically abundant in the early milk when significant functional immaturity of neonatal organ systems exists. Such temporal relationships suggest that bioactive milk constituents may be important in neonatal development. Available evidence to support the function of milk-borne cytokines and hormones as developmental regulators of the immune system in neonates is presented.

NEONATAL IMMUNODEVELOPMENT

The immune system of newborns is functionally immature and enters a state of extensive differentiation and reorganization in the early postnatal period (Xanthou 1993). A number of critical maturation events occur before T and B cells acquire specific effector functions. At birth, the thymus is fully colonized with hematopoietic stem cells that gradually mature into various T lymphocyte lineages with specialized functions, i.e., T helper (Th) or T cytotoxic (Tc) cells. Cell surface proteins, or cluster of differentiation (CD) markers, on T lymphocytes change; therefore, this pattern of expression is used to identify lymphocytes at various maturational stages (Fig. 1). Certain cell surface proteins, such as the T cell receptor (TCR), and CD3 and CD5 molecules are expressed by all or most mature T lymphocytes. Immature T lymphocytes also express the CD4 and CD8 molecules in tandem. At maturity, Th no longer express CD8 and become CD4+CD8− lymphocytes, whereas T cytotoxic cells (Tc) do not express CD4 and become CD4−CD8+. Following birth, B cell precursors enter a course of maturation entirely separate from that of T cells. As in T lymphocytes, the expression of specific cell surface proteins, in this case immunoglobulins, changes as B cells mature. The total cellularity of the spleen and lymph nodes increases dramatically postnatally as T and B cells begin to traffic from primary immune organs and establish residence in secondary immune organs (Fig. 1). Within the secondary immune organs, both T and B lymphocytes develop memory for specific antigens.

Lymphocytes from neonatal human and rats are not readily activated (Nehlese-Cannarella and Chang 1992); their ability to reject tissue grafts is poor, and clonal expansion (i.e., proliferation) of circulating lymphocytes, thymocytes and splenocytes following challenge with polyclonal mitogens in vitro is lower in comparison with adults (Grove et al. 1991, Middleton and Bullock 1984). The limited capacity of lymphocytes to become activated is a consistently observed feature of the immune response of neonates that is due in part to active suppression of lymphocyte proliferation and in part to the reduced capacity of immune cells of neonates to synthesize regulatory cytokines. Natural killer cells are present in small numbers and, similarly, their functional responses in neonates are very low.
in part to disorders of the immune system, i.e., autoimmune disorders (Crohn’s disease and diabetes mellitus) and lymphoma (Goldman and Goldblum 1995). Moreover, the protection afforded by maternal milk feeding is evident not only during infancy but also well into adulthood (Ellis and Picciano 1992).

### PROLACTIN IS AN IMMUNOMODULATORY AGENT

In adults as well as neonates, PRL is classified as a hormone with immunoregulatory activity. Prolactin exerts its effects through the PRL receptor (PRL-R), which is a member of the IL-2 cytokine receptor superfamily (Basan 1989). Prolactin influences lymphocyte maturation and function (Reber 1993), T cell–dependent macrophage activation and natural killer cell activity (Bernton et al. 1988). Prolactin can act in vitro to induce interleukin-2 and interleukin-2 receptors on lymphocytes from hyperprolactinemic rats (Viselli et al. 1991). Administration of PRL to adult rats enhances antibody production and acts as a potent vaccine adjuvant. Animals deficient in PRL—such as adult Snell and Ames dwarf mice strains with defective growth hormone, thyroid-stimulating hormone and PRL secretion—have involuted lymphoid tissues that are decreased in weight and contain few cells and cells with suboptimal activity (Murphy et al. 1995). Treatment of Snell mice (DW/J) with ovine PRL drastically reduces thymic cellularity and significantly increases the number and activity of circulating antigen-specific T cells. Thus, milk PRL may be capable of directing lymphocyte migration from primary to secondary immune organs and trafficking of immune cells in circulation.

### BIOLOGICALLY ACTIVE PROLACTIN IS PRESENT IN MILK

Human and rat milks contain abundant PRL bioactivity that is distributed among several variant isoforms differing in molecular weight, degree of glycosylation or phosphorylation (Ellis and Picciano 1995, Kacsoh et al. 1993). Although it is well accepted that modified forms of PRL are not equally detected in all assay systems, both bioassay and immunoassay methods underestimate total milk PRL content. For example, the Nb-2 lymphoma cell proliferation assay detects 1.4- to fourfold more total PRL in human milk and two- to sixfold more total PRL in rat milk than do immunoassays. Similarly, the level of PRL immunoreactivity present in maternal serum is similar to that in milk, but PRL bioactivity in milk is considerably greater than in serum. The mammary gland concentrates or selectively transfers PRL into milk. Prolactin secretion is apparently regulated, because the number of variants and their quantities are greatest during early lactation and decline with the progression of lactation. The biological activity of human and rat milk PRL is consistently distributed among four to six PRL variants during early lactation but in later lactation is present primarily as an unmodified 23- to 24-kDa form similar to PRL predominant in the pituitary (Ellis et al. 1996).

