Integrative analysis of the effect of an infant formula enriched in polyamines on the microbiome and host changes during lactation in BALB/c mice model

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INTRODUCTION AND OBJECTIVES

Breast milk **polyamines are considered essential for post-natal maturation of the gut and immune system in mammals.**

Infant formulas usually have lower contents of polyamines, and **this low polyamine intake** may be partially responsible of the differences in health outcomes between breast-fed and formula-fed infants.

As immune system development and microbial colonization pattern are closely related in newborns, the objective of this work was to utilize bioinformatics and integrate data-sets obtained using different methodologies.

MATERIAL AND METHODS

BALB/cOlaHsd mice pups (age 14 days, weight 7.85 g ± 1.06 g) were randomly separated in different experimental groups for a 4 days diet intervention. The study design includes four different treatment groups: 1) mice fed with normal lactation; 2) early weaned mice fed with commercial infant formula; 3) and 4) early weaned mice fed with infant formula enriched with polyamines. We have integrated the data from **microbial composition using fluorescent in situ hybridization and qPCR** and also, **immune cell populations analyzed by fluorescence activated cell sorting**, and **host response by transcriptomics focused in immune system pathways**. Heat map-based clustering analysis was performed using the *heatmap.2* function from *gplots* R package. Hierarchical clustering of individual samples was based on the Euclidean distance metric. R package *ade4* was used to assess differences between the feeding groups by performing a Between-Class Analysis (BCA) with all the data available. The statistical significance of the BCA was evaluated with a Monte Carlo test, based on 999 replicates.

RESULTS

BCA showed that normal lactation was completely separated from infant formula fed groups and polyamine-enriched formula (high concentration) from commercial infant formula and infant formula enriched with low concentration of polyamines on the basis of the first axis of the BCA. A Monte Carlo test showed that the differences between groups were statistically significant (p=0.001).

Similarly, **clustering analysis**, as shown in the heat map representation, groups the different individuals with similar results as in BCA.

Dysregulation of Cd40 expression is associated with gastrointestinal disorders. Cd1d1 can regulate mucosal commensalism and the colonization of the intestines through the activation of NKT cells. **The differences in Cd1d1 and Cd40 gene expression might explain why polyamine-enriched formula modulate immune system and microbial colonization patterns during lactation in a similar way to that seen in normal lactation.**

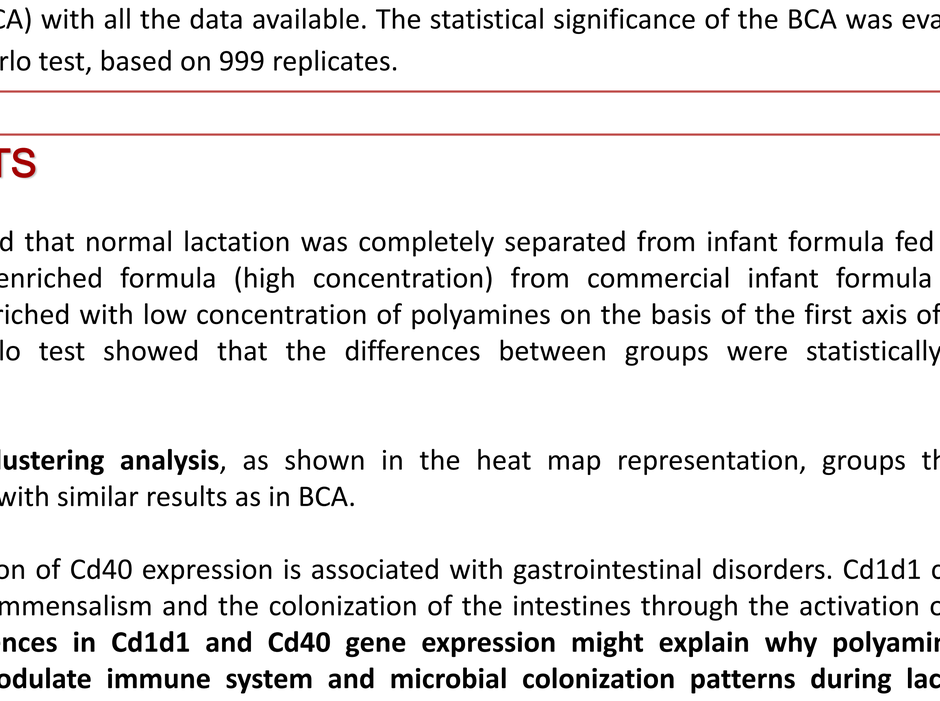


Figure 1 BCA clustering of different mouse feeding groups in the studies (coloured ellipses).

