

BSG 2016 - Abstract Submission

Inflammatory Bowel Disease

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CROHN'S DISEASE MUCOSA-ASSOCIATED *E. COLI* SHOW BETTER TOLERANCE OF A SUPEROXIDATIVE STRESS ENVIRONMENT, THAT MIMICS CONDITIONS INSIDE MACROPHAGE PHAGOLYSOSOMES.

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This abstract is: A basic science submission

Does your Endoscopy abstract include a video?: No

Preferred presentation type: Poster only

Introduction: Mucosa-associated *E.coli* are commonly found in patients with Crohn's disease (CD), colorectal cancer (CRC) and to a lesser extent in ulcerative colitis (UC)^{1,2}. They are able to replicate within macrophage phagolysosomes and are found inside CD tissue macrophages^{3,4}. Our aim here was to assess these mucosal *E.coli* isolates for ability to tolerate stress conditions characteristic of that found within the phagolysosome.

Methods: *E.coli* isolates from patients with CD (n=16), UC (6), CRC (7), and those obtained from other patients (n=7; IBS (2), sporadic polyps (3), piles (1), healthy individuals (3)) and laboratory strains (6) were grown in LB medium (OD_{600nm} 0.1). Ten-fold dilutions were plated under stress conditions; 100mM MES pH5 with or without 1mM NaNO₂ (low pH and nitrosative stress), 1mM H₂O₂ pH7 (peroxidative stress), 1mM methyl viologen pH7 (superoxidative stress) and compared to growth on LB agar pH7. Data was correlated with ability of isolates to replicate within J774 macrophages⁴. Host oxidative stress response of macrophages infected with either CD isolate HM605 or *E.coli* K12_{EPI300} (susceptible to macrophage killing) were studied. cDNA synthesised from isolated RNA, was subjected to Qiagen RT² PCR Oxidative stress arrays (n=3 replicates/arrays performed for condition).

Results: CD isolates showed greater tolerance to superoxidative stress than isolates from UC, CRC and other patients/laboratory *E.coli* strains (p<0.05 ANOVA; N=4 expts, n=3 replicates). In particular, 4/10 colonic CD *E.coli* isolates (HM413, HM427, HM605, HM615) and 2/6 ileal CD isolates (LF82, LF86), all high intramacrophage replicators [4.6±2.3 fold], showed high % survival under superoxidative stress (95.2±12.0% (mean±SD); versus laboratory strains [0.9±2.3fold replication], % growth 17.3±38.6%. No differences in tolerance were seen amongst isolates to other stress conditions, excepting all laboratory strains which were intolerant. No evidence was found to suggest that CD isolate HM605 could alter macrophage oxidative stress response to infection to promote its own intraphagolysosome growth; e.g. superoxide stress response genes *Ncf1*, *Nos2* and *Sod2* were all upregulated to similar levels at 6h (>2-fold) in both *E.coli* HM605- and K12_{EPI300}-infected macrophages compared to uninfected controls (p<0.05).

Conclusion: CD mucosa-associated *E.coli* are tolerant of superoxidative stress to support their survival and replication within host macrophages. Adaptation to the phagolysosome niche appears not to be through ability to alter host oxidative stress response to infection.

References: ¹Thomazini *et al.* Int J Med Microbiol 2011;301: 475-79

²Martin *et al.* Gastroenterology 2004;127: 80-93

³Bringer *et al.* Cell. Microbiol 2006;8:471-84.

⁴Subramanian *et al.* AAC 2008;52:427-34.

Disclosure of Interest: A. Tawfik: None Declared, J. Rhodes Consultant for: is/has been a member of advisory boards for Atlantic, Procter & Gamble and Falk, Conflict with: has received honoraria from Abbott, Falk, Ferring, Glaxo Smith Kline, Procter & Gamble and Schering Plough, B. Campbell Conflict with: has received honoraria from Amgen, Falk and Entero