Perinatal antibiotic treatment of sows affects intestinal barrier and immune system in offspring

European project INTERPLAY – WP6 task 6.1

Stéphanie Ferret-Bernard, Gaëlle Boudry, Laurence Le Normand, Véronique Romé, Gérard Savary, Cécile Perrier, Jean-Paul Lallès, Isabelle Le Huërou-Luron

INRA UR 1341 ADNC, Saint-Gilles, France
Association between early life nutrition and health later in life has emerged = perinatal programming

Neonatal period = key period in acquisition of gut microbiota and education of immune system, especially towards LPS

Disturbances of microbiota colonization -> alter mucosal and systemic immunity leading to asthma, allergy, obesity...
Hypothesis

Post-natal intestinal development

- Altering microbiota implantation during early life would change the post-natal development of enterocyte functions and local immune cell sensitivity to bacterial products.

- This will have consequences on their responses to a High Fat (HF) diet later in life.
Objectives

• Use of peripartum antibiotic treatment to alter piglet microbiota acquisition

• Investigate the post-natal development of the intestinal barrier function and transcriptome as well as GALT education towards LPS in piglets born to ATB sows

• Analyze their gut adaptation to a HF diet later in life
Experimental protocol

<table>
<thead>
<tr>
<th></th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal/g)</td>
<td>410</td>
<td>455</td>
</tr>
<tr>
<td>% of energy</td>
<td>17</td>
<td>14</td>
</tr>
<tr>
<td>protein</td>
<td>79</td>
<td>64</td>
</tr>
<tr>
<td>carbohydrate</td>
<td>4</td>
<td>22</td>
</tr>
</tbody>
</table>

Control sows (n=12)

Antibiotic-treated sows (n=11)

farrowing/birth

weaning

1 piglet per litter selected for slaughtering

PND14  PND21  PND28

d-10  d21

amoxyccillin 40 mg/kg/d per os

Diets for 4 weeks

PND14  PND21  PND28

amoxycillin 40 mg/kg/d per os
Maternal and offspring microbiota

Changes in microbiota diversity in collaboration with O. Perrez-Gutteriez, J. Zhang, H. Smidt (WUR, Netherlands)

• Peripartum antibiotic treatment modified sow faecal microbiota profile

• Maternal antibiotic treatment reduced piglet ileal microbiota diversity
The maternal antibiotic treatment was associated with early ileal barrier function defaults, as in rodents (Fåk et al 2008).
Transcriptomic analysis of laser-captured enterocytes from 21 day piglets

32 (29 annotated) differentially expressed genes in ATB vs CTRL (p<0.001) of which:
- 11 down-regulated genes (< 0.5)
- 16 up-regulated genes (> 2)

- protein catabolism,
- immune response,
- energetic metabolism

=> Changes on enterocyte transcriptome could orientate local macrophages towards a pro-inflammatory profile in ATB group
Isolation and purification of lamina propria mononuclear cells (LPMC) from PND21 piglets -> culture with LPS for 72hrs

=> LPMC were still tolerant to LPS in both groups

=> Cytokine responses seemed to be oriented towards a pro-inflammatory profile in LPS-challenged LPMC of ATB piglets
Peripartum antibiotic treatment affects the molecular cross-talk between commensal bacteria, epithelial barrier and immune cells in the early period of life.

Is the adaptation to a HF diet later in life modified?
Ileal barrier function in adults

Adult pigs deriving from CTRL or ATB sows given either a LF or a HF diet for 4 weeks

Paracellular permeability (FD4 flux)

Tissue conductance

=> CTRL pigs displayed increased ileal permeability under a HF diet
=> Such an increase was not observed in ATB pigs (Cani et al 2008)
Gene expression in adult ileum

qPCR on ileum extracted RNA

TNF
Diet x maternal treatment
p=0.055

=> TNFα gene expression tended to decrease in ATB-HF
TNFα secretion by ileal biopsies

Ileal biopsies cultivated for 20hrs with media, PWM or 100 µg/mL LPS

TNFα secretion in supernatant (ELISA)

=> TNFα secretion was blunted in ATB pigs
=> The sensitivity to LPS was altered in ATB-HF pigs
Summary and conclusion

Maternal antibiotic treatment

during the neonatal period:
- modified piglet ileal microbiota;
- increased ileal permeability;
- altered cross-talk between microbiota, enterocytes and immune cells (LPMC response to LPS) towards a pro-inflammatory profile;

later in life:
- did not modify ileal barrier function in LF-fed animals but prevented the HF diet-induced increase in permeability;
- blunted the TNFα response of ileal biopsies to inflammatory stimuli, especially in ATB-HF pigs (corroborating gene expression).

The gut response to a HF diet is dependent upon early-life microbiota colonization
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