Research Pathology Services

Questionnaire - Background Study Information for Histopathology Requests

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Basic concepts:

-What was the study's hypothesis?

- Mice colonized with fecal microbiota derived from human patients with autoimmune thyroiditis have a more severe thyroiditis compared to mice colonized with fecal microbiota from human healthy donors.

-What specific scientific questions would you like to answer with the histological staining?

- Severity of spontaneous autoimmune thyroiditis (SAT) scored of FFPE sections of the thyroid gland.

-What disease is your animal model intended to recapitulate?

- Autoimmune thyroiditis

-What are the model's strengths? Weaknesses?

- This is currently the only well-characterized murine model which spontaneously develops autoimmune thyroiditis.
- Weaknessess: 60% of female NOD.H-2h4 mice, 20–24 weeks of age, develop salivary gland infiltration (Sjogren's syndrome, SS). However, in human, Hashimoto's autoimmune thyroiditis can be present of 30% of SS symptoms.

-Any other important details?

Animal model:

-What is the model? NOD-H.2h4
-What species? Mice
-Age? 13 to 19 weeks old
-Gender? Female
-Genotype?
-If a unique genotype(s), what are knock out / knock in traits?

Treatments: (if any)

-What it the primary treatment?

- Group A) no antibiotic pretreatment, no fecal microbiota transplantations (FMTs)
- Group B) no antibiotic pretreatment, sham FMTs (PBS only)
- Group C) antibiotic pretreatment, autologous FMTs (e.g. murine feces)
- Group D) antibiotic pretreatment, FMTs from healthy human donors
- Group E) antibiotic pretreatment, FMTs from human patients with autoimmune thyroiditis

-What is the treatment regimen (route of administration, vehicle concentration & volume, number of treatments, treatment intervals, time interval between last dose and death)?

- Antibiotic pretreatment consists of 5 consecutive days of 10 mg/ml metronidazole, ampicillin, and neomycin, and 5 mg/ml vancomycin given via oral gavage (200 microL per gavage per mouse)
- FMTs treatment consists of once weekly gavage of 200 microL per mouse

-What is primary treatment mechanism of action? If unknown, what about MOA of another compound in the same drug family?

- A perturbed microbiome composition may promote the development of an autoimmune disease via a reduced integrity of intercellular junctions (leaky gut), via the generation of self-antigens by posttranslational modification of proteins, via lipopolysaccharide (LPS)-induced Toll-like receptor 4 activation, and/or induction of a type1 (Th1) to type 2(Th2) T helper cell shift. (ref: Fröhlich, E. & Wahl, R. Microbiota and Thyroid Interaction in Health and Disease. Trends Endocrinol. Metab. 30, 479–490 (2019)).

-What are the target organs of the drug?

- Thyroid
- Gut microbiome composition

-Are there known off target effects?

-What are the other study variables (i.e. time points, genotypes, diets)?

- At day 4 – all mice received 0.05% NaI (sodium iodine) in their drinking water for the rest of the study
- Normal chow diet

Study design:

-What were the in-life biomarkers?

- (Fresh) fecal samples are collected once weekly (on the day on the FMT treatment)

-Were there any clinical signs of toxicity/reaction to materials? Any clinical differences between the genotypes?

-What other endpoints were assessed? If so, what are your findings to date?

-Where there matched controls? (ie, normal diet, untreated and/or normal diet-sham injected, etc?)

- Matched controls are group A and group B (see above)

-How many tissue samples total? 96 mice

-How many of each tissue type? 6 tissues each: thyroid, salivary gland, thymus, spleen, liver, ileum, colon