Which factors in raw cow's milk contribute to protection against allergies?

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Several epidemiologic studies have shown that growing up in a farming environment is associated with a decreased risk of allergies. A factor that correlates strongly with this effect is the early ingestion of unheated cow's milk. Although, to date, no controlled studies on raw milk consumption have been performed to formally demonstrate this effect, several factors in bovine milk have been described that might explain how raw cow's milk consumption can decrease the risk of allergies. In addition, increasing knowledge on the immunologically active factors in breast milk have also contributed to our understanding of the effects of bovine milk in infants because many of the factors in bovine milk are expected to have functional effects in human subjects as well. Here we review these factors and their mechanisms of action and compare their presence in bovine milk and breast milk. A better understanding of these factors, as well as how to retain them, might ultimately lead to the development of mildly processed milk and infant nutrition products that could become a part of preventive strategies to reduce the incidence of allergic disease. (J Allergy Clin Immunol 2012;130:853-8.)

Key words: *Allergy, milk, breast-feeding, farm, processing*

Abbreviations used
DC: Dendritic cell
FcRN: Neonatal Fc receptor
SCFA: Short-chain fatty acid
Treg: Regulatory T

It is widely accepted that a dysbalanced immune response to harmless foreign proteins causes type I allergy. The most prominent factor in type I allergy is the presence of allergen-specific IgE antibodies. These antibodies bind to FcεRI on effector cells, most prominently on mast cells and basophils.[1] On allergen binding to the IgE in a multivalent manner, mast cells will be activated. After at least 100 to 1000 of these interactions, mast cells and basophils will degranulate and release their inflammatory mediators, such as histamine and cytokines, and this can induce a clinical allergic response.[2]

The T_H2 cytokines IL-4 and IL-13 are important for the induction of IgE by plasma cells, which is inhibited by the T_H1 cytokine IFN-γ. There is mutual inhibition even on the level of specific transcription factors: the T_H2-related transcription factor GATA-3 inhibits the T_H1-related transcription factor T-box transcription factor and *vice versa*.[3] It has been hypothesized that the final balance of T_H2 and T_H1 is essential for the induction of an allergic immune response. Following that line of thought, the underlying mechanism of allergen immunotherapy was hypothesized to act through induction of T_H1. However, this was shown to be too simple when a role for regulatory T (Treg) cells was identified.[4]

Treg cells control or stabilize the T_H1/T_H2 balance by excreting IL-10, which in turn leads to increased levels of allergen-specific IgG_4. The induction of IgG_4 has been related to the success of allergen immunotherapy. IgG_4 can diminish allergic effector responses in different ways, first by competing with IgE in the binding of allergens and second by binding to the inhibiting receptor FcγRIIb, which leads to specific inhibition of the signaling of FcεRI.

The strong increase in the prevalence of allergic diseases in westernized societies in the last 50 years, approximately doubling within every 15 years, has indicated that specific environmental factors have an influence on the outcome of the genetic predisposition for allergy in specific subjects. Examples of situations that are associated with a reduction in the prevalence of IgE-mediated allergic diseases are increased family size, birth order, and day care visits but also growing up on a farm. The environmental influences have been grouped under the name the hygiene hypothesis.[5] The immune mechanisms underlying the hygiene hypothesis are not yet fully elucidated. One of the explanations has been the reduced immune regulation caused by decreased infection stress and the infection-dependent counterregulatory role of IL-10.[6,7]

BREAST-FEEDING, FARM MILK, AND ALLERGY

Breast-feeding has been linked to a decreased risk of the development of allergic diseases.[8-10] A variety of factors present in breast milk have been associated with effects on children's immunity and the development of allergic diseases. Several cytokines are detectable in breast milk, including anti-inflammatory cytokines, such as IL-10 and TGF-β, which seem to vary in

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TABLE I. Typical concentrations of milk proteins in mature human and bovine milk

