WP 3.2: Probiotics in newborn mice

1. Related WPs, MG contact: Synergy with WPs 1.3a,1.4b,1.5,1.6b,2.2,3.1. 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:
We aim to establish whether administration of probiotic bacteria peri- and postnatally will accelerate maturation of the gut epithelium and affect early hemapoiesis.
Sub-aims are:
a) To identify relevant probiotic bacteria or mixtures hereof.
b) To test whether administration of probiotics may compensate for effects of antibiotics treatment on development of the immune system.

4. Background and a central hypothesis:
If perinatal antibiotic treatment of mice as anticipated shows to compromise immune development due to a decrease in the diversity of the microbiota and/or the absence of specific genera, one way to compensate may be administration of one or more probiotic strains during antibiotics treatment.
We hypothesize that administration of probiotic bacteria from birth, especially concomitant with antibiotic treatment may lead to a faster maturation of GI epithelium and well-balanced maturation of immunity.

5. Key analyses and methods:
Perinatal antibiotics and probiotics treatment of dams and offspring mice.
To eradicate major populations of the gut microbiota, dams are treated with an antibiotics, identified in WP3.1 as a potent manipulator of immune maturation, perinatally, and probiotics are administered concomitantly.
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: 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 stumuli.
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:
To establishment if probiotic administration early in life improves immune development in antibiotic treated mice pups.

7. Estimated time frame

Task	2013				2014				2015				2016				2017				2018			
Planning, protocol									x															
Sample collection										x	x													
Flow cytometry										x	x													
OPT-scanning										x	x													
16s seq/rtPCR											x	x												
rtPCR, microscopy, etc											x	x												
Publication work													x	x										

8. Estimated budget from NEOMUNE: 1.2 mio DKK

9. Estimated budget from elsewhere: 0.4 mio DKK
Salaries and equipment.

10. Additional comments:

- These activities will be based on results from WP 3.1. Therefore, protocols and planning are initiated late in project.