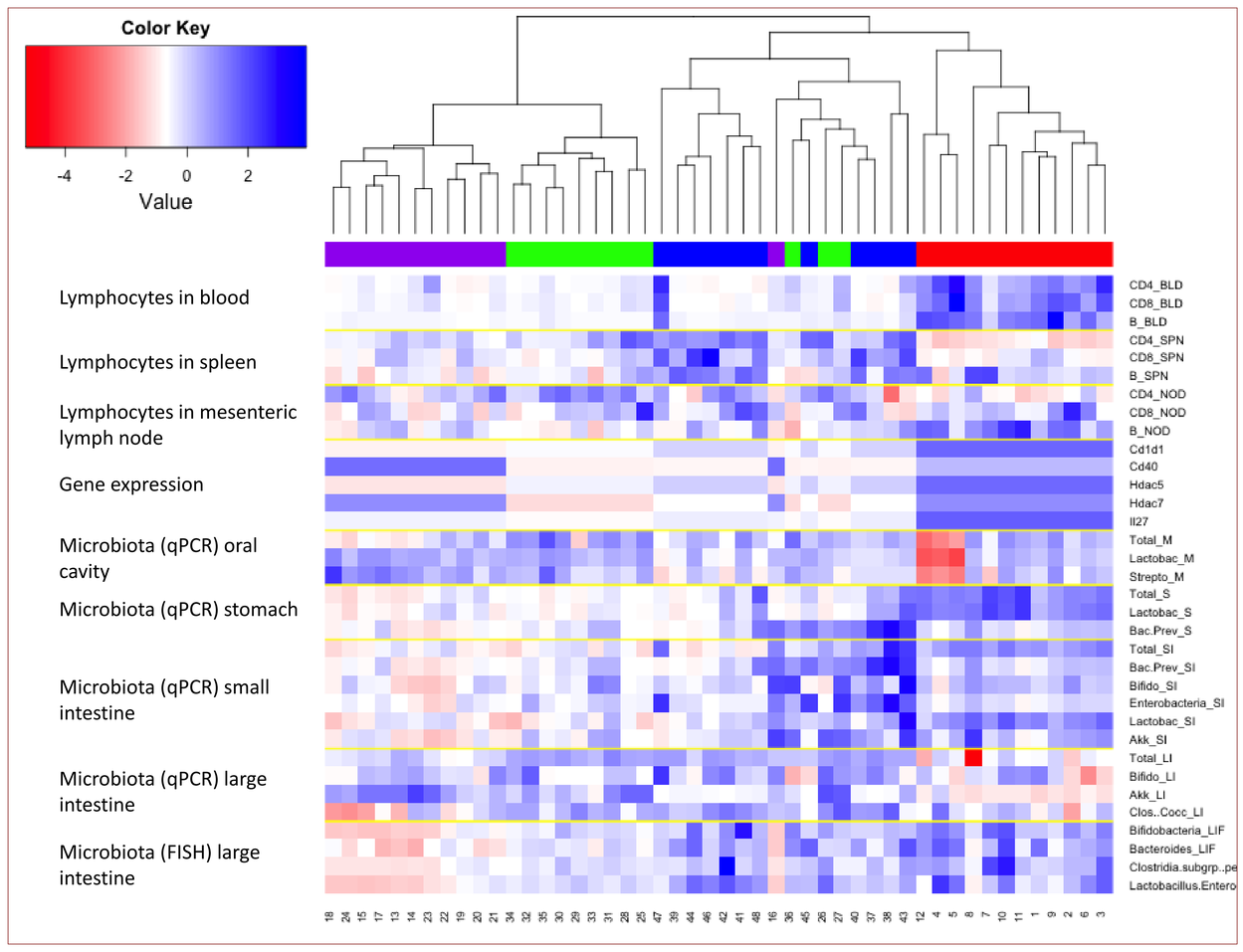
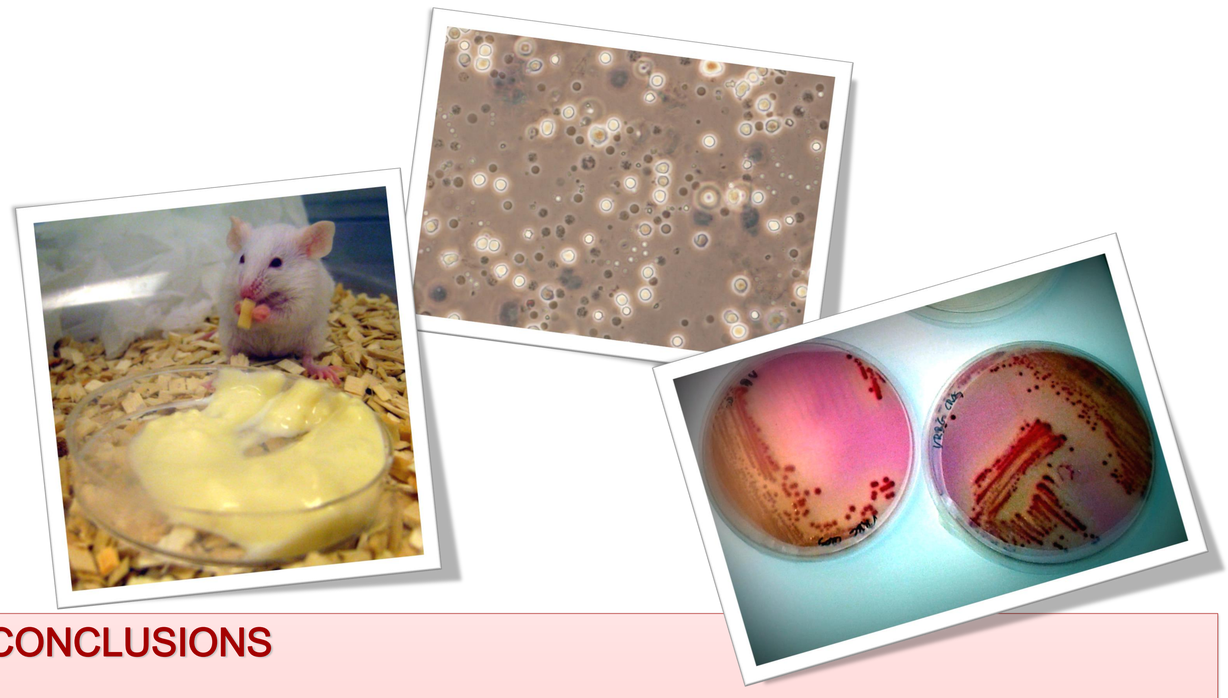


Figure 2. Heat map demonstrates relative differences between the results obtained in previous studies. Color breaks in heat map are adjusted to show normalized intensity values. Cluster colours on the top of the heat map represent the different diets of the mice pups in the study: unweaned pups receiving milk by normal lactation (red); early-weaned pups fed with commercial infant formula without polyamines (purple); early-weaned pups fed infant formula enriched with low (green) and high (blue) concentrations of polyamines.



CONCLUSIONS

Combined results of our previous studies on polyamine supplementation suggest **that polyamines added to formula or milk can modulate both immune system development and intestinal microbial colonization patterns in a manner similar to that observed by milk polyamines in normal lactation.**

This approach **potentially represents a simple solution for polyamine supplementation which could have substantial benefit promoting improved growth and development of infants.**

Further studies should be conducted in human subjects to verify our result**s.**

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