Lactating mice exposed to infant formula enriched with polyamines: impact on host transcriptome and microbiome.

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ABSTRACT

Recent evidence shows the impact that the supplementation of an infant formula in polyamines has in the modulation of microbial colonization and immune system development during lactation using a BALB/cOlaHsd model. To contribute to deciphering and identifying new complex interactions underlying the host response to polyamines, a systems biology approach integrating data from microbiota along gastrointestinal tract, lymphocyte populations and immune system gene expression analysis of a lactating mice model fed different diets was carried out. The study design included four different dietary regimens including the following: mice fed with normal lactation; early weaned mice given commercial infant formula; and early weaned mice fed with infant formula enriched with two different concentrations of polyamines. Cluster analysis by principal component analysis and heat map demonstrated that the bacterial communities and immune system status differed between groups. The assessment of the relationship between immune system developments, microbiota succession and polyamine supplementation in a global manner proved that the supplementation of an infant formula with polyamines promotes similar microbial communities along the whole gastrointestinal tract, and similar lymphocyte populations and expression of immune related-genes as during normal lactation and the results differ from those with the infant formula without polyamines. Further studies should be conducted in human subjects to verify the current results, as the supplementation of polyamines may resemble the effect of natural breastfeeding practices in the gastrointestinal microbiota and immune system development in a mouse model.

1. Introduction

Polyamines are polycationic compounds essential for cell growth. Their main sources include diet and production for the intestinal microbiota and biosynthesis from amino acids.[1] They are naturally present in human milk, displaying an important role in infants during intestinal maturation and development of intestinal nutrient absorption.[1] In addition, polyamines are linked to cell growth and differentiation in eukaryotic and prokaryotic cells. Hence it is natural to associate polyamine intake to the developmental status of early intestinal microbiota.[2] At present, there are no general recommendations for polyamine intake. However, epidemiological studies in lactating children and mothers suggest that low polyamine intake from breast milk or formula may increase the risk of allergic diseases.[3, 4]

Epidemiological studies have shown that breastfeeding is associated with a reduction in the risk of specific diseases such as infections, atopic dermatitis, asthma, obesity and diabetes[5] comparing with infant formula feeding. Infant formulas usually have lower contents of polyamines than breast milk.[6] Polyamines are essential for developing tissues in relation to their specific roles in stabilizing the negative charges of DNA and the structure of chromatin, the regulation of several transcriptional factors, and the regulation of the protein synthesis[1]. All these functions are fundamental for gut and immune system maturation, and the low polyamine intake may be partially responsible of the differences in health outcomes between breast-fed and formula-fed infants.

Previous studies have shown that supplementation of infant formula with polyamines may influence both gastrointestinal microbiota and immune system development.[7-9] As immune system development and microbial colonization pattern are closely related in newborns,[10, 11] the objective of this work was to use a systems biology approach integrating data-sets on microbiota and immune system obtained using different methodologies to understand in a global perspective the effects of polyamines in the pivotal relationships between immune system development, microbiota succession and health.

2. Materials & methods

2.1. Data sources

Recently published data in regards to microbial composition using fluorescent *in situ* hybridization (FISH)[7] and qPCR along the whole gastrointestinal tract;[9] immune cell population in blood, spleen and mesenteric lymph node analyzed by fluorescent activated cell sorting (FACS);[8] and host response by transcriptomics focused on key-genes related with immune

system development in small intestine[8] were included for integrative analysis. Details on the methodologies employed are described in the mentioned references.

In brief, 48 BALB/cOlaHsd mouse pups (age 14 days, weight 7.85 g ± 1.06 g) were randomly and blindly allocated in different experimental groups for a 4-day diet treatment. The study design included four different treatment groups including male and female animals in the same proportion: NL) mice fed with normal lactation; IF) early weaned mice fed with commercial infant formula; T1 and T2) early weaned mice fed with infant formula enriched with polyamines, respectively. The concentration of supplemented polyamines ingested for T1 mice was 2.10 µg, 22.05 µg and 38.00 µg of putrescine, spermidine and spermine respectively; and 8.40 µg, 88.20 µg, 152.00 µg of putrescine, spermidine and spermine respectively for T2 group.[8] Nonenriched formula and formula with polyamines were made with warm water following the manufacturer's instructions and given to the early weaned pups twice daily by gastric gavage.[7-9] Polyamines were added to the infant formula immediately before administering to the mice the concentrations of polyamines employed in this study were in a proportion similar to human milk and lower to the NOAEL (no-observed adverse effect level).[7, 12] All the mice were determined to be healthy on the basis of individual physical examinations and microbiological status of mice were determined to be healthy based on the results of routine microbiological screening carried out in the colony and maintained through the study in accordance with FELASA recommendations.[13, 14] The experimental protocol was approved by the National Ethics Committee for Animal Experiments in Finland (ESLH-2009-04 845/Ym-23).