Component	Concentration		Component	Concentration	
Proteins	Human milk (mg/mL)	Bovine milk (mg/mL)	Proteins	Human milk (μg/mL)	Bovine milk (μg/mL)
β-Casein	4.7	9.6	Lactoperoxidase	0.8	34
κ-Casein	1.4	3.4	sCD14	15	7
α_{S1}-Casein	0.9	10.6	Lactadherin	90	30
α_{S2}-Casein	Absent	2.8	β_2-Microglobulin	10	10
β-Lactoglobulin	Absent	3.1	Xanthine oxidase	10	10
α-Lactalbumin	3.0	0.9	IL-1β	0.0013	0.003
Serum albumin	0.5	0.3	IL-6	0.00015	0.00015
Lactoferrin	2.0	0.1	IL-10	0.00019	0.0003
Lysozyme	0.5	0.0004	TNF-α	0.0006	0.003
Osteopontin	0.14	0.02	TGF-β1	0.0008	0.005
IgG	0.04	0.63	TGF-β2	0.003	0.018
IgA	1	0.1	Alkaline phosphatase	Present	Present
IgM	0.1	0.09			
Mucin 1	0.73	0.01			

Shown are the major milk proteins, as well as selected additional proteins with possible immunologic relevance. Concentrations are expressed as concentration per milliliter of milk.

concentration according to the allergic status of the mother and the duration of lactation. TGF-β levels in breast milk have been correlated to the occurrence of allergies.[11] In addition, breast milk–induced tolerance might also depend on factors such as vitamin A and osteopontin, but the underlying mechanisms remain elusive.[12,13] In addition, the presence of bovine milk–derived vaccenic and rumenic acid in breast milk correlates with a decreased risk of allergic sensitization.[14]

In analogy to breast milk, several epidemiologic studies have identified that the consumption of untreated bovine milk can also reduce the risk of allergy, most prominently of allergic asthma.[15-19] Unfortunately, because raw milk can contain pathogenic organisms, no controlled intervention studies have been performed in young children to formally confirm this effect.

It is important to compare the composition of human and bovine milk to understand how the consumption of bovine milk can prevent the occurrence of allergies in human subjects. Milk is a complex fluid, containing a large (>400) number of different components designed to provide nutrition and protection to the neonate. The composition of milk depends on genetics (eg, species and breed), the food the mammal consumes, and other factors, such as lactation stage, parity, milking frequency, and the presence of disease (eg, mastitis). Human and bovine milk are very similar regarding the presence of the different components in milk. However, the concentration of the components differs between human and bovine milk.

Tables I and II show the concentration of a number of active immune components in human and bovine milk. The concentration is a typical value representing the concentration of mature human and bovine milk healthy subjects consume as part of a typical diet. The data in Tables I and II are mainly based on the work of Jensen,[20] Heck et al,[21] and Hettinga et al.[22] Regarding milk proteins, the most striking difference between human and bovine milk is the absence of β-lactoglobulin and α_{S2}-casein in human milk, which might contribute to their immunogenicity and allergenicity in patients with cow's milk allergy.[7] Furthermore, human milk contains an at least 10-fold higher concentration of lactoferrin, osteopontin, and lysozyme and an at least 10-fold lower concentration of lactoperoxidase and α_{S1}-casein compared with bovine milk. The fatty acid composition of human and bovine milk is similar, except for the higher concentration of linoleic acid (C18:2 cis 9,12) and conjugated linoleic and docosahexaenoic acid in human milk fat. However, it should be noted that bovine milk differs from human milk based on the relatively high concentration of very short-chain fatty acids (SCFAs; C4:0 and C6:0), which are absent in human milk fat. The concentration of polar lipids in human and bovine milk is also similar. Regarding the vitamin and mineral composition, human milk contains more vitamins A and D and less calcium and zinc compared with bovine milk. The major difference between human and bovine milk is the concentration and number of oligosaccharides present.

The oligosaccharides in bovine and human milk are quite different in concentration and composition (Table II).[7,23] Human milk contains a much higher number and concentration of oligosaccharides, leading to a microbiota composition with high numbers of bifidobacteria.[7] In addition, allergic children have lower numbers of bifidobacteria than nonallergic children.[24] Human milk oligosaccharides consist of neutral and acidic oligosaccharides. Many neutral oligosaccharides contain fucose, and most acidic oligosaccharides contain sialic acid.[25,26] Bovine oligosaccharides are present in milk at much lower concentrations and have virtually no fucose-containing oligosaccharides.[27]

Milk oligosaccharides are thought to play an important role in the prevention of adhesion of potentially pathogenic bacteria to the intestinal epithelium by acting as decoy receptors for lectins and sugars, thus preventing their infectivity.[23,28] In addition, they serve as a substrate for the intestinal microbiota and play a role in shaping the microbiota composition and have also been ascribed immunomodulatory function, although evidence for this is scarce.

