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Transforming growth factor-β_1 is elevated in unpasteurized cow's milk

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Unpasteurized milk consumption was associated with less atopy prevalence. Not only microbial load but also fatty acids and cytokines such as transforming growth factor-β_1 (TGF-β_1) may play a role on the effect of unpasteurized milk. Levels of TGF-β_1 in different cow's milk samples were evaluated: we consider raw unpasteurized milk before and after boiling, commercial pasteurized and micro-filtrated cow's milk and different commercially available cow's milk formulas. TGF-β_1 concentration in raw unpasteurized cow's milk was 642.0 ± 52.9 pg/ml before boiling and decreased significantly after boiling (302.7 ± 50.59 pg/ml) ($p < 0.05$). TGF-β_1 concentrations were also significantly lower in commercial pasteurized milk (246.2 ± 43.15 pg/ml) and in commercial micro-filtrated milk (213.0 ± 31.6 pg/ml) in comparison to unpasteurized unboiled milk ($p = 0.002$). The levels of TGF-β_1 in all formula samples were below the threshold of detectability for the assays. As TGF-β_1 in the milk may contribute to the development of the immature gastrointestinal tract by influencing IgA production and oral tolerance induction, we suggest to consider not only the microbial compounds but also the cytokine patterns to explain the protective effect of unpasteurized cow's milk on allergic disorders.

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In a recent paper, Perkin et al. have investigated the role of different aspects of the farming lifestyle and particularly dietary factors in the development of allergic disorders in childhood (1). In the study farmers' children have a reduced prevalence of current asthma and of seasonal allergic rhinitis symptoms. Among all the different considered environmental factors, only the current unpasteurized milk consumption was associated with less atopy and less eczema, independently by the farming status. This consistent protective effect was not apparent for any other food assessed by food-frequency questionnaire. The effect of unpasteurized milk consumption was associated with other objective measures such as skin prick test wheal size, serum total IgE levels, and interferon-γ production in stimulated whole-blood assay, leading authors to suggest that the protective effect was a genuine phenomenon (1). Another study has recently confirmed that farm milk consumption ever in life is significantly and inversely associated with asthma, rhinitis, and sensitization to allergens (2). Of particular importance in this last study is the consistency of the same findings across children from farming, rural non-farming, anthroposophic, and urban environment. This indicates that farm milk consumption since the first year of life may represent a route of exposure that is independent of concomitant exposure to microbial compounds present in animal sheds and farm homes (2). In both papers, authors speculate about the components of the farm milk responsible for the observed protective effect, focusing in particular on the different levels or different composition of pathogenic and non-pathogenic microbes compared with milk after pasteurization (1, 2). In fact unpasteurized milk is rich in a variety of gram-negative species and of their lipopolysaccharides, which could

influence from early age the developing immune system (3). Unpasteurized milk can also contain lactobacilli that could have a protective effect for eczema (4). However, it can be argued that also other compounds, different from microbes, and specifically fatty acids and/or cytokines may play a role for the effect of unpasteurized milk. Considering breast milk, a variety of different factors have been associated to the effects on the children's immunity and development of allergic diseases. Oddy et al. showed that fatty acid profiles (increased ratio of n6:n3 fatty acids) may be associated with non-atopic eczema in infants at 6 months (5). Different cytokines are also detectable in breast milk (IL-4, IL-5, IL-6, IL-10, IL-13, IFNγ, and transforming growth factor-β_1 [TGF-β]), which seem to vary in concentration according to the allergic status of the mother and to the duration of lactation (6). Indeed, epidemiologic studies focused on the role of TGF-β_1 in breast milk to provide protection against allergic diseases in infancy (7). TGF-β is a multifunctional cytokine involved in cellular proliferation, differentiation, extracellular matrix regulation and survival that is considered to be primarily involved in the development of the infant immune responses (8). We have recently observed that in breastfeeding mothers TGF-β_1 was significantly higher in colostrum compared with mature milk, and it was significantly lower in atopic vs. non-atopic mothers (9). Furthermore, in a recent animal model study, it has been demonstrated that orally administered TGF-β_1 retains biological activity in the intestinal mucosa and enhances the induction of oral tolerance to a high-dose antigen (10).

Therefore, aim of the present study was to verify the presence and to quantify the levels of TGF-β_1 in different treated cow's milks to add further informations on the cow's milk composition in relation to development of allergic diseases.

