AB184. 237. Development of a novel methodology to assess the microbiome of mesenteric lymph nodes incorporating culture and culture-independent methods

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Background: Mesenteric lymph nodes (MLNs) provide a platform on which translocated bacteria incite intestinal immunological responses.1,2 To fully elucidate these processes, it is essential that we comprehensively understand their microbiome. We have developed a novel work-flow to determine the MLN microbiome in inflammatory bowel disease (IBD), using culture and culture-independent methods.

Methods: Where appropriate, IBD patients in University Hospital Limerick undergo resections that include the mesentery, allowing greater MLN yields. In our novel process, nodes were harvested from resection specimens [Crohn's disease (CD), n=10; ulcerative colitis (UC), n=10] using aseptic techniques. MLNs were washed in sterile phosphate buffered saline and swabbed. Culture and culture-independent methods (i.e., DNA extraction) were used to identify microbes on the MLN surface (i.e., controls). Total DNA was subsequently extracted from node tissue. Extracted DNA then underwent PCR amplification of 16S DNA to confirm the presence or absence of translocated bacterial DNA.

Results: This is a non-laborious methodology which takes approximately 20 (DNA) and 48 (culture) hours to obtain results. Researchers should expect to spend 1 h processing nodes, 3 h for DNA extraction and 30 minutes assessing agar plates. To date, DNA has been extracted from MLNs of thirteen patients (CD, n=5; UC, n=8) and bacterial DNA has been confirmed in CD (100%) and UC (63%) patient nodes. Surface-derived bacteria were confirmed in sample controls using both methods (culture-independent, n=3; culture, n=5).

Conclusions: This process can be used to provide a comprehensive overview of the MLN microbiome, differentiating between bacteria present on the MLN surface and the node interior.

Keywords: Mesentery; node; microbiology; novel; culture; technique

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