Milk-Derived Transforming Growth Factor-β and the Infant Immune Response

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Breast milk cytokines have the potential to regulate the immune response to food antigens in infants. Cytokines are present in all mammalian milks and are capable of inhibiting excess inflammation and modulating epithelial proliferation. There are a range of candidate cytokines in milk such as transforming growth factor-β (TGF-β), the major cytokine present, and interleukin-10, which play a role in immune regulation in the developing infant. This article will be a review of the current literature with regard to TGF-β in infant immune development. Our data on supplementation of formula with rTGF-β2 will be discussed in view of the current literature. Oral antigen exposure also plays an important role in priming the developing immune response. The influence of early introduction of oral β-lactoglobulin in allergy prone rat pups will also be discussed. *(J Pediatr 2010;156:S21-5)*.

Transforming growth factor-βs (TGF-βs) are a family of multifunctional cytokines involved primarily in regulation of cell proliferation and differentiation, stimulation of extracellular matrix synthesis and deposition, as well as modulation of inflammation and allergy. In the suckling and weaning period of life, early nutritional events have the potential to influence the developing immune response. In infants, the gastrointestinal tract is exposed to food, bacteria, and environmental antigens at a time when the gut mucosal immune system is still developing.[1] Breast milk is not only a source of nutrition for the infant during this time, but it also provides antigens for the developing immune system to learn and develop appropriate immune regulatory mechanisms for maintenance of intestinal homeostasis.[2] Milk provides bioactive factors that directly modulate immune response development (**Figure**) as well as factors that promote colonization of the intestine by bacterial flora, which in turn influence immune response development.

Bioactive cytokines, including TGF-β, are present in all mammalian milks.[3-5] TGF-β and interleukin-10 (IL-10) are thought to be the key cytokines responsible for maintaining normal homeostasis in the adult gut by creating a cytokine milieu for appropriate antigen processing and promoting the development of oral tolerance.[6] In infants, breast milk TGF-β and IL-10 are thought to play a role in programming the developing infant immune response for this important function. TGF-βs control the initiation and resolution of immune responses through the recruitment of cells, and their activation and survival. TGF-βs act on lymphocytes, natural killer cells, dendritic cells, macrophages, mast cells, and granulocytes. Their regulatory action is complex and dependent on the differentiation state of the cell and the surrounding cytokine milieu. TGF-β and IL-10 play an important role in maintenance of intestinal homeostasis and tolerance induction, as illustrated by the finding that TGF-β1 is involved in inducing T-regulatory cells expressing Foxp3, which in turn produce IL-10.[7] Both cytokines work together to control immune responses in the intestine.

Maternal TGF-β: Exogenous Source of TGF-β1 and TGF-β2

There are 3 highly related mammalian isoforms: TGF-β1, TGF-β2, and TGF-β3.[13,14] The amino acid homology between isoforms ranges from 70% (β1 versus β2) to 79% (β2 versus β3). Also, each of the TGF-β1, TGF-β2, and TGF-β3 isoforms display greater than 98% of amino acid sequence homology between species.[8] TGF-β2 is the predominant cytokine present in human milk; however, there is considerable variability in concentration between women and daily variation between milk samples from an individual.[3,9] We have also shown that TGF-β2 is the predominant isoform present in rat milk and TGF-β receptors, which facilitate uptake of milk TGF-β are present in the rat pup intestine.[10-12]

The significance of the relative concentrations of the different isoforms of the TGF-βs in milk, particularly in the recipient infant, is unknown. However, relative concentration may not be the critical factor in determining function of the TGF-βs. Function is dependent on strength of signals from the cells to the nucleus irrespective of the concentration of TGF-βs present,

IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
LAP	Latency-associated peptide
TGF-β	Transforming growth factor-β
TNF	Tumor necrosis factor
TGF	Transforming growth factor

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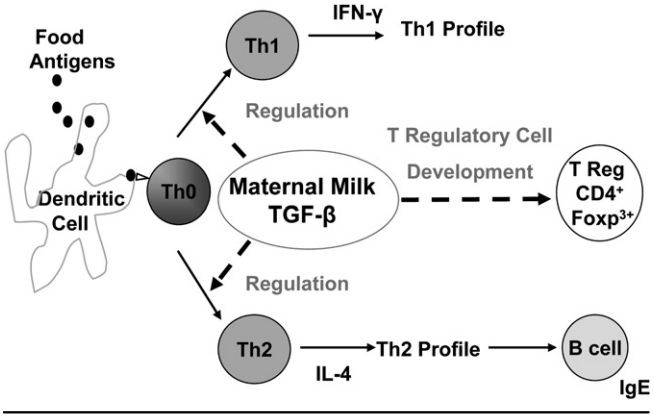