2.2. Data synthesis and analysis

Heat map-based clustering analysis was performed using the *heatmap.2* function from *gplots* R software package.[15] Hierarchical clustering of individual samples was based on the Euclidian distance metric. R package *ade4* (v. 1.6.2)[16] was used to assess differences between the feeding groups by performing a principal component analysis (PCA).

3. Results & discussion

Multivariate analysis displayed that normal lactation was completely separated from infant formula groups in both, cluster analysis and Principal Component Analysis (PCA) (Figure 1 and 2). It is thus demonstrated that different diets during lactation may have a significant impact on immune system gene expression, lymphocyte populations and microbial colonization. The observed differences contribute to the accumulated published data suggesting a higher

prevalence of certain diseases when breast milk is replaced by infant formula during lactating period.[5]

Moreover, the addition of polyamines to the infant formula is able to modulate immune system parameters and gastrointestinal microbiota. Although PCA (Figure 2A) shows that pups fed with an infant formula supplemented with polyamines shape in groups different to normal lactation pups; the clustering (Figure 1) and the Gauss scores (Figure 2B and 2C) demonstrates that the polyamine supplementation modulates immune system development and microbial colonization pattern in a similar manner to normal lactation when compared with infant formulas.

There is a close relationship between microbiota and immune system development.[17] Microbes promote development of B lymphocytes in Peyer's Patches, the development of intraepitelial lymphocytes and the amount of NK cells.[17] In relation to the latter, the host–bacterial interaction in early stages has been reported to be key modulators of disease risk in early and later stages according with the Early Programming Theory[18] and the Developmental Origins of Health and Disease (DoHD) approach.[19] In this study, the differences in gene expression include differences in *Cd40, Cd1d1* and *Hdac5* genes.[8] Those genes have crucial roles in the development of immune system.[8] Differences in the expression of *Cd40* gene in antigen presenting cells were recently positively correlated with changes in mucosal populations of some *Prevotella* species and negatively correlated with *Ruminococcus bromii.*[20] Our previous results reveal that lactating mice and early weaned mice fed with infant formula supplemented with polyamines have similar *Bacteroides-Prevotella* populations,[7, 9] similar expression of *Cd40* and other immune parameters,[8] which could explain the proximity in the clustering and PCA analysis between NL, T1 and T2 groups.

High epithelial expression of *Cd1d1* in intestine in response to microbial lipid antigens activates NKT-cells.[21] Recent evidence suggests that NKT-cells modulates host and commensal microbiota interactions, regulating intestinal homeostasis and preventing inflammation,[22] helping to explain similarities between NL, T1 and T2.

Commensal microbiota can modulate histone deacetylases influencing epigenetic status of the host immune system and affecting the risk of the development of some disorders.[23] Both normal lactation and polyamine supplementation of infant formula increase the expression of *Hdac5,* which codes the enzyme histone deacetylase 5, while subjects fed infant formula without polyamines exert a low expression in this gene.[8] Differences in epigenetic status between breastfeeding and formula feeding can explain why the risk of some diseases associated with formula feeding in early life is extended to adulthood.

Considering all our results together, this data demonstrates that supplementation of polyamines resembles the effect of natural breastfeeding practices as observed in the microbial communities along the whole gastrointestinal tract, lymphocyte populations and expression of immune related-genes in a mouse model. These changes might reduce the susceptibility to some diseases associated to infant formula feeding and their effect could be extended to adulthood through epigenetics mechanisms. This study provides the bases to go one step-forward on the potential use of polyamines in infant food and their effects on infant health is pendant to be demonstrated through further studies in human populations.

4. Conclusions

Further studies should be conducted in human subjects to verify the present results. Even with the approach of added polyamines formula milk does not completely reproduce the effect of normal lactation on microbiota and immune system parameters, when breastfeeding is not possible, the supplementation of infant formulas with polyamines in a similar concentration to human milk may be a simple approach which potentially brings great benefits in growth promotion and development during lactation.

Conflict of interest

The authors have no conflict of interests to report.

