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Oropharyngeal administration of colostrum to extremely low birth weight infants: theoretical perspectives

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Studies in adults have shown that the oropharyngeal route can be used to effectively and safely administer interferon-α, an immune cell-derived cytokine, to patients who are unable to tolerate its parenteral administration. The mechanism for this appears to be the stimulatory effects of the cytokine, on the oropharyngeal-associated lymphoid tissue system. Own mother's colostrum (OMC) is rich in cytokines and other immune agents that provide bacteriostatic, bacteriocidal, antiviral, anti-inflammatory and immunomodulatory protection against infection. OMC may be especially protective for the extremely low birth weight (ELBW) infant in the first days of life; however clinical instability typically precludes enteral feedings during this period. Oropharyngeal administration is a potential alternative method of providing OMC. Oropharyngeal administration of OMC may have immunomodulatory effects on the recipient infant, and would be especially beneficial to the ELBW infant who would otherwise remain nil per os during the first days of life.
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Introduction

Numerous studies have linked own mother's milk (OMM, mothers' milk) feedings with a lower incidence and severity of nosocomial infection or late-onset sepsis in premature (<37 weeks gestation) infants.[1–5] Although not all of these investigations have focused specifically on the extremely low birth weight (ELBW; birth weight <1000 g) population, biochemical and immunologic data suggest that mothers' milk feedings may provide the greatest protection from infection for the most immature ELBW infants. In comparison to larger preterm infants, ELBW infants are the most immunocompromised, are exposed routinely to invasive, lifesaving procedures and remain in the pathogen-laden neonatal intensive care unit (NICU) for the longest period of time, typically between 12 and 16 weeks. These factors significantly increase the risk of acquiring a nosocomial infection.

Colostrum is the early milk that is produced when the tight junctions in the mammary epithelium are open, allowing paracellular transport of many immunologically derived protective components from the mother's circulation into the milk.[6] These tight junctions close gradually over the first days post birth, and fuse with the onset of lactogenesis II, also known as the 'milk coming in'.[6,7] Thus, colostrum is a very different milk product than is mature milk that flows after the closure of tight junctions. Colostrum is rich in cytokines and other immune agents that provide bacteriostatic, bacteriocidal, antiviral, anti-inflammatory and immunomodulatory protection against infection.[8–12]

Whereas studies from early in the last decade documented higher concentrations of many protective factors in the colostrum of mothers who delivered preterm as compared to term infants,[13–15] most recent work has further associated the *degree* of prematurity with the composition of maternal colostrum. These studies suggest an inverse relationship between duration of pregnancy and the concentration of protective factors in colostrum.[7,16–19] Thus the milk produced by mothers of the least mature infants contains the highest concentrations of protective factors.[16–19] Similarly, findings from a small group of studies suggest that closure of the tight junctions in the mammary epithelium may be delayed following preterm birth, resulting in prolonged availability of these protective products in the early post-birth period.[7,19] The gestation-specific trends in the composition and duration of colostrum suggest an immaturity in the mammary gland that parallels that of the infant, and may have physiologic significance for protecting the infant from infection.

The immune components that are unique to preterm colostrum may be especially protective during the first week of life when ELBW infants are the sickest and at highest risk for infection. However, the immature gastrointestinal tract and the presence of comorbidities that cause bowel hypoperfusion usually preclude enteral feedings during this time. Prolonged nil per os (NPO)

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status and the use of antibiotics lead to intestinal atrophy[20] and an abnormal pattern of intestinal colonization,[21] factors that significantly increase the risk of feeding intolerance and nosocomial infection. Thus, there is an urgent need to identify safe and efficacious alternative methods for administering preterm colostrum to ELBW infants in the first days of life, when they cannot be fed enterally. Oropharyngeal administration of colostrum is one potential option.

Previous studies in adult populations have shown that the oropharyngeal route can be used to effectively and safely administer interferon-α (IFN-α), an immune cell-derived cytokine, to adults who are unable to tolerate its parenteral administration.[22–26] Oropharyngeal administration is not the same as oral administration. Oral administration involves swallowing a liquid, with resultant gastrointestinal absorption. Oropharyngeal administration involves placing small amounts of a liquid directly onto the oral mucosa with expectation that the liquid, or any of its components, is absorbed by the mucous membranes.

In adults, oropharyngeally-administered IFN-α is thought to have a stimulatory effect on the oropharyngeal-associated lymphoid tissue (OFALT) system.[26,27] Theoretically, providing colostrum to ELBW infants by the oropharyngeal route during the first days post birth would similarly influence the OFALT system.[28] However, this hypothesis has not been tested previously. The purpose of this paper is to review the evidence that supports oropharyngeal administration of own mothers' colostrum (OMC) to ELBW infants during the first days post-birth.

