

Milk: An epigenetic inducer of FoxP3 expression



To the Editor:

Kirchner et al¹ recently implicated microRNAs of unprocessed cow's milk in mediating the allergy-preventive effect of farm milk.² On the basis of translational evidence, we predicted that the transmission of milk-derived immune-regulatory exosomal microRNAs including microRNA-148a, microRNA-155, microRNA-29b, and microRNA-21 prevent atopy by inducing FoxP3+ regulatory T (Treg)-cell differentiation.³ Notably, farm milk intake is associated with higher *FOXP3* demethylation and higher Treg-cell numbers.⁴ Stable expression of FoxP3 in Treg cells depends on DNA demethylation at the Treg-cell-specific demethylated region, a conserved CpG-rich region within the *FOXP3* locus.⁵ DNA methylation is mediated by DNA methyltransferases (DNMTs). Both DNMT1 and DNMT3b are associated with the *FOXP3* locus in CD4+ cells. DNMT1 deficiency results in highly efficient FoxP3 induction following T-cell receptor stimulation.⁶ Importantly, DNMT1 is a direct target of microRNA-148a,⁷ which is abundant in bovine colostrum, mature cow's milk, and human breast milk.³ MicroRNA-148a is highly expressed in bovine milk fat and milk fat globules of human breast milk.

Boiling of cow's milk results in substantial loss of microRNA-148a-3p.¹ Remarkably, nucleotide sequences of microRNA-148a-3p of *Homo sapiens* and *Bos taurus* are identical. MicroRNA-148a directly downregulates the expression of DNMT1 and DNMT3b,⁷ whereas microRNA-21 indirectly inhibits DNMT1 expression by targeting RASGRP1.⁷ MicroRNA-29b, which dose-dependently

increases in human serum after intake of pasteurized cow's milk, targets DNMT3a and DNMT3b. Milk exosomes not only transfer microRNAs but also TGF- β , which plays a pivotal role in FoxP3 expression as well as downregulation of DNMT1, DNMT3a, and DNMT3b expression (Fig 1).

Thus, milk transmits crucial microRNAs that may be critical effectors for Treg-cell-specific demethylated region FoxP3 demethylation by targeting DNMTs, thereby stabilizing FoxP3 expression. Milk exosomes are of functional importance for the appropriate development of the infant's immune system via transfer of maternal microRNAs and TGF- β , which are derived directly from mammary gland epithelial cells or extruded from milk fat globules. Boiling of milk may disrupt the protective lipid bilayer of milk exosomes, accelerating microRNA degradation. Furthermore, heat-induced alterations of exosomal membrane proteins may disturb the intestinal uptake of milk exosomes.

In accordance with our hypothesis,³ it has been demonstrated that incubation of PBMCs with isolated human breast milk exosomes increases the number of FoxP3(+)CD4(+)CD25(+) Treg cells. Furthermore, bovine milk exosomes induce FoxP3 expression and Treg-cell differentiation in murine splenocytes. It is of most critical concern that current infant formula contains only negligible amounts of bioactive microRNAs. In contrast to formula feeding, the presence of maternal milk in β -lactoglobulin-sensitized rat pups exhibits an immune response profile similar to that of unchallenged dam-reared rats but with greater FoxP3 mRNA expression and CD4(+) FoxP3(+) cells.

In summary, there is accumulating evidence that milk-derived exosomes via transfer of DNMT-targeting microRNAs and exosomal TGF- β stimulate the expression of FoxP3, the