Inclusion of prebiotics or synbiotics (prebiotics and probiotics) in infant nutrition to replace the function of human milk oligosaccharides in breast milk has demonstrated a reduction in the development of atopic dermatitis but not atopic sensitization caused by synbiotics[29] and an effect of prebiotics alone on atopic dermatitis, recurrent wheezing, and allergic urticaria,[30] as well as on infections, but no reduction in allergen-specific IgE levels has been shown.[31] An effect on atopic dermatitis but not on atopic sensitization is puzzling but could be linked to the earlier finding that low levels of bifidobacteria are associated more strongly with atopic dermatitis but not with asthma and rhinitis.[32]

TABLE II. Typical concentrations of fatty acids, minerals, vitamins, and oligosaccharides in mature human and bovine milk

Component	Concentration		Component	Concentration	
	Human milk	Bovine milk		Human milk	Bovine
Lipid–fatty acid	**g/100 g of fat**	**g/100 g of fat**	**Lipid–polar lipids**	**mg/100 g**	**mg/100 g**
C18:2 cis 9 trans 11 (CLA)	0.4	0.5	Phosphatidylethanolamine (PE)	6.9	7.9
C18:2 cis 9,12	10.8	1.3	Phosphatidylinositol (PI)	1.4	1.3
C18:3 cis 9,12.15	1.0	0.5	Phosphatidylserine (PS)	2.3	2.1
C22:6 cis4,7,10,13,16,19 (DHA)	0.25	0.01	Phosphatidylcholine (PC)	6.2	6.7
C22:5 cis 7,10,13,16,19 (DPA)	0.19	0.09	Sphingomyelin (SM)	8.1	7.1
C20:4 cis 5,8,11,14 (ARA)	0.46	0.20	Gangliosides	0.28	0.14
C20:5 cis 5,8,11,14,17 (EPA)	0.12	0.05			
Vitamins	**mg/100 g**	**mg/100 g**	**Minerals**	**mg/100 g**	**mg/100 g**
Vitamin A	0.061	0.028	Calcium	28	120
Vitamin D	0.0001	0.0001	Iron	0.03	0.05
Vitamin C	4.5	1.5	Zinc	0.12	0.35
			Selenium	0.002	0.002
Oligosaccharides	**mg/100 g**	**mg/100 g**	**Oligosaccharides**	**mg/100 g**	**mg/100 g**
Total (without lactose)	700	10	Lactose	7000	4500
3′-Sialyllactose	15	4	2′-Fucosyllactose	184	Absent
6′-Sialyllactose	42	2	Lacto-N-tetraose (type 1)	86	Absent
6′-Sialyl lactosamine	present	2	Lacto-N-fucopentaose-I	67	Absent

The components shown are selected components that might be relevant for immune function. The oligosaccharides shown are the 3 major oligosaccharides (and lactose) present in human and bovine milk. Component concentrations are expressed as mg/100 g of milk fat (solid) or as mg/100 g of milk (components are typically measured in dry matter).
CLA, Conjugated linoleic acid; *DHA*, docosahexaenoic acid.

WHICH BOVINE MILK FACTORS MIGHT PROTECT AGAINST ASTHMA AND ALLERGY DEVELOPMENT?

As discussed above, apart from apparent differences, bovine milk contains a wide variety of ingredients that are similar to the molecules present in breast milk. We will discuss in more detail which of these factors might be linked mechanistically to a protective effect of raw farm milk on the development of allergy and asthma. It should be noted that although epidemiologic studies have been performed with raw farm milk, with commercially available milk as a reference group, mechanistic research has been performed with isolated milk ingredients. In addition, commercial milk products can be subjected to varying degrees of heating, resulting in different amounts of intact milk proteins. Furthermore, some of the milk ingredients (especially heat-insensitive ingredients, such as fat, lactose, and spore elements) with functional activities are present in all types of milk, and some are only retained in unprocessed farm milk. All of the factors that might play a functional role in the protective effects of raw farm milk are discussed as part of a unifying hypothesis, as shown in Fig 1.

Because the presence of nondenatured milk proteins correlates with the functional effects of farm milk, native milk proteins can be considered of crucial importance for the protective effect of farm milk.[18] Together with the fact that the digestive process in infants is much milder when compared with that seen in adults, quite a proportion of the ingested native milk proteins is expected to reach the small intestine in small children.