OFALT and GALT: Implications for the ELBW infant

The mucosa-associated lymphoid tissue (MALT) system consists of strategically placed lymphoid structures that protect the respiratory and gastrointestinal tracts from pathogens. The MALT system is comprised of (1) OFALT, which consist of the palatine tonsils and adenoids, (2) bronchial-associated lymphoid tissues (T), which lines the respiratory epithelium and (3) gut-associated lymphoid tissues (GALT), including the appendix and Peyer's patches, which are aggregated lymphoid nodules that line the distal ileum.[29,30] The surface area of the MALT system is extensive, facilitating direct and more immediate contact between external pathogens and immune cells such as T and B lymphocytes and monocytes located within these lymphoid organs.[29]

Proposed mechanisms for cytokine activation of OFALT and GALT

Lymphoid immune cells within the MALT system can interact with exogenous cytokines, such as those present in human colostrum.[26–28] Cytokines are messenger molecules that can be synthesized and rapidly secreted by many cells, including immune cells, in response to an exogenous antigen or cytokine stimulus.[29] Once synthesized, the cytokine can activate or suppress the function of its cell of origin or neighboring immune cells.[30] Cytokines can also stimulate or inhibit the production of other cytokines, and function synergistically or antagonistically to form a complex interrelated network of communication, known as signaling, among cells during an immune response.[29] Among their many functions, certain cytokines stimulate T and B cell activity and the production of immunoglobulins, including secretory immunoglobulin A (sIgA).[30]

Different sets of immune cells release different types of cytokines. Most notably, a subset of T-helper lymphocytes, TH1 cells, secrete IFN-γ, tumor necrosis factor-β (TNF-β) and interleukin-2 (IL-2) that promote cell-mediated immune reactions. On the other hand, TH2 lymphocytes produce IL-4, IL-5 and IL-13, which typically support humoral (antibody) immune responses. TH1 cytokines tend to decrease the activities of TH2 cells and vice versa.[29]

When lymphoid cells within GALT are exposed to certain exogenous cytokines, the cytokine to cytokine–receptor interaction stimulates T lymphocytes, which transfer this message to other gut lymphocytes.[31,32] These gut lymphocytes may migrate to distant sites, transmitting a cytokine-mediated activation signal to cells within inflammatory sites or other immune tissues.[31,32] Once reaching their destination in the lymph nodes, spleen, brain or other end organ, these activated lymphocytes may release anti-inflammatory cytokines that inhibit inflammation in neighboring tissues.[31] Thus, a small GALT-associated stimulus can result in either enhanced immune activation at distal organs or an anti-inflammatory response,[31] dependent upon the cytokines released and the types of immune cells along the signaling pathway.[26] These messages are amplified over the course of cytokine to cell communication.[26]

Evidence suggests that minute concentrations of cytokines, such as amounts present in colostrum drops, are extremely potent. This body of evidence is drawn from studies involving IFN-α, an antiviral cytokine that has been extensively studied in animal and human subjects.[22–25,33–36] IFN-α activates natural killer lymphoid cells, and has been demonstrated effective in extremely small concentrations.[26,28] For example, a single small dose ($1\,\text{pg} = 10^{-12}\,\text{g}$) of IFN has been shown to protect one million cells from ten million virus particles in a tissue culture experiment.[29] Theoretically, the systemic immunomodulatory effect of these extremely low dose cytokines works in the following manner. A small dose (50 U) of human IFN-α, that has a specific activity of $10^9\,\text{U mg}^{-1}$ protein and an average molecular weight of 20 kDa, contains 1.5×10^9 molecules.[27] Assuming each OFALT/GALT lymphoid cell will bind 100 molecules, such exogenous IFN-α dose would potentially activate 1.5×10^7 cells.[27] This original 'activation' message would be amplified as a result of cell-to-cell cytokine signaling[37,38] and message dissemination to distant sites.[27] The migration of activated immune cells occurs by lymph and blood to distant (lymphoid and nonlymphoid) organs[39] where they may release cytokines[40–42] and stimulate organ-localized as well as circulating cells.[27]

Although oropharyngeal administration of colostrum assumes absorption of cytokines by the OFALT system[26,27] it is likely that oropharyngeal administration also activates responses in GALT, because of some swallowing of the colostrum. Thus, questions as to whether these cytokines are stable in the gastrointestinal tract and whether they interact with immune cells in GALT are relevant to the discussion of oropharyngeal administration. For many years it was assumed that an orally administered cytokine, such as IFN-α, would not be efficacious because of proteolysis in the gastrointestinal tract.[27] However, research with animals[33–36] and adult humans[22–25] has demonstrated that orally administered IFNs have potent antiviral activity,[36] can exert both local and systemic effects, and yet be nearly undetectable in the serum[43] with often no more than 1% of the administered dose recovered in serum samples.[44,45] Colostral cytokines likely remain stable in the gastrointestinal tract of infants, as they appear to escape proteolysis in the immature gastrointestinal tract of the ELBW infant.[46–48] This stability allows for potential interaction with GALT tissues.