In addition to PRL variants present in the glycosylated/phosphorylated fraction of milk, high-molecular-weight forms of PRL are detected in milk of rabbits (Postel-Vinay et al. 1991), humans (Mercado and Baumann 1994) and rats (Ellis et al. 1996). Very-high-molecular-weight or “big-big” and “big” forms of PRL have been described in other biological fluids and are known to originate from the association of 23–24-kDa PRL or its variants with PRL-R, PRL-binding proteins or immunoglobulin or through PRL dimerization. The secretion of PRL into milk

**FIGURE 1** Key stages in hemopoietic stem cell differentiation. Abbreviations used: S, stem cells; MK, megakaryocyte; LS, lymphoid stem cell; ES, erythroid stem cell; HS, hemopoietic stem cell; GM, granulocyte-monocyte-monocyte precursor; T, T lymphocyte; B, B lymphocyte; T_h, helper cell; T_c, cytotoxic cell; CD, cluster of differentiation marker; TCR, T cell receptor.

Intraepithelial lymphocytes and Peyer’s patches reside within the small intestine (Guy-Grand et al. 1993) and differentiate extensively in the postnatal period as neonates are exposed to environmental antigens. Cells maturing in the Peyer’s patches are capable of migrating from the intestine to secondary immune organs (Ottaway et al. 1987), whereas the intraepithelial lymphocytes do not migrate but respond to antigenic substances present locally in the intestine.

**MATERNAL MILK CONSTITUENTS WITH IMMUNOMODULATORY ACTIVITY AND THEIR POTENTIAL ROLES IN NEONATES**

Maternal milk contains a wide array of biologically active agents (i.e., cytokines, certain endocrine hormones and growth factors) with known immunomodulatory activity including at least 10 classical cytokines: interleukins 1, 2, 6, 8 and 10, colony-stimulating factors M and G, transforming growth factors α and β, tumor necrosis factor α, and interferon γ (Goldman et al. 1996). Although originally classified as an endocrine hormone, 23–24-kDa prolactin (PRL) and its several variants are listed among the potential immunomodulatory agents present in milk because a growing body of research has detailed the cytokine-like actions of PRL upon immune cells in neonates and adults (Reber 1993, Reichlin 1993).

If milk constituents with immunomodulatory activity are important for neonatal system maturation, one would expect neonates deprived of these factors to exhibit impaired immune system function. At least two lines of evidence suggest that the neonatal immune system may be influenced by immunomodulatory agents in maternal milk. First, in vitro and ex vivo studies showed that several aspects of the acquired or antigen-driven immune response differ between human neonates fed maternal milk and those fed substitutes devoid of immunomodulatory activity (Stephens 1991). Second, results of epidemiological studies suggest that human milk feeding confers protection against acute and chronic diseases such as respiratory syncytial virus, otitis media, diarrhea and diseases related at least
IMMUNOMODULATORY AGENTS IN MATERNAL MILK

complexed with binding proteins/receptors may be an important mechanism for protecting milk PRL from proteolytic degradation and for preserving PRL biological activity in the intestinal tract of neonates.

DEMONSTRATION OF MILK PROLACTIN ACTIVITY IN NEONATES

To affect immune cell development in neonates, milk PRL must retain its bioactivity or become activated at the target site. Milk PRL bioactivity is detected in stomach milk of mice neonates (Gala and Shevach 1993), and PRL bioactivity of the phosphorylated fraction of human milk is enhanced by treatment with the intestinal enzyme alkaline phosphatase (Ellis and Picciano 1995). Studies in rat neonates showed that milk PRL is absorbed from the jejunum and ileum as intact and/or low-molecular-weight processed forms (Gonnella et al. 1989, Whitworth and Grosvenor 1978). The transfer of milk PRL from the intestine of neonates into serum is believed to occur through receptor-mediated endocytosis across the mucosal cell, and PRL-R is abundantly expressed by these cells in neonatal animals (Nagano et al. 1995). High PRL biological activity is detected in serum during d 2–5 of life (Kacsoh et al. 1993), although endogenous production of PRL by the rat pituitary does not occur until d 5 of life (Hoeffler et al. 1985). Biologically active PRL present within the small intestine may modulate differentiation and/or function of gut-associated lymphoid tissue such as the intraepithelial lymphocytes that express PRL-R at a quantitatively greater density compared with PRL-R expressed on neonatal splenocytes (Mastro et al. 1995).

EVIDENCE THAT MILK-PROLACTIN INFLUENCES NEONATAL IMMUNE CELL FUNCTION

Milk PRL is experimentally reduced in lactating animals by the inhibition of pituitary PRL secretion with the dopamine agonist bromocriptine (Shyr et al. 1986). Milk production is maintained with bromocriptine doses of approximately 1.25 mg/kg maternal body wt per day. Splenocytes and thymocytes of neonates ingesting PRL-poor milk from bromocriptine-treated dams exhibit markedly different proliferation responses and cluster of differentiation marker expression than cells from neonates ingesting PRL-sufficient milk (Grove et al. 1991). Typically, the proliferation of neonatal thymocytes and splenocytes challenged with polyclonal mitogens in vitro is markedly reduced before d 15 of age in rats. However, in neonatal rats ingesting PRL-poor milk (d 2–5), splenocytes and thymocytes respond precociously to mitogens, with significant proliferation observed as early as d 10 of life. Consumption of PRL-poor milk also reduces the percentage of thymocytes expressing the CD4 and CD5 differentiation markers and the percentage of splenocytes expressing the emigration marker, THY1.