The major milk proteins α-lactalbumin, β-lactoglobulin, and the caseins are all proteins that cannot be linked directly to immune functioning. However, because milk is intended to feed the infant, as well as protect it, less abundant milk proteins, such as IgA, IgG, IgM, TGF-β, IL-10, lactoferrin, lactadherin, and lysozyme, do have immune-related functionalities that could be linked to the functional effects of farm milk. In addition, milk-derived fatty acids, vitamins, lactose, and oligosaccharides present in bovine milk can also play an important role, especially when it is taken into account that these are not quite as heat sensitive as the proteins.

These factors might work on separate levels; that is, they could contribute to the induction of adaptive immune responses, create a microenvironment that favors Treg cell development, modulate microbiota composition, and contribute to overall intestinal barrier function. These mechanisms are summarized in Fig 1 and discussed in more detail below.

Mosconi et al[33] elegantly demonstrated that the presence of immune complexes of allergen and allergen-specific IgG confers oral tolerance to the allergen and induces allergen-specific Forkhead box protein 3–positive $CD25^+$ Treg cells in a murine model. This effect was shown to be dependent on the neonatal Fc receptor (FcRN), which is expressed in the neonatal intestine. Although this route of oral tolerance induction is independent of TGF-β, TGF-β is crucial in the induction of oral tolerance when the allergens are present in breast milk in the absence of specific IgG.

Can bovine milk immunoglobulins also play a role in immune complex–dependent tolerance induction? Human IgG, as well as IgA, can be transported over the epithelium in a bidirectional fashion, indicating that immune complexes can be taken up from the gut lumen and transported into the mucosa or Peyer patches. Likewise, bovine IgG can bind to human FcRN, and binding to FcγRII on human phagocytes has also been demonstrated.[34,35]

In a recent study, Qiao et al[36] demonstrated that human dendritic cells (DCs) expressing FcRN can indeed selectively bind IgG-antigen immune complexes and facilitate antigen presentation to antigen-specific T cells. This reversed transport of IgG by FcRN appears after complexing of IgG with luminal antigen, resulting in transport of the immune complexes into the lamina propria, after which the antigen is presented by DCs to $CD4^+$ T cells in regional lymphoid tissues.[37] This results in efficient immune responses as evidenced by cellular proliferation and cytokine production. As seen in Table I, mature bovine milk contains IgG_1, IgA, and IgM. Interestingly, bovine milk contains inhalational allergen–specific IgG to house dust mite, *Aspergillus*