Animal experiments

Studies with mice have demonstrated that TH1 cytokines such as IL-2, or granulocyte-macrophage colony-stimulating factor (GM-CSF), administered to the oral mucosa interact with cells in the lymphoid or epithelial tissue of the oropharyngeal cavity, and result in systemic antiviral activity.[49] When human IFN-α8 is administered by the oral–mucosal route to mice, it can be detected in the serum within 5 min of administration and locally activates gene transcription in lymphoid tissue of the oropharyngeal cavity, accompanied by a systemic antiviral response.[50] Observation of the effects of orally administered IFN-α and IFN-γ can be transferred by injection of blood, but not plasma, from an IFN-α or -γ treated mouse to a non-treated mouse.[43] This finding suggests that such systemic effects result from cell-to-cell transmission rather than a direct effect of IFN-α and IFN-γ in the blood.[43,51] The distinction between plasma and cell-to-cell transmission is an important one because these findings support the premise that sequential amplification of the original activation message is a result of cell-to-cell signaling. It is probable that oropharyngeally administered colostral cytokines would exert a similar amplification process by interacting with the lymphoid tissue in both the OFALT and GALT.

The effectiveness of oropharyngeally administered low dose IFN-α and IFN-β has been demonstrated in animal models of autoimmune disease, including multiple sclerosis and insulin-dependent diabetes mellitus.[31] For example, in non-obese mice with insulin-dependent diabetes mellitus, the oral administration of low dose IFN-α was shown to decrease islet inflammation, suppress diabetes and increase levels of anti-inflammatory TH2 cytokines, such as IL-4 and IL-10.[31] Similarly, in mice with encephalitis and multiple sclerosis, the oral administration of low dose IFN-α/-β was shown to decrease CNS inflammation and decrease the secretion of pro-inflammatory cytokines IL-2 and IFN-γ.[52]

Veterinarians and researchers have administered IFN-α orally for many years, and have noted efficacy in the treatment of viral, bacterial and parasitic infections in larger animals such as cats, cattle, swine and horses.[33–36]

Trials with human subjects

In adults, the oropharyngeal administration of IFN was first evaluated in persons with AIDS,[22] with promising results. However, subsequent studies[53–55] yielded inconclusive findings. In one related study, 28 adult patients with chronic hepatitis B were treated with IFN-α, administered oropharyngeally in the form of lozenges. The treatment periods and patient numbers varied as follows: 13 patients over 300 days, 2 over 180 days, 2 over 120 days and 11 for less than 120 days. Of the initially viremic subjects, 52% seroconverted (that is, generated circulating antibody) in response to treatment.[24] Similarly, a study of 14 randomly selected children and adults with chronic active type B hepatitis revealed that all subjects seroconverted in response to treatment with oropharyngeally administered IFN-α.[25] Immune stimulation was noted in all subjects and lasted several weeks beyond the treatment period, as measured by various immunological parameters and markers of hepatitis infection. More recently, oral–mucosal IFN therapy was found to be safe and efficacious in the treatment of measles in a pediatric population,[56] an age group for which the oropharyngeal route is increasingly of interest because of ease of administration and few untoward effects. A limitation of these studies is that they were largely anecdotal and did not include a placebo control group.

Despite these limitations, clinical and scientific interest in the use of oral–mucosal IFNs persists, because of potential effectiveness and ease of administration. IFNs, previously considered to be exclusively antiviral when administered parenterally, recently have been shown to be efficacious in treating many conditions including malignancies, multiple sclerosis and other immune disorders.[31] However, this potential efficacy has been limited by untoward effects, poor patient compliance and prohibitive cost associated with parenterally administered high dose IFNs. Oropharyngeal administration of cytokines does not appear to be associated with these limitations, in that it requires only small doses to stimulate OFALT cells, with subsequent intense cell-to-cell amplification, and an associated systemic effect.[27]