In another study, the proliferation response and CD marker expression of thymocytes and splenocytes of 5-d-old mice ingesting PRL-poor milk since birth were also examined (Gala and Shevach 1993). Comparison of these results to those for neonatal rats is confounded because immune endpoints were examined at an earlier age (d 5) in neonatal mice than in rats (d 10). Nonetheless, neonatal mice fed PRL-poor milk showed significantly reduced splenocyte numbers of d 5, although differences in proliferation and differentiation marker expression were not yet evident. Administration of anti-PRL serum directly to neonatal mice and rats has also been used to reduce levels of serum PRL while maintaining pituitary PRL synthesis.

Thymocytes and splenocytes of suckling mice treated with PRL-antiserum on d 1–3 of life were characterized by significant reductions in the percentages of CD4+ T-helper lymphocytes and the percentage of B cells expressing immunoglobulin G (Russell et al. 1988). Thus, data from neonatal rats and mice suggest that ingestion of PRL-poor milk may affect differentiation of immune cells in the thymus, migration of thymocytes to secondary immune organs as well as proliferation of thymocytes and splenocytes when challenged with polyclonal mitogens in vitro. The manifestation of these alterations are unknown; they may not be evident during or immediately following the period of reduced milk PRL ingestion and may be evident only as the neonate matures.

Prolactin exerts its biological effects through PRL-R signal transduction. Both thymocytes and splenocytes express long and short forms of the PRL-R at birth (Gunes and Mastro 1996). Prolactin receptor expression in the thymus does not change significantly during the period from birth to early adulthood, but in the spleen it increases during the first 2 wk of life (Fig. 2 A, B). The expression of PRL-R in both the spleen and thymus of neonates is down-regulated by ingestion of milk (Fig. 2 C, D). Levels of PRL-R in the spleen decrease 7 h after birth in neonates ingesting milk compared with littermates not ingesting milk (Fig. 2 C, D). The down-regulation of PRL-R expression in the spleen of neonates by milk feeding may be one mechanism by which milk PRL regulates immune cell function and influences lymphocyte trafficking. Most recently, we observed that intraepithelial lymphocytes express high levels of PRL-R in the neonatal period, and we are investigating whether ingestion of milk PRL influences PRL-R expression in this cell population.

SUMMARY AND FUTURE DIRECTIONS FOR RESEARCH

In summary, several lines of evidence collectively support the case that milk PRL is a modulator of the neonatal immune

FIGURE 2 Expression of cell surface prolactin receptor (PRL-R) on thymocytes and splenocytes of neonatal rats. Panels A and B show the ontogeny of PRL-R expression from birth to 60 d; panels C and D present data (means ± SEM) on the down-regulation of PRL-R expression following milk ingestion. An asterisk denotes a significant (P < 0.05) decrease in PRL-R compared with results for milk-fed groups. Thymocytes or splenocytes were stained with antibody to the PRL-R and leukocyte common antigen for normalization of total lymphocyte cell number. The PRL-R positive cells were identified and quantified as a percentage of total lymphocytes by flow cytometry.
system of neonates. Prolactin modulates immune responsiveness of lymphocytes and their accessory cells in adults. Many varieties of lymphocytes express PRL-R in the early neonatal period, and this expression is regulated by milk ingestion. Several forms of biologically active PRL are concentrated in maternal milk during a period of dynamic specialization of lymphocytes in neonates. In neonates, prolactin biological activity is retained and possibly enhanced within the intestine and may locally direct maturation of intraepithelial and Peyer’s patches lymphocytes. Moreover, milk PRL is transferred to the circulation and, in its absence, splenocyte and thymocyte populations manifest altered patterns of lymphocyte differentiation and proliferative responses.

Although the role of immunomodulatory agents in the development of the immune system of neonates is presently awaiting detailed exploration, there is sufficient evidence to warrant such exploration. Prolactin is just one of many agents with immunomodulatory activity in maternal milk, and it is likely that other milk cytokines exert their pleiotropic actions on neonatal target tissues. The immediate goals for defining the immunomodulatory roles of milk cytokines and hormones, however, include isolating the factors from milk, defining target cells affected by these agents in neonates, and establishing in vitro model systems for their study. The form or activity of milk-borne cytokines and hormones in blood of neonates may differ from their form or activity present in maternal milk, and therefore the role of the gastrointestinal tract as a possible modifier of milk cytokines and hormones must be evaluated. Until these goals are met, the potential effect of milk immunomodulatory agents in human neonates, particularly those born prematurely, cannot be assessed.

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LITERATURE CITED


