A systems biology study of mice raised in different enviroments: Investigation of the effect of gut microflora and global metabolome.

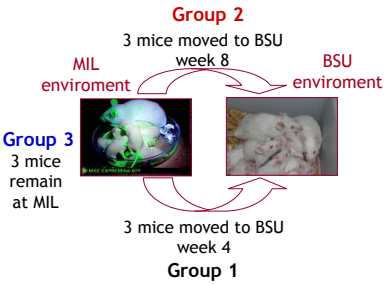
H. Gika[1,2], M.K. Friswell [3], G. Theodoridis[1,2], P. Gilbert [3], Ian D. Wilson[1]

[1] AstraZeneca, DMPK, Alderley Park, UK., [2] Department of Chemistry, Aristotle University of Thessaloniki, Greece.
[3] School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, UK.

Introduction

The microbial flora associated with host animals, commensal or pathogenic, is collectively term the microbiome and contributes both positively and negatively to health. Healthy female C3H strain mice were used to investigate the impact of a change upon both individual gut flora and metabolite profiles. Of a litter of 9 mice, 3 were separated at weaning and transferred to a different environment with common food and bedding. Three further siblings were relocated at immunological maturity (8 weeks), whilst three remained. Faecal and urine samples were collected weekly and frozen. Faecal pellets were subject to **PCR-DGGE analysis** (utilising primers specific for V2-V3 region of eubacterial 163rDNA) and Gel images were analysed to produce similarity dendrograms based on banding patterns. Urine samples were analysed by **UPLC-ESI-MS** on a C18 column using a 10 min gradient and LC-MS profiles were processed by multivariate statistics.

Study Overview

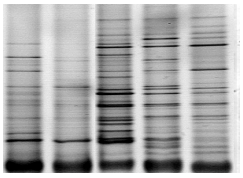


9 female C3H mice were divided into 3 groups. Group 1: moved to alternative environment at 4 weeks of age (immediately after weaning-immunologically immature); Group 2: moved to an alternative environment at 8 weeks of age (immunological maturity); Group 3: remained at the original environment. All mice were age and gender matched and fed identical food with identical bedding provided.

Experimental

PCR-DGGE analysis

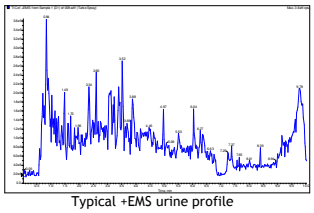
DNA was extracted from the faecal pellets using a DNA stool mini kit (Qiagen, UK) and was subject to PCR utilising primers specific for the V2-V3 region of eubacterial 16srRNA. Amplicons were run on a polyacrylamide gel, with a denaturing gradient to separate out bands of differing sequence. Gels were visualised under UV light and digitally photographed. Gel images were analysed to produce similarity dendrograms based on banding patterns using Bionumerics software (Applied Maths, Belgium) (1).



Typical DGGE gel. Dna is run on a 30-60% formamide.urea denaturing gradient. The Gel is stained with Sybr Gold (Invitrogen)

Urine UPLC-MS analysis

20 uL of urine were diluted with 60 uL of water. UPLC (Waters Acquity) - MS analysis was performed on a Q-Trap 4000 (AB SCIEX). Raw LC-MS data were extracted with MarkerView. Multivariate data analysis was performed with Simca P.



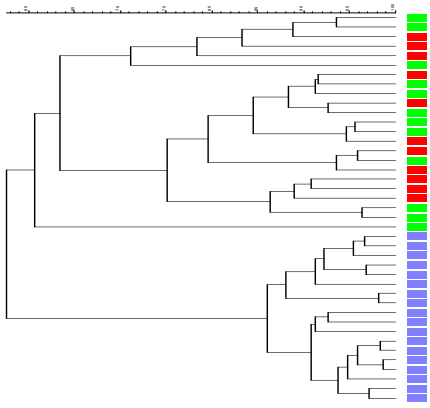
Typical +EMS urine profile

- Column: Aqcuity /Hypersil at 50 °C, Flow rate : 0.4 mL/min, gradient elution of acetonitrile over water (0-0.5 min): 5% AcN, (0.5-1.5): 10 %, (1.5-4.5): 25%, (4.5-8): 55%,(8-9): 100%, (9-9.5): 100%, (9.5-9.51): 5%, (9.51-12): 5%. QC (pooled samples) were analysed between the samples. EMS data were collected in +ve and -ve ESI mode (100-850 amu)