Figure. Maternal milk containing TGF-β provides an immunoregulatory environment for antigen presentation to prevent excess activation of Th1 or Th2 pathways and promotes the development of T-regulatory cells (T reg).

thus highlighting the importance of receptor interactions. TGF-β predominately signals through the Smad family of proteins. Monteleone et al[13] propose that the strength of signals from the cells to the nucleus and not the concentration of TGF-β1 determines the effect of TGF-β. The signals are controlled by inhibitory *i*Smads, that is, *i*Smad 7. Oral administration of TGF-β in vivo has been shown to result in biological activity sufficient to promote oral tolerance.[14] Increased serum TGF-β, Smad2 phosphorylation, and TGF-β and Smad7 mRNA expression in the intestines were all increased after oral administration of TGF-β. We have also shown that TGF-β retains biological activity when given as a supplement in infant formula and that oral tolerance was induced to the cow's milk antigen, β-lactoglobulin.[15,16]

TGF-β and Receptor Interactions: Endogenous Production and Secretion of TGF-βs

In the adult intestine, TGF-β1 is the predominant isotype present in cells and in the extra cellular matrix. Localization patterns show TGF-β1 present in epithelial cells as well as cells in the crypt and lamina propria. Inflammation results in an increase in TGF-β1 intensity of staining in the epithelium. TGF-β2 is detected in a similar distribution pattern but at a lower intensity of staining. TGF-β1 expression is low in rat intestine after birth, but expression increases toward the weaning period.[10]

Factors influencing production of the TGF-βs are not well understood.[17] Exogenous TGF-βs added to cells in priming cultures has been shown to significantly enhance the production of TGF-βs on restimulation. Therefore, TGF-βs have a role in self-regulating their own production. Interferon (IFN)-γ has also been shown to be important because an inhibitory effect on TGF-β2 production can be induced by IFN-γ. IFN-γ also differentially regulates constitutively expressed and cytokine-induced TGF-β1 and TGF-β2 mRNA levels as well as secretion of TGF-βs from retinal epithelial cells.[18]

Endogenous production of TGF-βs in the gut is also influenced by genetic predisposition and antigen exposure. Duodenal biopsy samples from children with delayed cow's milk allergy who were continuously exposed to cow's milk secreted less TGF-β than biopsies from children with delayed cow's milk allergy who avoided cow's milk, demonstrating that restoration of regulatory mechanisms occurred.[19] Atopic mothers also have a reduced TGF-β2 concentration in their breast milk that may influence the developing infant immune response to food antigens.

The actions of TGF-β1 and TGF-β2 are mediated by binding to cell surface receptors. Cross-linking studies with iodinated TGF-β1 or TGF-β2 show that most cells have 3 size classes of binding subunits. These are designated TGF-β receptor type I (TβRI, 53 kDa), type II (TβRII, 70-85 kDa), and type III (TβRIII, 250 to 350 kDa).[8] Signal transduction occurs via TβRI and TβRII. TGF-β1 binds TβRI, and TβRII forms directly, which then leads to signal transduction. TGF-β2, however, cannot bind directly to TβRI and TβRII. TβRIII expression on cells makes the cells more responsive to TGF-β2. In cells expressing TβRIII, TGF-β binds TβRIII; the resulting complex is then presented to the TβRII, leading to the formation of a high-affinity complex consisting of TGF-β, TβRII, and TβRIII. TβRIII is then displaced from this complex. Under these conditions, cells are equally responsive to TGF-β1 and TGF-β2. TβRIII greatly enhances the affinity for TGF-β2 binding to the signaling receptor.[20]

TGF-β receptor expression in the intestine is important for uptake of breast milk–derived TGF-β2. In our studies on the ontogeny of TGF-β receptor expression in the postnatal rat small intestine, we showed that TβRIII, TβRI, and TβRII proteins are abundantly expressed throughout the mucosa during the suckling period.[11] Throughout the weaning period, expression of TβRI and TβRII remain on the gut epithelium, whereas TβRIII markedly diminished from the epithelium toward weaning as the levels of TGF-β2 decreased.[11] We have also shown that soluble TβRIII is present in breast milk; its presence further enhances the binding affinity of milk-derived TGF-β2.[12]