Evidence for OFALT and GALT in ELBW infants

GALT structures, including Peyer's patches, are fully developed at birth with T and B cell areas noted as early as 19 weeks of gestation;[57,58] however, antigenic exposure is necessary for immune activation of GALT.[57] As the intrauterine environment is sterile, the ELBW infant is 'inexperienced' with respect to antigenic challenge, as evidenced by the paucity of immunoglobulin A (IgA)-producing B cells in the peripheral blood of neonates ($<8/10^6$ lymphocytes)

and the significant increase noted ($\sim 600/10^6$ lymphocytes) at 1 month of age.[57,59]

Although the immune response of the preterm infant is immature, recent research suggests that intricate feedback mechanisms, which regulate the production of pro-inflammatory and anti-inflammatory cytokines, are operational in infected very low birth weight (<1500 g) infants.[60] Although this finding has important implications for OFALT and GALT stimulation, further inquiry has been slow because the rodent, the most appropriate animal model for this research, does not develop lymphoid cells in Peyer's patches until after birth.[61] In the absence of suitable animal models, some studies have focused on human fetuses from *in utero* gestations that would be comparable to ELBW infants after birth[62,63] Studies, involving samples of fetal ileal segments[62] and/or fetal epithelial cells,[62,63] provide suggestive evidence that the GALT system in ELBW infants may be functional and capable of responding to cytokine or antigenic stimulation.

Toll-like receptors are found on cells of the innate immune system and play an important role in regulating intestinal homeostasis.[64] These receptors recognize and respond to bacterial cell wall products by increasing the production of inflammatory cytokines, including IL-8, through activation of an intracellular signal.[62] It has been noted that when human fetal intestinal epithelial cells are stimulated with exogenous cytokines, such as TNF-α or IL-1-β, they produce significantly more IL-8 than comparable human adult intestinal epithelial cells.[63] This finding suggests that fetal intestinal epithelial cells, whereas immature and undifferentiated, are not only capable of responding to cytokine stimulation, but also may do so in an exaggerated fashion.[63] Some investigators have proposed that the OFALT and GALT systems in the ELBW infant may be more receptive to cytokine stimulation precisely because of extreme prematurity and concomitant immunologic inexperience.[28]

Mothers' milk and OFALT

Mothers' milk contains numerous anti-inflammatory and pro-inflammatory cytokines, including IL-1-α, IL-1-β, IL-2, IL-3, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, IFN-γ, TNF-α, GM-CSF, M-CSF and transforming growth factor (TGF)-α and -β.[8–12,28] Although mothers' milk contains pro- as well as anti-inflammatory cytokines, the overall effect of mothers' milk is anti-inflammatory in nature.[8–10] Although these anti-inflammatory effects may result solely from anti-inflammatory cytokines, such as IL-10,[8,65] other non-cytokine agents including platelet activating factor acetylhydrolase, soluble TNF-α receptors and epidermal growth factor may contribute to the observed anti-inflammatory effects.[9,10,66,67]

Although it is well-documented that mothers' milk contains cytokines, which have a role in protecting the breastfeeding infant against infectious organisms, the mechanism underlying this effect is not well-understood. Most research in this area has focused on the protective role of milk-derived cytokines within the gastrointestinal tract. Given that exogenous cytokines in mothers' milk may also be absorbed oropharyngeally there is potential for activation of the infant's OFALT system.[27,28] During the act of breastfeeding, mothers' milk cytokines theoretically stimulate both OFALT and GALT, with a combined response greater than that for a singular component. Although the term infant who feeds at breast would be the recipient of this combined effect, the ELBW infant in the NICU receives enteral feedings by a nasogastric gavage tube. Thus, mother's milk does not typically come into contact with OFALT, a deficit that could be corrected with oropharyngeal administration of colostrum.

Although more research is needed to understand the precise function of milk cytokines in breastfeeding infants, *in vitro* studies have shown that milk leukocytes readily produce cytokines when stimulated,[27,68,69] that these cytokines may influence the development and maturation of the infant's immune cells, and that they may be sequestered and protected from digestion until they reach the intestine.[48,68] The fact that cytokines are present in mother's milk suggests an important biologic function. Furthermore, many of these cytokines are found in higher concentrations in preterm than term colostrum, indicating they may have an important role in protecting the premature infant, especially in the first days of life.