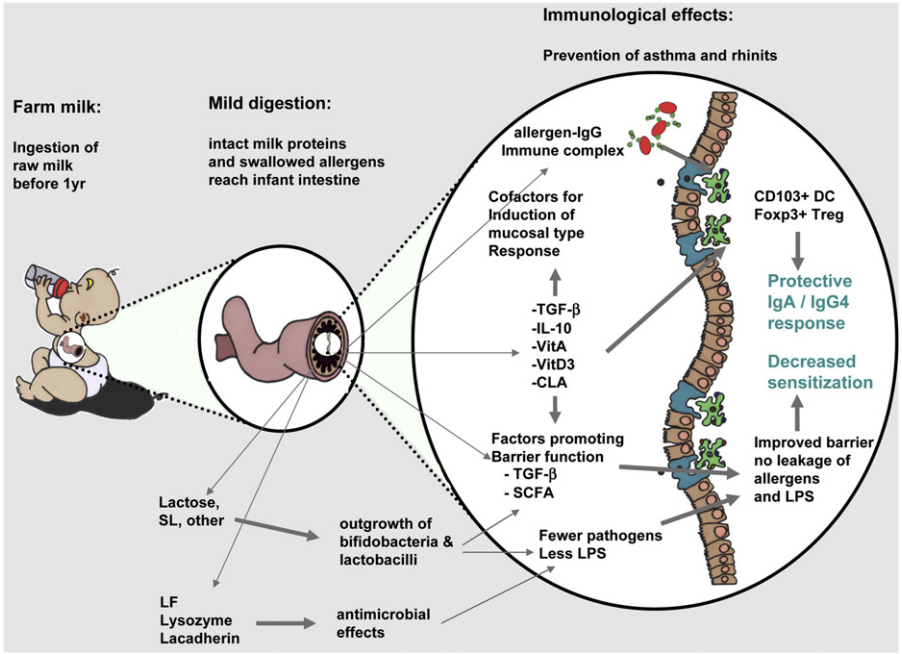

FIG 1. Allergenic proteins, as well as milk proteins, are swallowed and reach the gastrointestinal tract partly intact because of milder digestion in infants compared with that in children and adults. On simultaneous ingestion of raw milk, the allergens come into contact with allergen-specific IgG or IgA in cow's milk, form immune complexes, are actively taken up into the Peyer patches through Fc receptors, and thus induce efficient immune responses to these allergens. Milk also contains vitamins A and D, milk-derived conjugated linoleic acid *(CLA)*, and immunomodulatory cytokines, such as TGF-β2, osteopontin, and IL-10 (low levels), which all contribute to a regulatory environment in which preferentially IgA and not IgE and Treg cells are induced. Antimicrobial proteins, such as lactoferrin *(LF)* and lysozyme, as well as nondigested lactose and milk oligosaccharides, such as sialyllactose *(SL)*, might modulate the microbiota composition and metabolic activity (SCFA production) of bifidobacteria. TGF-β and SCFAs promote epithelium differentiation and barrier function, preventing the leakage of water-soluble allergens into the intestinal mucosa. All these factors are needed in concert, and on processing and heat treatment of milk, some of these factors are denaturated, depleted, or both, thus removing the effects of unprocessed raw farm milk.

species, grass pollen,[38] and birch pollen (R.J.J.v.N. unpublished results).

On inhalation, a large part of the inhaled allergens will be cleared from the upper airways by means of mucociliary motion and be swallowed. Therefore it can be envisaged that these swallowed allergens will be bound by bovine immunoglobulins after the ingestion of raw milk, and targeted uptake and presentation of the allergens through Fc receptors will occur. Because other milk components create a Treg cell–favoring microenvironment (discussed below), this should in theory lead to the production of (neutralizing) allergen-specific IgA and IgG_4 immunoglobulins.

The presence of TGF-β in breast milk confers a protective effect against allergy-related outcomes in infancy and early childhood.[11] In a mechanistic study Verhasselt et al[39] have shown that TGF-β in murine breast milk is of crucial importance in inducing oral tolerance to allergens. The TGF-β variant found in bovine milk is predominantly TGF-β2, but TGF-β1 is also present at relatively high levels. Both bovine TGF-β2 and TGF-β1 have a very high sequence identity with their human counterparts (almost 100% for TGF-β2) and can thus be expected to have the same activity as TGF-β in breast milk.

Likewise, den Hartog et al[40] recently showed that bovine IL-10, although it only has 70% sequence identity with human IL-10, has a similar immunomodulatory effect on human DCs and monocytes compared with human IL-10. IL-10 is also present in breast milk, and IL-10 levels negatively correlate with the prevalence of necrotizing enterocolitis in young infants,[41] suggesting that orally delivered IL-10 is functional in infants *in vivo* and contributes to the regulation of immune responses.

Thus because bovine milk contains immunomodulatory cytokines, such as TGF-β2 and (very low levels of) IL-10, its consumption might results in a regulatory environment in which Treg cells are induced, leading to the production of IgA and IgG_4 but not IgE. The presence of vitamins A and D in milk can also play an accessory role here. Vitamin A is metabolized in intestinal epithelial cells to retinoic acid, which in turn is needed for Treg cell development. Deficiency of vitamin D, which is well known for its immune-suppressive effects, is a risk factor for allergy development.

In addition, the presence of bovine-derived fatty acids, such as the conjugated linoleic acids rumenic and vaccenic acid, in breast milk was shown to be protective against eczema, atopic dermatitis, and sensitization to food allergens.[14] Thus the rumenic and vaccenic acid present in cow's milk might have a similar protective effect when consumed in bovine milk during infancy. These fatty acids are capable of inhibiting proinflammatory cytokine production and might thus, in concert with TGF-β, vitamin A, vitamin D, and IL-10, create a microenvironment that promotes the differentiation of Treg cells.

