Abstracts of Distinction

AWE-09 CHARACTERIZATION OF NEURONAL INNERVATION & ITS SUPPORTING STRUCTURES IN GERM-FREE, CONVENTIONAL & GERM-FREE-MICE RECOLONIZED WITH BACTEROIDES-THETAIOAOMICRON

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Introduction The enteric nervous system (ENS) is located throughout the length of the gastrointestinal (GI) tract as a series of interconnected ganglionated plexi. It has the unique ability to orchestrate GI behavior independently of the central nervous system. The ENS has two major cell populations: Enteric neurons and enteric glial cells. While enteric neurons regulate gut reflexes with the help of neuropeptides such as substance P, it is becoming evident that enteric glial cells also actively participate in this process via bidirectional signaling with neurons and other cells in the gut wall. Enteric glial cells are also critical for maintaining epithelial barrier function (Yu, 2014).

Given the proximity of the intestinal microbiota to the ENS, it is perhaps not surprising that the function of the ENS is under partial control of gut microbes and the host immune system through complex mechanistic processes. The complex signaling between microbes and their human hosts, and the resulting effect on normal physiologic functions and GI anatomy, is still poorly understood. Given the number of bacterial species inhabiting the gut, identifying specific contributions by individual species is a major challenge. Mono-colonization of Germ Free (GF) mice allows identification of strain specific influences on GI anatomy. We set out to investigate the role of *Bacteroides Thetaiotaomicron* (Bt), a major and stable endosymbiont of the human gut (Wexler and Goodman, 2017), in influencing the components of the ENS. Our aims were to find out whether Bt alters;

- 1) Neuronal budding using neuronal marker GAP-43,
- 2) Glial cell density using glial cell marker S100β, and
- 3) Substance P expression using substance P antibody.

Methods 3 mice groups were used: Germ-free (GF) N=5, Conventional (CONV) N=5 and germ-free mice recolonized with Bt (Bt-RECOL) N=5. Colonic sections from each mouse were mounted onto slides. 5 slides from each mice group were stained via indirect immunofluorescence to investigate the expression of biomarkers GAP43, S100β and substance P separately. 5 fields of view of the mucosa and myenteric plexus per slide for every marker used were observed under an epifluorescence microscope. 150 digital images per marker were captured and saved [Mucosa: GF (n=25), CONV (n=25) and Bt-RECOL (n=25); Myenteric plexus: GF (n=25), CONV (n=25) and Bt-RECOL (n=25)]. Quantitative

assessment of the digital images was carried out using imageJ software. Positive staining was represented as number of pixels per image and this data was saved in MS Excel. PRISM 7 (GraphPad software) was used to calculate statistical significance. Comparison between groups was performed using One-Way ANOVA. All values are mean ± standard error of mean (SEM). $p < 0.05$ was considered statistically significant.

Results Neuronal budding, measured by GAP-43, displayed no significant changes in GF vs. CONV group within the mucosa (GF: 3696.96 ± 258.06 vs. CONV: 4202.63 ± 336.34 pixels/image). However, a significant increase in GAP-43 immunostaining within the myenteric plexus was observed in GF vs. CONV (GF: 11519.42 ± 1547.95 vs. CONV: 7280.97 ± 894.46 pixels/image, $p = 0.0218$). Bt-RECOL significantly increased mucosal GAP-43 (Bt-RECOL: 4975.71 ± 447.65 vs. GF: 3696.96 ± 258.06 pixels/image, $p = 0.0169$) and myenteric plexus GAP-43 immunostaining (Bt-RECOL: 13958.66 ± 1931.47 vs. CONV: 7280.97 ± 894.46 pixels/image, $p = 0.0029$).

Glial cell density measured by S100β, was significantly reduced in the mucosa of GF mice compared to CONV mice (GF: 3031.12 ± 437.99 vs. CONV: 5508.13 ± 507.96 pixels/image,

$p = 0.0006$). No significant difference was observed within the myenteric plexus (GF: 6562.18 ± 803.85 vs. CONV: 7919.89 ± 659.24 pixels/image). Bt-RECOL restored mucosal (GF: 3031.12 ± 437.99 vs. Bt-RECOL: 5820.71 ± 356.06 pixels/image, $p = < 0.0001$) and myenteric plexus (GF: 6562.18 ± 803.85 vs. Bt-RECOL: 11537.75 ± 998.50 pixels/image, $p = 0.0003$) glial cell density.

The expression of substance P was non-significant between CONV and GF mice groups within the mucosa. A significant increase in SP immunoreactivity was observed in Bt-RECOL group when compared to both GF and CONV groups (Bt-RECOL: 1629.46 ± 151.95 vs. GF: 546.96 ± 94.15 pixels/image, $p < 0.0001$; Bt-RECOL: 1629.46 ± 151.95 vs. CONV: 578.498 ± 79.36 pixels/image, $p < 0.0001$). In the myenteric plexus, there was a significant increase in SP immunoreactivity in the Bt-RECOL group compared to GF group (Bt-RECOL: 7296.502 ± 726.54 vs. GF: 5004.698 ± 529.96 pixels/image, $p = 0.0228$). Bt-RECOL group also displayed a significant increase in SP immunostaining relative to the CONV mice group (Bt-RECOL: 7296.502 ± 726.54 vs. CONV: 5080.508 ± 516.14 pixels/image, $p = 0.0287$). The differences in immunostaining between CONV and GF groups were not significant.

Conclusion Conventionalization with *Bacteroides Thetaiotaomicron* (Bt) restored glial cell population in addition to increased neuronal budding. We also show that Bt enhances substance P expression. Our data shows that Bt is required for normal neuronal innervation in the colon suggesting its critical role in maintaining normal colonic function. We hypothesize that functional activity of the ENS can be regulated by Bt through direct or indirect mechanisms. Future studies on how Bt interacts with the enteric nervous system (pathway interactions and key molecules involved) can help us develop tools to greatly impact intestinal health.