

# FORALLVENT

## Position paper on potential avenues for the development of allergy protective milk formulas

Partner 8: University of Natural Resources and Applied Life Sciences Vienna (BOKU), Austria

Over the last decades a strong increase in asthma and allergic diseases has been documented. This increase indicates the presence of strong environmental factors underlying disease susceptibility. Moreover, the onset of these diseases is due to a combination of environmental and genetic factors. In 1989, David Strachan was the first who observed an association between unhygienic conditions and protection from the development of allergic illness (D.P. STRACHAN, Br.Med.J. 299:1259-60, 1989). Since then a large number of studies have confirmed this “hygiene hypothesis“, and especially the growing up of a child in a farming environment was linked to an allergy-protective effect (RIEDLER et al., The Lancet 358: 1129-33, 2001). In 2007, Waser et al. have demonstrated that there is an inverse association of farm milk consumption with asthma and allergy in rural populations across Europe, independent of farm-related co-exposures (WASER et al., Clin.Exp.Allergy 37: 661-70, 2007).

All these findings formed the basis in the FORALLVENT project for the idea to develop an allergy-protective milk formula.

In the FORALLVENT milk workshops, experts from different fields such as allergology, immunology, epidemiology, food science, food technology, nutrition and also communication discussed the potential avenues and the possibility of developing such an allergy-protective milk or milk product.

Results from previous milk analyses indicate that the protective effect of farm milk consumption is more pronounced, if it is consumed as raw and unskimmed milk, without having undergone any heat treatment. On the other hand, raw milk consumption undoubtedly bears some risks, as described below.

Milk in the udder of healthy cows can be regarded as sterile. Contamination occurs after milking, originating from air and/or surfaces (production lines, milking machines, storage and cooling vessels etc.), whereas the type of contaminant usually reflects the contamination source and the environment.

Pathogens in raw milk may originate from the cow (e.g., cow's diseases), from pathogens in the environment and/or humans. As milk may be utilised as a substrate by almost every pathogen, it may cause a wide range of outbreaks (examples: streptococci, staphylococci, *E.coli*, *Brucella*, *Salmonella*, *Listeria*, *Yersinia*, *Mycobacterium tuberculosis*, etc.). The consumption of raw milk is especially hazardous to so-called "potential high risk consumers" like pregnant women, infants, children, elderly persons and people with a weakened immune system. Milk pasteurization is well-known to form an acknowledged basis for the production of pathogen-free milk of sufficiently high hygienic quality. At the moment, different approaches of milk technology do not provide any alternative method (e.g. microfiltration) to guarantee pathogen-free milk.

If milk is to be foreseen for an intervention study, it has to be safe in all cases. The question how we could assure a totally safe product without pasteurisation is difficult to answer. One possibility could be to use milk from certified farms which is carefully analysed prior administration. However, even if it is controlled with several modern and sensitive methods used for the detection of pathogens, it cannot be fully guaranteed as being safe. In addition, all these methods take time until a result will be obtained (usually more than 24 hours, rather more than 48 hours), and this causes some delay in the delivery to subjects. Another risk may be the presence of dormant and/or partially damaged microbes, which cannot reliably be detected with the available methodologies. Last but not least it has to be taken into consideration that only limited number of samples can be examined, in which potential pathogens may not be found by chance.

Due to these facts and also to the experience of scientists from different disciplines, direct administration of raw milk to subjects in an intervention study still bears risk which cannot be accepted. Hence we must first identify the ingredients/compounds /contaminants of raw milk which are responsible for the immuno-modulating, and thus, allergy-preventive effects.

In this context, the microbial load of milk including viable, dormant and uncultivable microorganisms or bacteria with probiotic properties has to be considered. Another approach suggests to study the effect of ingredient molecules such as fatty acids (e.g. conjugated linoleic acid-CLA) or proteins (such as whey proteins, e.g. immunoglobulins,  $\beta$ -lactoglobulin,  $\alpha$ -lactalbumin and bovine serum albumin, or also lactoferrin) and even peptides. As it still remains unclear, which aspect of the milk

processing makes the difference, more studies will be needed to elucidate this phenomenon.

It has been demonstrated that the spectrum of microorganisms in raw milk differs from that in pasteurised or (recontaminated) UHT-milk. Gram-positive microorganisms in milk originate from the farm environment, Gram-negatives from recontamination at processing level. However, a recent study (GEHRING et al., *Environment International*, 34(8): 1132-6, 2008) showed that there is no significant difference in endotoxin levels between shop and farm milk samples. Therefore, the protective effect of farm milk consumption against allergy development cannot be explained by a higher load of Gram-negative bacteria.

Probiotics can also modulate the immune response. However, the cellular and molecular mechanisms used by probiotics to modulate the immune response are largely unknown. Possibly, interaction of (compounds of) bacteria with members of the TLR-(toll-like receptors) family or C-type ( $\text{Ca}^{++}$ -dependent) lectins on the cell surface of dendritic cells are important (NIERS et al., *Ernährungsmedizin* 2: 32-35, 2008).

Lactoferrin, an iron-binding glycoprotein of the transferrin family, is known as a bioactive compound in human and also bovine milk. Besides its ability to iron absorption, Lactoferrin acts as antioxidant, antimicrobial, antiviral, anti-inflammatory and also immunomodulating protein. The latter is due to LPS-(lipopolysaccharide) binding, stimulation of NK (natural killer) cells, reduction of pro-inflammatory cytokines and T-cell maturation (STEIJNS and HOOIJDONK, *BJN* 84, Suppl. 1: S11-17, 2000). Due to its heat-sensitivity, Lactoferrin is partially or even completely destroyed during pasteurisation or UHT-treatment.

Whey also contains a number of peptides with immuno-modulatory properties, which are naturally present or are part of the primary sequence of whey proteins. Unfortunately, the knowledge of the mechanisms of whey protein-based immuno-modulating peptides is limited as these compounds are difficult to characterize and also due to the absence of clinical data on the physiological effects.

Conjugated linoleic acid (CLA), a mixture of positional and geometric isomers of linoleic acid, which contain a conjugated double-bond system, is reported to possess anti-carcinogenic, anti-atherosclerotic, anti-diabetic and immuno-modulating effects. CLA supplementation in young healthy men affected the immune function in terms of increased plasma IgA and IgM, and the anti-inflammatory cytokine IL-10, and

decreased levels of IgE and the pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\gamma$ . However, also in this context the mechanisms of beneficial effects of CLA are still unknown.

As long as the mechanism of the allergy and asthma preventive effect is not completely clarified, advanced intervention studies with humans will not be feasible. Nevertheless, experimental in vitro studies in combination with animal based experiments with mice or piglets may be a useful alternative.

The strategy to decompose milk into its major and minor components (proteins/peptides, carbohydrates, lipids/fatty acids) and to subsequently identify which compounds in raw milk are exerting effects should be followed. Microbes from raw milk as well as probiotic lactobacilli and bifidobacteria of intestinal origin should also be investigated. Initially, bioassays should be developed to monitor cellular responses to raw versus pasteurized milk. In a second step, animal models as the NF $\kappa$ B mouse model or the piglet model can be applied.

Steps will then be taken to either preserve the compound(s) during pasteurisation, or re-introduce them to milk after pasteurisation.

These strategies may be considered as prerequisites for intervention studies.

*Wolfgang Kneifel, Silvia Apprich*