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Refinement of animals models influenced by the intestinal microbiota

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Background & Aim

The composition of the intestinal microbiota (IM) plays an important role in the pathogenesis and etiology of several human autoimmune as well as metabolic diseases. Therefore the IM is an important factor when aiming for reduction and refinement of animal models.
The aim of this study was to clarify if a breeding set-up with increased genetic homogeneity would increase the similarity in the IM between offsprings. Furthermore to uncover if early bacterial exposure can prime the intestinal colonization later in life.

Materials and Methods

Caecum samples from eight week old C57BL/6Sca and five week old NMRI (Karolinska Institute, Sweden) were used. The samples were analyzed with a culture independent approach based on the 16S rRNA gene derived amplicons, followed by DGGE (denaturing gradient gel electrophoresis) to determine and compare the composition of the intestinal microbiota. Cluster analysis and three dimensional principle component analysis (3D PCA) was performed.

Results

Increasing the genetic homogeneity of the female breeders by using sisters, reveals higher similarity of the IM between the offspring as illustrated in figure 1.
In the breeding set-up with dams being sisters (Fig. 1A), no clear separation between the samples is seen, which represents a homogeneous IM within the offspring. In the breeding set-up with dams not being sisters (Fig. 1B), a clear separation between samples is seen, which represents a clustering reliant on which cage the animals came from.

Figure 2 shows the cluster analysis based on DGGE data of NMRI offspring inoculated at different postnatal age. Five main clusters (A-E) are seen, reflecting the different groups the animals were divided in for inoculation. Group A (inoculation day 0) has most in common with group E (no inoculation). Group B and C (inoculation day 3 and 7) are most distinct from group E (no inoculation). This observation indicates that priming the intestinal colonization might be within the first two weeks of age.

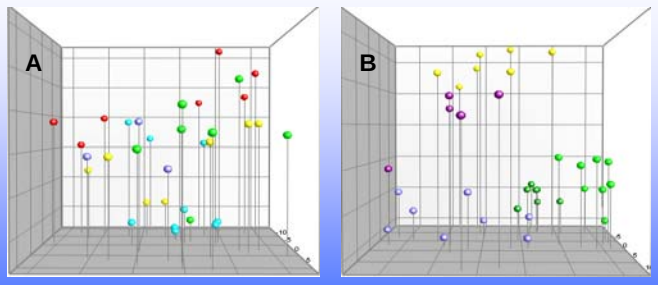

Figure 1
3D PCA based on DGGE data representing the composition of the caecum microbiota in eight week old C57BL/6Sca. Every cage is marked with a different colour. Animals coming from the same cage have the same coloured dots.
A: Dams being sisters in the breeding set-up. No clear separation between the samples is seen.
B: Dams not being sister. A clear separation between the samples is seen.

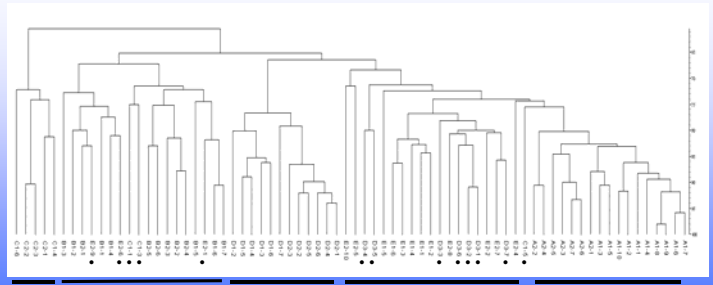

Figure 2
Dendrogram analysis based on DGGE profiles representing the composition of the caecum microbiota in five week old NMRI mice (100 % indicating complete similarity and 0 % indicating complete dissimilarity). The black bars represent the five main clusters. The black dots indicate the outliers in each group.
A: Offspring inoculated day 0 postnatal B: Offspring inoculated day 3 postnatal
C: Offspring inoculated day 7 postnatal D: Offspring inoculated day 21 postnatal
E: Offspring which were not inoculated. Bacteria from the surroundings colonized the IM

Summary and Perspective

- Increasing the genetic homogeneity in breeding C57BL/6Sca can increase the similarity in the IM in the offspring.
- Early bacterial inoculation can prime the IM in NMRI mice.

A higher similarity in the composition of the IM might entail a reduced variation in disease expression and smaller group sizes when using animal models influenced by the IM.

Furthermore assuming that different bacteria groups influence disease expression either in a positive or negative way, a carefully selected postnatal bacterial exposure might refine these animal models.