The presence of *Clostridium difficile* in infants is associated with an increased risk for atopic sensitization, wheeze, and atopic dermatitis, and a decrease in bifidobacteria is associated with eczema.[32,42-44] These differences might be linked to the immunoregulatory environment in the intestine. Thus factors that modulate microbiota composition can contribute to a balanced immune response to allergens. Interestingly, children with cow's milk allergy have a different microbiota after 6 months of infant formula with hydrolysates compared with nonallergic infants receiving regular formula that has not been hydrolyzed. This differential microbiota development induced by hydrolysates compared with intact proteins might suggest that the digestion status of protein could also contribute to the outgrowth of bifidobacteria. Alternatively, the allergic status itself can influence the microbiota composition. Some of the proteins involved in this process might be the antimicrobial proteins present in cow's milk. Lactoferrin has an iron-scavenging function in the intestine and can prevent the growth of iron-dependent pathogens. Lactoferrin has been shown to have a protective effect in necrotizing enterocolitis in infants with very low birth weight.[45] Other milk enzymes, such as lysozyme, also have an antimicrobial activity and thus can influence the microbiota composition in the infant intestine.[46]

As shown in Table II, the oligosaccharide composition of breast milk is far more complex than the composition in bovine milk. However, bovine milk contains saccharides that can promote the growth of bifidobacteria in the infant intestine, such as sialyllactose and lactose. Lactose is normally not completely digested by infants, and therefore certain amounts of lactose can reach the large intestine, where it can be metabolized by sugar-fermenting bacteria, such as bifidobacteria, that in turn produce SCFAs, such as as acetate, butyrate, and propionate. These SCFAs decrease the colonic pH, as a result of which some pathogens are decreased.

SCFAs are also known to enhance the epithelial barrier function. An intact epithelial barrier is essential for gut health because it prevents invasion by pathogenic bacteria and their degradation products, as well as entrance of intact proteins. In addition to SCFAs, the calcium present in milk and TGF-β, which is an important differentiation factor for intestinal epithelium, and sialyllactose, which has been shown to promote the differentiation of intestinal epithelial cells, as evidenced by the production of intestinal alkaline phosphatase,[47] all contribute to epithelial differentiation and intestinal barrier function.

Thus even though no solid evidence is available, we hypothesize that raw farm milk might, through regular lactose and oligosaccharide contents but also through the presence of intact milk proteins, promote the selective outgrowth of bifidobacteria. This, in combination with inhibitory effects on pathogens by the SCFAs produced, as well as by the presence of antimicrobial proteins, might result in a microbiota composition that is similar to that of breast-fed infants (ie, high bifidobacteria and lower *C difficile* and *Escherichia coli* levels).

An improved barrier function prevents leakage of allergens into the mucosal circulation and also prevents the leakage of food or microorganisms and their derived components, such as LPS. Thus barrier function is improved, and the proinflammatory effect of intestinal LPS is reduced.

FUTURE DIRECTIONS

Because ingestion of raw farm milk inversely correlates with the development of asthma, raw farm milk consumption can in theory be considered a preventive measure. However, because raw milk can contain pathogenic organisms, raw milk consumption as such cannot be recommended. As described by Loss et al,[18] the protective effect of farm milk on asthma development correlates with the amount of nondenaturated milk proteins in the milk consumed by the children in the study. Therefore mild processing of milk to preserve the functionality of milk proteins might be an excellent alternative to provide a microbiologically safe alternative to raw farm milk.

Even though only α-lactalbumin, β-lactoglobulin, and high levels of bovine serum albumin were statistically significantly correlated with the prevention of asthma in the article by Loss et al,[18] a dose-dependent tendency could be seen in that the odds ratios for asthma and current asthma decreased for all milk proteins measured but not for bacterial counts and fat content.

One important point should be made here in relation to the milk fat fraction. The milk fat globular membrane contains many proteins (eg, lactadherin, which prevents rotavirus binding to epithelial cells), as well as triglycerides and phospholipids. On milk collection, the milk is first separated into a cream fraction and a skim fraction, and the cream fraction is exposed to a much higher temperature treatment than the skimmed milk. When raw farm milk is compared with pasteurized shop milk, the functionality of proteins that are present in the cream fraction has thus largely been inactivated through denaturation. This might explain why the fat content of milk does not correlate with a decreased prevalence of asthma in the study by Loss et al,[18] whereas the consumption of farm butter does.[19]

CONCLUSIONS

Raw milk contains many proteins and other constituents that might help in preventing asthma in infants and young children. These findings are highly relevant and could lead to the development of mildly processed milk products and toddler and infant nutrition, which could become a part of preventive strategies to reduce the incidence of allergic disease.

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