Protection against Allergy: Study in Rural Environments (PASTURE)

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BACKGROUND
School-age children growing up on a farm have significantly less asthma, hay fever and atopic sensitisations as compared to their peers not living on a farm[1-3]. Possible exposure factors inducing tolerance towards environmental allergens are contact to microbial components in stable or consumption of unpasteurized milk[2]. The immune system recognizes microbes via receptors of the innate immunity such as CD14 or Toll-like receptors (TLR). In earlier studies, we showed that the genes for CD14, TLR 2, and TLR 4 are significantly stronger expressed in school-age children living on a farm. Retrospective analyses furthermore indicated that exposure to a farming environment as well as consumption of unpasteurized milk was most effective in conferring protection if occurring in pregnancy or the first year of life[5]. However, the protective immunological mechanisms triggered by maternal or early life exposure to microbial compounds are still unclear.

OBJECTIVE
The aim of the European PASTURE study is to investigate how prenatal or early life exposure to farming environment influences the development of childhood allergies. Here, we measured expression of innate immune response genes in farmers' and non-farmers' children in the cord blood and at one year of age to assess possible changes in the immune system correlating with exposure to microbial products.

METHODS
This study comprises over 1100 farming and non-farming families from rural environments of five European countries (France, Finland, Austria, Germany and Switzerland). The families participating in the study were recruited during pregnancy. Questionnaires concerning their lifestyle including contact to animals were completed by parents. Blood samples of the children were collected after birth (cord blood) and at one year of age for, among others, studies of gene expression. In 943 cord blood and 778 one year samples enough RNA was detected for subsequent quantitative real time PCR (TaqMan®). The gene expression values were normalized to the expression of a house-keeping gene. We analyzed data using the comparative Ct method according to manufacturer's instructions (Applied Biosystems).

RESULTS
We assessed the gene expression of TLR 1-9 and CD14 in cord blood and one year blood samples. In cord blood we found that the expression of the genes for TLR 7 and 8 was significantly higher in farmers' than in non-farmers' children. Furthermore, expression of TLR 2 and 4 was higher in farmers' children but not reaching statistical significance.

We also analyzed the relation between specific farm exposures, such as contact to cow, sheep/goat, or compost, during pregnancy and the gene expression of TLR and CD14. Cord blood leukocytes of children whose mothers had contact to cows during pregnancy had significantly reduced expression of TLR 1, 7, and 8. However, contact to sheep/goat or compost led to a significant up-regulation of TLR 4, 5, and 9 in cord blood.
In a next step we investigated if consumption of farm milk during pregnancy had an influence on the expression of TLR at birth and one year of life. One year old children of mothers consumed unskimmed farm milk during pregnancy expressed significantly more TLR 1, 2, 4, and 8. These effects could only be observed in one year blood samples but not in cord blood.

CONCLUSIONS

Not only exposure of the children to farming environment in school-age is associated with altered expression of genes of the innate immunity, but also prenatal exposure left a footprint in the immune system of the children.

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