Acknowledgements

C. Gómez-Gallego is a recipient of the Seneca Postdoctoral Grant (19818/PD/15) from the Seneca Foundation, the Regional Agency of Science and Technology of the Region of Murcia through the program "*Talento Investigador y su Empleabilidad*" (funded by the Education and Universities Council - Autonomous Community of the Region of Murcia, Spain).

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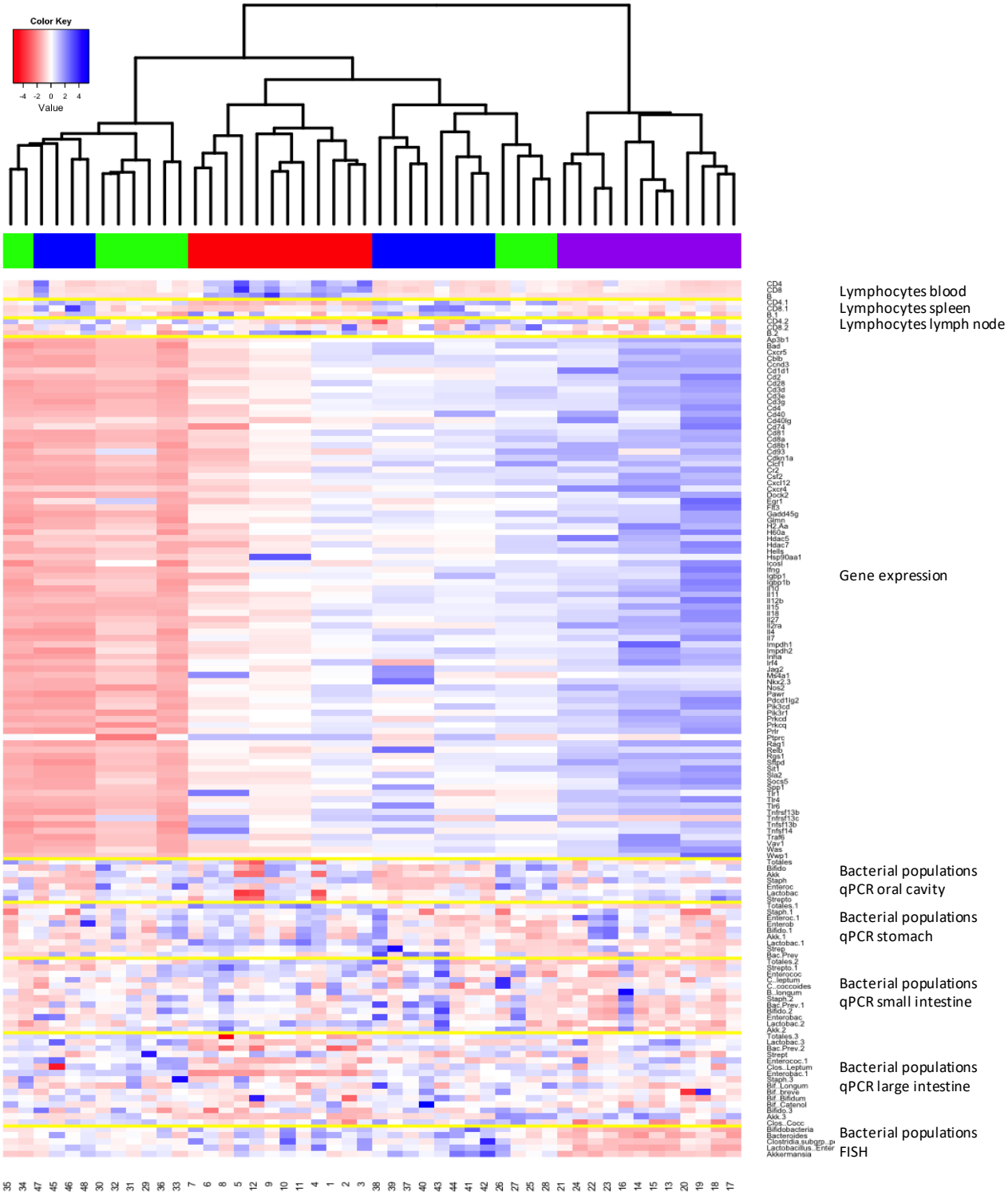

Figure 1. Heat map and cluster of the different subjects. Samples from normal lactation (NL) group were represented in red, from infant formula-feeding were represented in purple (IF), and from infant formula supplemented with low amount of polyamines (T1) and high amount of polyamines (T2) were represented in green and blue respectively. Samples were clustered according to feeding practices and polyamine supplementation.