TGF-β Signaling and Regulation of Inflammation

The role of TGF-βs in inhibition of inflammation has been assessed in vitro using human intestinal biopsies cultured in the presence of neutralizing anti–TGF-β antibody.[21] T-bet (a T-box transcription factor required for T helper cell type [Th]1 differentiation) expression was significantly higher in biopsies cultured with anti-TGF antibody. TGF-β blockade also reduced T-cell apoptosis and induced a significant increase in pro-inflammatory cytokines IFN-γ, tumor necrosis factor (TNF)-α, IL-2, IL-6, IL-8, and IL-17, further highlighting the role of TGF-β in regulation of inflammatory responses in the intestine. TGF-βs inhibit inflammation and dampen down inflammatory responses in the intestine of

both adults and infants.[6,25] TGF-β production is therefore an important negative regulator of inflammation. Infant rat pups fed formula supplemented with physiological levels of TGF-β2 have a normalized (reduced) IL-18 pro-inflammatory cytokine profile and reduced allergy associated responses compared with those fed formula alone.[6]

Early Postnatal: Effects of TGF-β on the Developing Immune Response

Human Th cells can be divided into major subtypes defined by the pattern of cytokines that are secreted on stimulation: Th1 (IFN-γ, inflammation), Th2 (IL-4, allergy), Th17 (IL-17, antimicrobial defence and autoimmunity), Tr1 (IL-10, immune regulation), and Th3 (TGF-β, immune regulation).[22] In the neonate, the immune system is dominated by a preference for the production of Th2 cytokines.[23] Th2 cells promote humoral immunity and classic allergic responses, such as immunoglobulin (Ig)E production and eosinophilia, by secreting IL-4, IL-5, and IL-13. During infancy, the ability to mount a Th1 immune response is important in preventing persistent Th2 immune responses, which are thought to be associated with development of allergic disease.[24] The maturation of naïve T cells into committed effector and regulator cells depends on complex interactions between the antigen, the antigen-presenting cell, and the milieu of cytokines in the immediate environment and cell surface receptors.

TGF-β plays a major role in the development of T-cell lineage. The action of TGF-β alone on naïve cells helps to drive the development of T-regulatory cells.[25] In contrast, TGF-β, together with IL-23, IL-6, and IL-1β, drives Th17 differentiation.[26] IL-4 and IFN-γ have been shown to antagonize the action of TGF-β on naïve cells, which provides a potential mechanism for the divergence of Th1, Th2, and Th17 cell lineages.[27] In addition, IL-4 in the presence of TGF-β inhibits the generation of Foxp3(+) T-regulatory cells and results in a population of Th cells that produce IL-9 and IL-10 but have no regulatory function.[28] Therefore, TGF-βs have direct effects on dendritic cells and on the differentiation and homeostasis of effector and regulatory T cells.[25] One of the main functions of TGF-βs in the immune system is to promote and maintain T-cell tolerance to self, food, and harmless environmental antigens, not only during infancy but throughout life to maintain normal homeostasis.

Oral Tolerance and Prevention of Allergy

Although the Th1/Th2 imbalance is considered important in development of allergy or inflammation, induction of oral tolerance is also an important component in appropriate mucosal immune function. Oral antigens, such as food or commensal bacteria, are normally processed in a manner that results in a regulated immune response. This response does not injure the host and results in systemic hyporesponsiveness on subsequent oral or systemic challenge with the same food antigen.[29] Oral tolerance induction is thought to occur through a number of possible mechanisms including anergy, receptor downregulation, or active cellular suppression by T cells. IL-17 produced by Th-17 cells also plays a role in oral tolerance induction, and impaired oral tolerance development is associated with increased production of IL-17 within draining lymph nodes of the gut.[30,31] It is widely thought that if the normal processes in establishing oral tolerance are defective, then food-allergic diseases can result.

Oral tolerance to food antigens can be induced experimentally, but optimization of the dose used for sensitization is critical.[32] Animals fed high doses of ovalbumin secreted more IL-4 (associated with allergy) and less TGF-β, whereas those fed low doses secreted more TGF-β and less IL-4. The different doses of antigen resulted in different patterns of cytokine secretion. The authors proposed that high doses of antigen induce cells to be anergic. Low doses of antigen induce antigen-driven active suppression with increased secretion of TGF-β.[33] Allergic children have a reduction in the number of TGF-β1–producing cells in the intestine and impaired generation of Th3 cells in both the epithelium and lamina propria.[34]