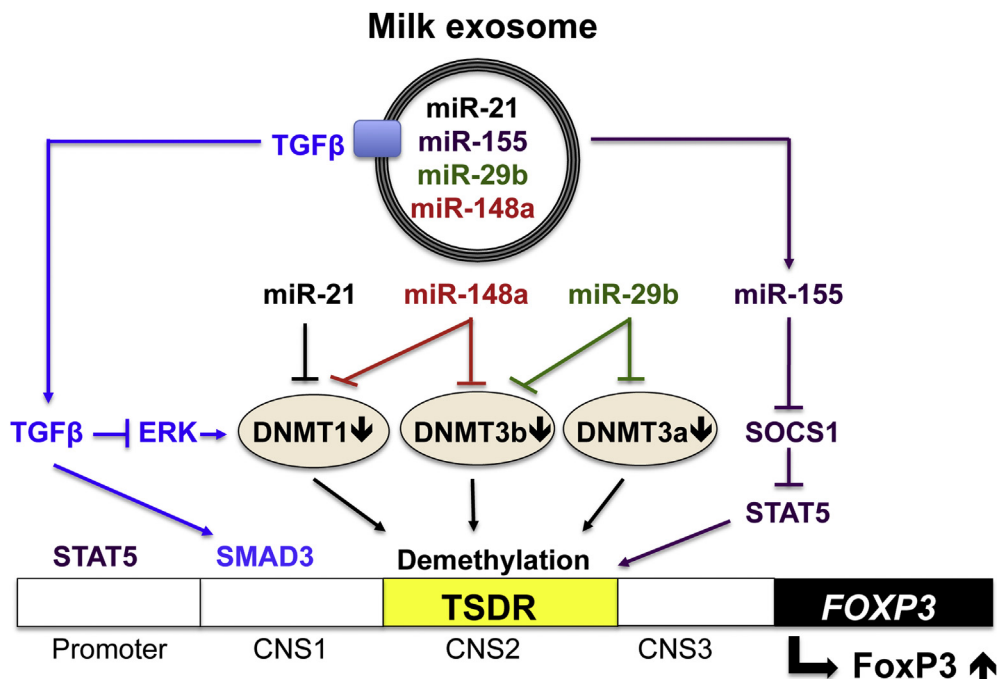


FIG 1. Working model for milk-driven epigenetic regulation of FoxP3 expression. *CNS*, Conserved noncoding sequence; *ERK*, extracellular signal-regulated kinase; *miR*, microRNA; *SOCS1*, suppressor of cytokine signaling 1; *STAT5*, signal transducer and activator of transcription 5; *TSDR*, Treg-cell-specific demethylated region.

peacekeeper of the immune system. A deeper understanding of milk's immunologic functions in postnatal epigenetic imprinting of *FOXP3* will enhance our understanding of the pathogenesis and prevention of atopic diseases and offer new strategies for functional improvements of infant formula.

Bodo C. Melnik, MD^a

Sven Malte John, MD^a

Gerd Schmitz, MD^b

From ^athe Department of Dermatology, Environmental Medicine and Health Theory, University of Osnabrück, Osnabrück, Germany; and ^bthe Institute for Clinical Chemistry and Laboratory Medicine, University Clinics of Regensburg, Regensburg, Germany. E-mail: melnik@t-online.de.

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Reply



To the Editor:

The beneficial farm effect on asthma and allergies has been consistently found across many countries; the underlying mechanisms, however, are largely unknown though they are increasingly being deciphered. The induction of A20 in lung epithelial cells, for example, may explain effects triggered by endotoxin exposure.¹ Nevertheless, many other contributing pathways have not yet been verified. The persistence of the farm effect into adulthood² suggests the involvement of epigenetic mechanisms as already discussed in the context of prenatal exposures.³ The major exposures determining the farm effect are regular stays in animal sheds and consumption of unprocessed cow's milk; the latter is partially related to fat content, particularly the proportion of ω -3 polyunsaturated fatty acids, and partially to heating of milk during processing or before consumption.⁴

In the article by Kirchner et al,⁵ we have taken up the idea that heat-sensitive milk ingredients beyond proteins may contribute to the farm milk effect and focused on a class of molecules highly

involved in immune regulation, that is, microRNA (miRNA). Given the high promiscuity of miRNA, we cautiously interpreted our findings with respect to possible biologically plausible mechanisms and concentrated only on genes robustly targeted by at least 3 miRNA species.

We appreciate the enthusiasm with which Melnik et al⁶ discuss further biologic pathways potentially involved in the protective effect of farm milk. The idea that milk-born miRNAs cause demethylation of *FOXP3* is definitively interesting, and the experiment by Admyre et al⁷ inducing T regulatory cells by incubation with breast milk in a dose-dependent manner is compelling. However, we refrained from such speculation and relied only on experimentally validated miRNA-mRNA interactions; the only validated miRNA species targeting *FOXP3* are hsa-miR-31-5p and hsa-miR-210-3p, which were not differentially expressed in our studies. Although the DNA methyltransferases 1, 3a, and 3b are validated targets of single miRNA species differentially expressed in our analysis, they do not specifically regulate demethylation of the *FOXP3* gene. In a pilot study including about 200 schoolchildren of the GABRIELA (A Multidisciplinary Study to Identify the Genetic and Environmental Causes of Asthma in the European Community - Advanced Study) study,⁸ we did not find any impact of raw milk consumption on demethylation or mRNA expression of *FOXP3* (Ege M, Jurinovic V, 2013, unpublished data).

Furthermore, Melnik et al suggest a potential transfer of relevant quantities of TGF- β by raw milk contributing to *FOXP3* expression. However, this assumption is contradicted by experimental data derived from 800 milk samples of the GABRIELA study: The geometric means of TGF- β in high heat-treated and pasteurized milk were 2.97 μ g/L and 8.63 μ g/L, whereas that of raw milk ranged in between with 5.71 μ g/L; in addition, there was no effect of TGF- β levels on asthma and atopy.⁸

Taken together, the evidence assembled by Melnik et al relies on extrapolation of *in vitro* experiments and murine models to the human situation, which warrants caution. Evidence from population-based analyses might here be advisable; therefore, we are currently assessing miRNA expression in milk samples from a cross-sectional and a longitudinal study including children with known asthma and allergy status to confirm our hypothesis. We hope that the emerging data will help unraveling the biologic mechanisms underlying the beneficial effect of farm milk in the near future.

Markus J. Ege, MD, MPH^a

Michael W. Pfaffl, PhD^b

From ^aDr von Hauner Children's Hospital, LMU Munich, Munich, Germany; and ^bAnimal Physiology & Immunology, TUM School of Life Sciences Weihenstephan, Freising, Germany. E-mail: markus.ege@med.lmu.de.

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