Methods

Levels of TGF-β_1 in different cow's milk samples were evaluated: we consider raw unpasteurized milk before and after boiling, store-bought pasteurized and commercial micro-filtrated cow's milk (LatteBlu®, Parmalat, Parma, Italy) and different commercially available cow's milk formulas (Nidina 1® and Alfare'®, Nestle, Vevey, Switzerland).

At least 10 samples of each milk were obtained, filtered and prepared for TGF-β_1 assay as previously described (9). Briefly, whole milk aliquots were centrifuged at 500 g for 12 min, after which lipid layer and aqueous fraction were removed. Aliquots of the aqueous fraction were filtered (0.45 μm) and stored at −70°C until assayed. ELISA assays for TGF-β_1 were performed according to manufacturers' instructions performing a dual antibody sandwich analysis after spiking experiments (Biosource, Camarillo, CA, USA). Threshold of detectability for the assay was 31.2 pg/ml, with $< 5\%$ intra and $< 7\%$ interassays coefficient of variation. Before assay, sample was treated with 1 normal HCl to adjust to pH 3 (20 μl 1 normal to 500 μl sample); the acidified sample was incubated for 15 min at room temperature and neutralized with 1 normal NaOH (15 μl) and immediately tested. The treatment was performed to activate latent TGF-β_1 to the immunoreactive form.

Results

Concentration was expressed in picograms per milliliter (pg/ml) of milk (median ± standard deviation, s.d.). TGF-β_1 concentration in raw unpasteurized cow's milk was 642.0 ± 52.9 pg/ml before boiling and 302.7 ± 50.59 pg/ml after boiling, respectively. After boiling, a significant decrease of the levels of the cytokine was observed ($p < 0.05$). TGF-β_1 concentrations were also significantly lower in commercial pasteurized milk (246.2 ± 43.15 pg/ml) and in micro-filtrated milk (LatteBlu, Parmalat®) (213.0 ± 31.6 pg/ml) in comparison with unpasteurized unboiled milk ($p = 0.002$).

The levels of TGF-β_1 in all the samples of formulas were below the threshold of detectability for the assays.

Discussion

Our results demonstrate that TGF-β_1 levels are significantly higher in the raw unpasteurized cow's milk and that home (boiling) or industrial (pasteurization or micro-filtration) manipulation of the raw cow's milk can significantly decrease the content of this cytokine. All the cow's milk formulas investigated had undetectable levels of TGF-β_1 leading to consider that the industrial process to obtain formula milks can decrease TGF-β_1 levels. We have previously demonstrated that TGF-β_1 is present in the breast milk at high levels, particularly higher levels were observed in non-allergic mothers and in the first phases of lactation (9). Furthermore, we showed that, even if limited to a small population, lower levels of TGF-β_1 in breast milk may be associated to development of atopic dermatitis (9).

It is tempting to speculate that the presence of this and probably other cytokines in milk can influence maturation and function of epithelial, inflammatory and structural components of the developing intestinal system (11). Indeed, high levels of TGF-β_1 in breast milk can prevent sensitization to food allergens, acting synergistically with interleukin-10 to promote specific IgA production and inhibit T cell activation (11, 12). Thus, the cytokine pattern in the milk may contribute to the development of the immature gastrointestinal tract by inducing oral tolerance and promoting IgG–IgA antibody production and inhibiting IgE- and cell-mediated reactions to milk (13). The results by Ando and co-workers in an animal model reinforce this hypothesis as oral administration of the molecule was able to produce immune responses in the intestinal mucosa and to induce oral tolerance (10). TGF-β_1 is secreted in a latent form and then is activated extracellulary by extremes of pH, heat, proteases (8). The passage through infant stomach might free active form of TGF-β_1 in human milk, determining high-concentration exposure to the activated molecule in the infant intestine (14). For these reasons, we evaluated in the study the activated form of TGF-β_1 that is responsible for the multifunctional effects of the cytokine (14).

All these evidences lead to consider a possible role for TGF-β_1 in contributing to the observed protective effect of consumption of unpasteurized milk on prevalence of allergic disorders (1). Therefore, further analyses of the unpasteurized milk compounds responsible for the protective effect on allergic disorders have to consider not only the microbial compounds but also the cytokine patterns that could contribute to explain its beneficial effect.

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