During oropharyngeal administration of colostrum, IL-6 may stimulate immune cells in the OFALT system and provide an immunomodulatory effect. This notion is supported by the fact that mother's milk will preferentially stimulate the growth and differentiation of B lymphocytes to IgA-secreting plasma cells,[11] which has been attributed to the activity of cytokines, especially IL-6.[11,57] The fact that IL-6 is found in colostrum in significantly higher concentrations ($978.80 \pm 86.80\,\mathrm{pg\,ml^{-1}}$) than those found in transitional ($162.90 \pm 29.67\,\mathrm{pg\,ml^{-1}}$) and mature ($86.92 \pm 2.47\,\mathrm{pg\,ml^{-1}}$) milk[70] suggests it has an important biological function for the recipient infant in the first days of life. It is plausible that oropharyngeal administration of colostral IL-6 may stimulate the production of sIgA in mononuclear cells of the OFALT system and lead to enhanced mucosal immunity.

When OMC is administered oropharyngeally, it is possible that some factors may travel to the gastrointestinal tract instead of being absorbed by the oropharyngeal route. For example, insulin-like growth factor (IGF)-1 is highly concentrated in colostrum[71,72] and stimulates the production of IL-10, a potent anti-inflammatory cytokine, in T cells.[73] Animal studies have demonstrated both the presence of intestinal receptors for IGF-1[74,75] and its resistance to digestion,[72] suggesting that the IGF-1 in OMC may potentially stimulate enhanced production of IL-10 in GALT tissues.

Other cytokines contained in OMC including TGF-β, IL-6 and IL-10, stimulate the development and differentiation of IgA-producing cells.[68,76,77]

IFN-γ, which is also a colostral cytokine, upregulates the production of secretory component by epithelial cells.[78] Collectively, these colostral cytokines may increase sIgA production in GALT tissues, thus enhancing mucosal immunity.

Although the *in vivo* effects of OMC cytokines are not yet determined, the premise that they may provide immunomodulatory effects is supported by the presence of cytokine receptors in the fetal intestine[79,80] and by evidence that some of these cytokines are resistant to *in vitro* digestion,[48] that suggests they have an important biological function in the fetal intestine. Additionally, animal studies have demonstrated that cytokines, such as TGF-β, survive passage through the entire colon.[81] Taken together, these findings from previous studies support the hypothesis that OMC cytokines may exert immunomodulatory effects in the OFALT and GALT when administered by the oropharyngeal route.

Other non-cytokine immune factors in OMC may also potentially stimulate the OFALT/GALT system. For example, prolactin, once thought to have only endocrine functions, is now also recognized as a potent immunomodulatory factor, with cytokine-like influence on neonatal immune cells.[82] Prolactin promotes lymphocyte maturation, may direct trafficking of immune cells, and could potentially influence lymphocyte migration from primary to secondary immune organs.[82] It is also possible that prolactin may facilitate the migration of activated immune cells from OFALT/GALT structures to distant lymphoid sites.

In addition to cytokines, mother's milk contains a multitude of immune factors that have antimicrobial, anti-inflammatory and immunomodulatory properties and are more concentrated in the milk of women who deliver extremely premature infants.[7–19] A recent review paper[12] details the mechanisms of protection afforded by these various agents, which include barrier protection, bacterial cell wall lysis, anti-inflammation, immunomodulation and the creation of a gastrointestinal milieu that is hostile to the growth of pathogenic microorganisms. During the oropharyngeal administration of colostrum, it is likely that non-cytokine immune factors, which are highly concentrated in preterm colostrum, also may be absorbed mucosally. Of particular interest are sIgA and lactoferrin. Both of these antimicrobial factors are excreted in the urine[83–85] and stool[86] of OMM-fed infants, which suggests systemic absorption and a potential for systemic protection against infection.

Oropharyngeal administration of OMC may therefore promote immunocompetence by two distinct mechanisms; immunomodulation of cells within the OFALT and GALT systems, and the mucosal absorption of factors that interfere with bacterial colonization, such as sIgA and lactoferrin. On the basis of these theories, oropharyngeal administration of colostrum could have protective effects for an ELBW infant who would otherwise remain NPO during the first days of life.

Conclusion

In summary, the literature supports a role for OMC as a potential immune therapy for the ELBW infant. There is substantial evidence from animal and human studies to support the concept that oropharyngeally administered colostrum interacts with the recipient infant's OFALT and GALT systems, and provides protection against infection. Using mother's colostrum in this manner requires a paradigm shift, to view colostrum not simply as a feeding, but instead as a potential immune therapy and a *complement* to trophic feedings. It is plausible that some colostrum administered oropharyngeally could reach the gastrointestinal tract and be absorbed by the intestinal mucosa. The evidence and theoretical perspectives in this article can be used to develop and conduct subsequent prospective studies that examine the safety and efficacy of this inexpensive therapeutic intervention.

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