Investigation of Novel biomarkers and Definition of the role of the microbiome in Graves’ Orbitopathy ‘INDIGO’

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Introduction
- Graves’ disease (GD) & orbitopathy (GO) are autoimmune conditions. The TSH receptor (TSHR) is an autoantigenic target; thyroid stimulating antibodies (TSAB) cause GD (& GO?).
- Autoimmunity develops in people with a genetic predisposition but the environment also plays a role, e.g. the gut microbiota influences the balance between regulatory T cells (Treg) and TH17 cells (figure 1) which in turn affects development of autoimmunity (figure 2).

Figure 2: Mice lacking TH17 cells do not develop an autoimmune disease (MS), addition of bacteria leads to MS.

Hypothesis & Aims of the Study
- We hypothesize that in GD patients either gut microbiota which induce local tolerance (more Treg) are under-represented or microbiota generating a pro-inflammatory cytokine milieu are over-represented. In GO the TH17:Treg balance is even more tilted in favour of autoimmunity. INDIGO will test these hypotheses.
- INDIGO will investigate whether modification of the gut microbiota in a TSHR induced GD mouse model can lead to an improved and more reproducible model of GO.
- INDIGO will also seek biomarkers to identify GD patients most likely to develop severe GO.

Work Packages
- INDIGO comprises 9 work packages with distinct but complementary goals, as summarised in figure 3.

WP1: Management Structure (figure 4)

WP2 & 5: In Vitro Models & probiotic formulation
- Human model will use CaCo2 gut epithelium & THP1 monocyte cell lines.
- Mouse model will use MDIE-K gut epithelium & RAW-264 monocyte cell lines.
- In each case gut bacteria from GD or GO patients will be added, or from mice with TSHR induced GD/GO. These cultures will be produced in WP5.
- Cytokines released will be analysed using high-throughput platforms at PTP in WP6.

WP3: Patient Studies & Probiotic Trial
- Recruitment of GD/GO patients, questionnaire about diet & lifestyle, samples of blood, tears, faeces & nasal swabs for analysis in WPs 2, 5, 6, 7, 8
- Probiotic Trial; GD patients, standard block/replace alone or supplemented with probiotic &/or antibiotics. Microbiome analysis at various time points.

WP4: In Vivo model effect of modifying microbiome
- TSHR introduced into mice by electroporation
- Mice also treated with segmented filamentous bacteria or gut cultures from GD/GO patients
- Microbiome in mice developing GO/not compared in WP8

WP6 & 7: Proteomics; Immune Response to food
- Proteomic profiling of GD/GO sera & tears & mouse sera
- miRNA analysis of GD/GO sera & tears
- Measurement of cytokines from in vitro models
- Antibodies to antigens from foods and gut microbiota

WP8: Microbiome Analyses
- Results from human/mouse microbiome will:
  - Identify differences in GD vs GO vs healthy
  - Identify differences in mice with GO vs not
  - Does hyperthyroidism alter microbiome?
  - Can GD microbiome be modified by probiotic?
  - Is in vivo model improved by modifying microbiome?

WP9: Consolidation
- This WP will consolidate outputs, analyse correlations & identify areas for future research or clinical trials.
- Figure 8 summarises INDIGO & the personnel involved.

Funded by: EU Marie Curie IAPP