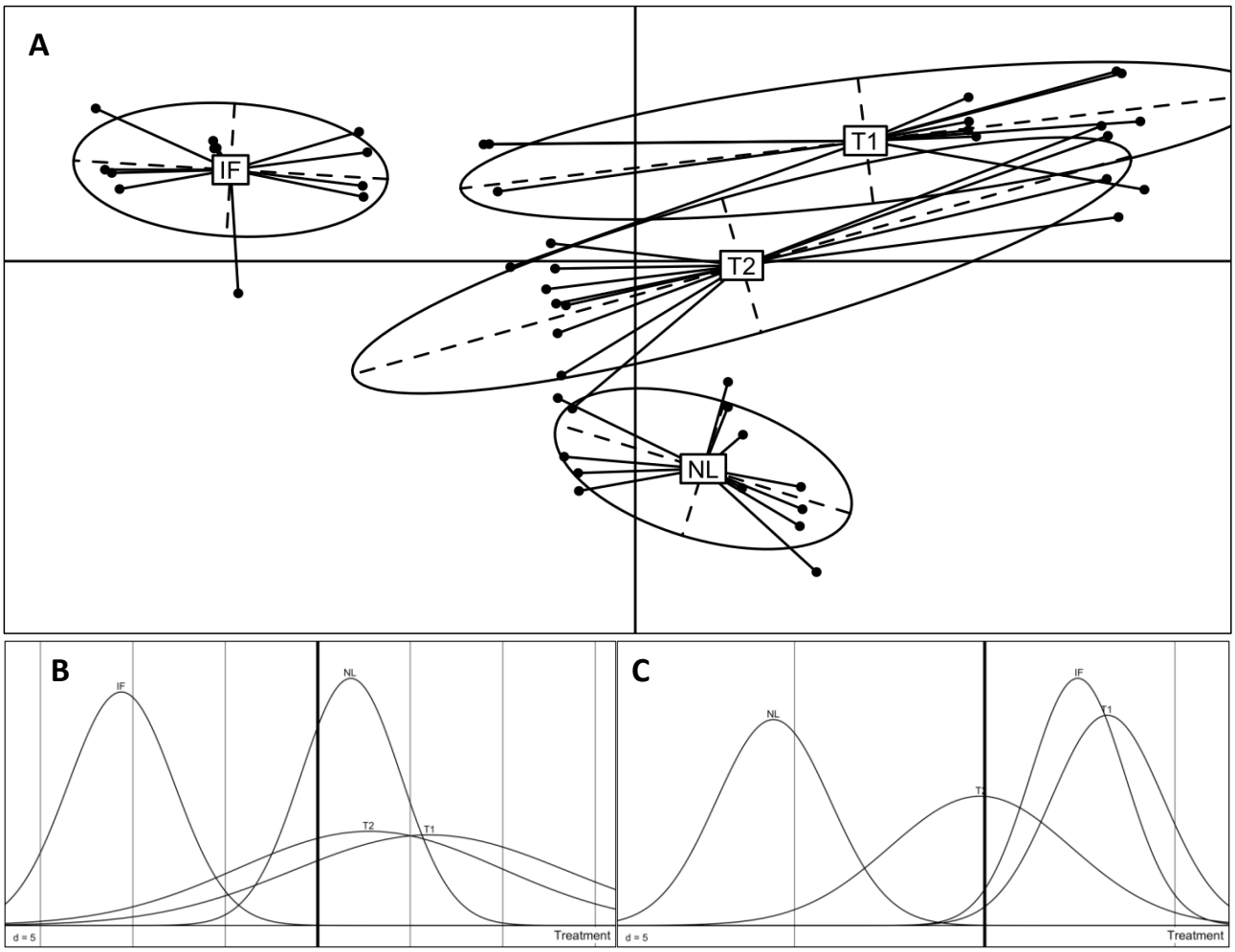

Figure 2. Impact of different diets and polyamine intake during lactation on gastrointestinal microbiota populations, lymphocyte population and gene expression. A) Results from the principal component analysis. B) 1D representation of Gauss scores of the first principal component in the PCA. C) 1D representation of Gauss scores of the second principal component in the PCA. NL= normal lactation group; IF-group fed with commercial infant formula; T1- group fed with an infant formula supplemented with low polyamine concentration; T2-group fed with an infant formula supplemented with high polyamine concentration.