T-regulatory cells are critical in regulating the immune response and maintaining immune homeostasis in the intestine. Oral tolerance development is associated with Foxp3-positive ($CD4^+CD25^+Foxp3^+$) regulatory cells.[35-38] Transcription factor Foxp3 is critical for the development and function of T-regulatory cells. A variant of IPEX syndrome, which is characterized by autoimmune and severe immuno-allergic symptoms, including severe enteropathy, food allergies, atopic dermatitis, hyper-IgE, and eosinophilia, is associated with a mutation within an upstream noncoding region of Foxp3 that affects its function.[37] Conversion of $CD4^+CD25^-$ T cells into $CD4^+CD25^+$ T cells by the expression of FoxP3 involves TGF-β2[39] Furthermore, a membrane-bound form of TGF-β (containing latency-associated peptide [LAP]) has also been described, and these LAP^+ $CD4^+$ T cells mediate suppression in the gut by a TGF-β2–dependent mechanism.[40] TGF-β production in the intestine is critical not only for oral tolerance induction but also for regulating intestinal immune responses in general after food ingestion.[7] Faria et al[7] provided evidence to suggest that TGF-β may be the primary link between distinct populations of regulatory T cells that are induced by feeding and that conversion of $CD4^+CD25^-$ into $CD4^+CD25^+$ T cells by the expression of FoxP3 involves TGF-β. Faria et al[7] proposed that T-regulatory cells act to suppress immune responses in the intestine and that macrophages that phagocytose apoptotic cells in the intestine or dying T cells themselves also produce TGF-β.

In support of the role of TGF-β in induction of tolerance in infancy, we show that supplementation of formula with physiological levels of TGF-β2 downregulates the β-lactoglobulin–specific IgG1 response as well as total IgE and mucosal mast cell activation as measured by RMCPII in recipient rat pups. Supplementation of formula also resulted in an increase in Th1 cytokines, IL-18, IL-12, and IFN-γ and an

increase in IL-10 in allergy-prone rat pups.[16] TGF-β2 supplementation of formula moved the immune response profile of allergy-prone (Th2 type) rat pups toward a Th1 profile in the suckling period. Importantly, this immune profile persisted after weaning when TGF-β2 was no longer present in the diet.[16] These findings support the role of milk-derived TGF-β in programming the developing immune response.

Early Oral Antigen Introduction, Maternal Milk, and Tolerance

Animal studies have demonstrated that tolerance is an antigen (allergen)-driven process in which maternal milk and TGF-β play an important role.[41] Early exposure to repeated doses of food proteins (allergens) can induce oral tolerance during an early age, which coincides with the establishment of healthy gut colonization with commensal bacteria, shown to be essential in promoting tolerance to allergens.[29,42] There are only a few studies in infants, whether in animals or humans, undertaken to validate the effects of antigenic dose and timing in inducing oral tolerance. Strobel et al[43] showed that feeding OVA within the first week of life to mice resulted in priming for both humoral and cell-mediated immunity. More recent studies link early antigen exposure with tolerance. Introduction of increasing amounts of cow's milk protein (starting at 0.6 mg) in children with severe IgE-mediated cow's milk allergy resulted in 71.4% achieving desensitization to cow's milk protein in a period of 6 months.[44] More research is required to determine the optimum intervention strategies to promote tolerance.

Maternal Milk, Early Antigen Feeding, and Development of Food Allergy

Although the relationship between breast-feeding and allergy prevention is controversial,[45,46] there is little doubt that breast-feeding should be promoted for its many other benefits. More recently, there has been growing interest in the role of breast milk on promoting tolerance to food antigens as new foods are introduced. At this stage, there is some evidence that continued breast-feeding during introduction of complementary foods is important for promoting tolerance.[47] There is growing support for the concept of inducing oral tolerance by early oral antigen exposure (reviewed by Lack et al[48]). The duration of exclusive breast-feeding and the timing of the introduction of allergens into the diet are key factors that are currently the focus of much debate and controversy.[49-51]

We investigated the immune response profile after early oral β-lactoglobulin (major allergen in infant formula) exposure during breast-feeding or formula-feeding in a rat pup model. Early oral antigen exposure during formula-feeding significantly increased serum allergy makers, intestinal mast cell numbers, and their activation. In contrast, early oral antigen exposure in the presence of maternal milk resulted in an immune response profile not significantly different from that of unchallenged rat pups. Early food antigen exposure in the presence of maternal milk promotes a regulated immune response and tolerance induction when compared with early antigen exposure during formula-feeding.

Conclusion

TGF-βs are an important family of growth factors involved in maintaining homeostasis in the intestine, regulating inflammation and allergy development, and promoting oral tolerance development in infants. Early oral antigen exposure may be beneficial for promoting tolerance development during introduction of food antigens. ■

Author Disclosures

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