Childhood stunting model induced by chronic undernutrition in gnotobiotic mice

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INTRODUCTION

• Childhood chronic undernutrition affects metabolism and hormonal signaling leading to stunting syndrome, which is associated with higher juvenile mortality rate and correlate with immune, cognitive and gut mucosal barrier deficits in adulthood (1, 2).
• Recent studies have highlighted the role of gut microbiota on juvenile growth (3). Germ-free mice colonized with undernourished child microbiota recapitulate the impaired growth phenotype (4). Besides, mono-colonized mouse models have been used to demonstrate that specific bacterial strains can promote juvenile growth, interact with the somatotropic hormone axis and buffer the adverse effects of childhood chronic undernutrition (5). Moreover, another preclinical study has shown that gut microbiota, and possibly short-chain fatty acids produced by bacteria, restores bone mass, which is likely mediated by the insulin-like growth factor 1 (IGF-1) (6).
• Taken together, these findings suggest that specific bacterial strains can reshape undernourished children microbiota and offer opportunities to improve juvenile growth and health.
• Here we introduce a representative preclinical model of childhood stunting induced by chronic undernutrition in gnotobiotic mice featuring a simplified and controlled murine microbiota, which recapitulates the phenotype observed with a complex murine microbiota. We believe this gnotobiotic mouse model is of interest to further decode the impact of gut microbiota and specific bacterial strains on juvenile growth, and develop novel microbiota-directed therapeutics.

MATERIALS & METHODS

• Animal experiments were approved by the Committee for Protection and use of Experimental Animals of the ANSES Laboratory of Lyon.
• C57BL/6J mice were housed in isolators, supplied with autoclaved tap water and irradiated sterile feed ad libitum. Germ-free (GF), gnotobiotic (GNOTO) and specific opportunistic pathogen free (SOPF) male pups were weaned at day 21 and randomly distributed in subgroups, either fed with depleted diet (DD: 4% proteins by weight, 2.5% fat, 3.5 kcal/g) or control breeding diet (BD: 21% proteins, 5% fat, 3.4 kcal/g). Body weight and size were recorded weekly using isoflurane anesthesia, as well as feed and water intake. Animals were sacrificed at D56 for the collection of blood, cecum, femur and tibia. GF mice were monitored weekly to confirm the absence of microbes, and GNOTO mice colonized microbiota was assessed by strains specific qPCR.
• Serum IGF-1 levels were measured in triplicate by ELISA kits (R&D systems) according to manufacturer’s instructions.
• Experimental analysis was performed with GraphPad Prism 7.0 software using Mann-Whitney test for comparison between two groups.

CONCLUSION

• Our results confirm that GF mice are more altered by chronic undernutrition than SOPF mice. Gut microbiota is therefore beneficial to buffer the deleterious effect of chronic undernutrition on juvenile growth.
• GNOTO mice recapitulate the stunting phenotype observed in SOPF mice, especially for:
  - Body weight
  - Body size
  - Bone size
  - IGF-1 levels
• GNOTO mice, featuring a simplified murine gut microbiota, are thus necessary and sufficient to reproduce the stunting model obtained with a complex microbiota.
• This new gnotobiotic mouse model offers the potential to study the mechanistic basis of the interactions between diet, microbiota and the host during chronic undernutrition, and allows the development of novel microbiota-directed therapeutics (such as probiotics, metabolites produced by bacteria, vaccines, etc.) to target childhood stunting.

REFERENCES

5- Schwarzer et al. Lactobacillus plantarum strain maintains growth undernutrition than SOPF mice. Gut microbiota is therefore beneficial to buffer the deleterious effect of chronic undernutrition on juvenile growth.

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Experimental design

<table>
<thead>
<tr>
<th>Condition</th>
<th>Breeding Diet</th>
<th>Depleted Diet</th>
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<tbody>
<tr>
<td>GF (N=4)</td>
<td>21</td>
<td>21</td>
</tr>
<tr>
<td>GNOTO (N=8)</td>
<td>10</td>
<td>10</td>
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</tbody>
</table>

Results

- Body weight and size growth curves during the juvenile period and associated daily gain
- Normalized daily feed and water intake
- Serum IGF-1 levels at D56
- Water intake (g/kg of body weight)
- Body weight (g), femur and tibia size at D56

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