Results

- DGGE analysis of faecal samples revealed that mice moved before immunological maturity (4 weeks of age) do not retain their faecal bacterial community. Mice moved after immunological maturity (8 weeks of age) retain their developed flora. When the results are shown using dendrogram analysis, mice moved at 4 weeks of age cluster independently to mice moved at 8 weeks of age. Mice moved at 8 weeks of age cluster together (show band similarity) to those mice that remained in the original environment for the duration of the study.
- Urine analysis showed as above, that mice moved at 4 weeks of age, displayed an altered metabolome, whereas mice that were moved at 8 weeks of age displayed a highly similar metabolome to those animals that were not moved.

Dendrogram analysis of faecal samples

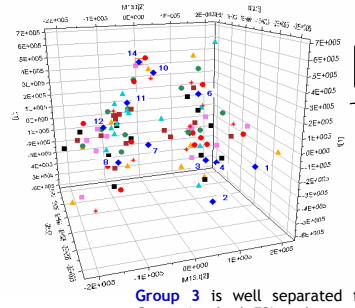


Key

- Group 1:Mice moved to an alternative environment (BSU) at 4 weeks of age (immaturity)
- Group 2: Mice moved to an alternative environment (BSU) at 8 weeks of age (maturity)
- Group 3: Mice that remained in original environment (MIL) throughout the experiment

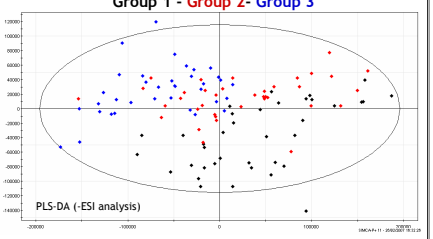
Each coloured box represents a different sample

PCA Plots of urinary Metabolite profiles



Urine profile change with **ageing**, colouring according to animal

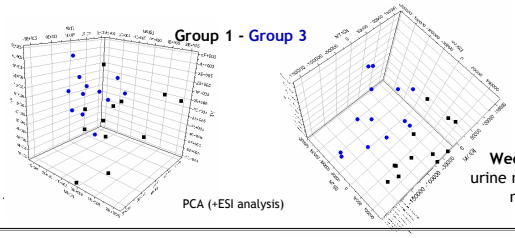
Group 1 - Group 2- Group 3



Group 3 is well separated from **Group 1** in both ESI modes.
There is overlap between **group 3** and **group 2** in both ESI modes.

The urine metabolome changed when siblings were moved before attaining immunological maturity (4 weeks), whereas remains similar when siblings move after this point.

Group 1 - Group 3



Week 4-8: No change of urine metabolite profile after relocation.

Week 14-18: Change of urine metabolite profile of mice moved before 8 weeks of age

Conclusions

- Siblings that co-habited until week 8 retained a common flora and urine metabolite profile, regardless of environment, or subsequent relocation for up to 1 year.
- Siblings that were relocated after weaning, but before attaining immunological maturity (4 weeks), underwent changes in their gut flora that were not mirrored by their siblings but which were associated with changes in urine metabolites.
- Mice harbour a stable, colonisation-resistant gut flora at 8 weeks of age. Relocation before attaining immunological maturity allows the gut flora to dysbiose with concurrent changes in urine metabolite profile.
- The change in the gut flora can be clearly linked to the metabolite profile shown in the mice.

Literature Cited

(1) McBain, A. J, *et al* (2003) Growth and molecular characterization of dental plaque microcosms. Journal of Applied Microbiology **94** (4) 655-64.

Acknowledgments

Dr Andrew McBain for help and advice and Professor Ian Stratford and Mr Brian Telfer for providing the samples.
EU TOK-IAP 2960 Grant for funding