WP 3.1: Antibiotics in newborn mice

1. Related WPs, MG contact: Synergies with WPs 1.1,1.2b,1.4b,1.5,2.2,3.2. MG contact: Hanne Frøkiær

2. Key involved personnel, their institution, mail address (project leader + main study site underlined):
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3. Main aim and sub-aims:
To obtain a thorough understanding of the influence of the microbiota on early events in immune development of importance for establishment of long term immunity. This will be done by treatment of different antibiotics (e.g. Ampicillin, Gentamycin, Metronidazol and to a less extent Vancomycin) and at different perinatal periods. The studies will provide key basic knowledge to support and guide the pig and human infant studies.
Sub-aims are:
a) To evaluate variations in early hematopoietic events after antibiotics-induced variation in gut microbiota.
b) To evaluate how variations in early hematopoietic events affect the establishment of adaptive immunity.
c) To establish an influenza challenge model to test long term immunity effects of perinatal antibiotics.
d) Evaluate how variations in early hematopoietic events affect propensity to infectious diseases later in life.
e) To identify key genes/molecules/cells that can be used as markers in pig and human infant studies.
f) To evaluate how variations in early hematopoietic events affect specific parameters in brain development.

4. Background and a central hypothesis:
We and others have previously demonstrated that the microbiota plays a key role in establishment of early hematopoietic events, i.e. differentiation of HSC into myeloid derived suppressor cells and other neutrophil-like cells, of importance for development of adaptive immunity and accordingly may influence long-term immunity, e.g. resistance towards viral infections and development of autoimmune diseases. In addition, a diverse microbiota accelerates the development/maturation of the gut epithelium and the kinetics of the epithelial maturation may be determined by the composition of the gut microbiota postpartum, which in turn may be a key determinant for development of mucosal and systemic immunity. Despite many studies of the effects of antibiotic treatment in early life, the effects on the very early events in immunological maturation have not been addressed.
We hypothesize that peri-/postnatal treatment with antibiotics will decrease the diversity of the microbiota and this will impact the early hematopoiesis and, accordingly, maturation of the immune system causing a weak or skewed immune system with less resistance towards infections and higher risk of autoimmune disease development.

5. Key analyses and methods:
Perinatal antibiotics treatment of dams and offspring mice: To eradicate major populations of the gut microbiota, dams are treated with e.g. vancomycin, gentamycin or ampicillin during gestation until few days after birth or treatment is initiated few days postpartum.
Flow cytometry: to investigate the proportion and composition of CD11b+ cells in spleen during the first weeks of life.

Microscopy: to assess the efflux of differentiating HSC from liver and influx of cells to spleen and other organs.
RT-PCR: to assess effects of microbiota on maturation of gut epithelium and cell migration in liver and spleen (up- and down regulation of chemokines, specific enzyme markers such as arginase, elastase).
16s sequencing and RT-PCR of gut contents.
ELISA: e.g. cytokine/chemokine measurement of ex vivo stimulated spleen cells to assess the responsiveness to microbial stimuli.
Western blotting: e.g. assessment of enzyme (elastase, myloid peroxidase) production in cells.
Optical Projection Tomography Scanning: to assess localization of HSC in liver, spleen and gut and to identify cells in different organs expressing specific proteins, e.g. Cxcl2R.

6. Expected results:

a) Establishment of mechanisms involved in the early maturation of the mucosal and systemic immunity.
b) Demonstration of a link between perinatal events involving the microbiota and early hemapoietic events and resistance towards e.g. viral infection later in life (microbial resistance?).
c) Establishment of an influenza challenge mouse model.
d) Identification of key markers of early life immune maturation (e.g. specific cell populations in blood, +/- expression of certain genes, e.g. in the brain.

7. Estimated time frame

Task	2013				2014				2015				2016				2017			
Planning, protocol			x	x																
Sample collection				x	x	x	x	x	x	x	x									
Flow cytometry of spleenocytes				x	x	x	x	x	x	x										
OPT of spleen, liver, gut, brain					x	x	x	x	x	x										
16s sequensing/rtPCR gut microbiota						x	x	x	x	x										
rtPCR, microscopy, ELISA etc					x	x	x	x	x	x	x	x								
Publication work(a)												x	x							
Publication work (b+c)											x	x	x							
Publication work (d)												x	x	x						

8. Estimated budget from NEOMUNE: 3.5 mil DKK
Running costs, phd student, lab/animal tech.

9. Estimated budget from elsewhere: 1.5 mill DKK
Salaries Hanne Frøkiær, Axel Kornerup, Stine Metzdorff, Dina Malling, Dennis Nielsen, equipment.

10. Additional comments:

- This work may provide important knowledge to support pig studies, particularly for longer time-frame outcomes that the pig model may not be able to focus on, and might also lead to insight of value for preterm infant treatment/feeding.