

Identifying the molecular mechanisms of interaction between colonic Crohn's disease mucosa-associated *Escherichia coli* and the intestinal epithelium.

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I) Lay summary

There is now very strong scientific evidence that bacteria living naturally in the gut are a causative factor in inflammatory bowel diseases. Recent studies by ourselves and at least six other groups have identified an increased number type of adherent ('sticky') bacteria called *Escherichia coli* (*E. coli*), on and inside cells lining both the small and large bowel of patients with Crohn's disease and ulcerative colitis. Strains of the same type of bacteria also cause a similar colitis in boxer dogs. These *E. coli* have the ability to cause inflammation but they lack known genetic markers of disease causation and differ from the "hamburger" *E. coli* and from other *E. coli* that cause epidemics of diarrhoea.

For many of the *E. coli* isolates studied by our research group, it is the bacterial flagellae, the wavy 'hairs' that allow some bacteria to move around, in association with vesicles, little blebs that can form on and be shed from the surface of the bacteria, that are responsible for initiating the inflammatory response. We now propose to conduct laboratory studies to discover the molecular factors possessed by the bacteria which enable them to stick to and invade the cells lining the inner surface of the bowel. In many ways, the attachment and entry of the Crohn's disease *E. coli* to the cells that line the bowel, in the absence of any of the typical markers of disease causation, is similar to the properties of group of *E. coli* which infect and inflame the bladder, known as uropathogenic *E. coli* (UPEC). Indeed, they may be the same organisms. There is already a considerable literature on how UPEC interact with cells lining the bladder and much of this may be relevant in the bowel.

The first part the study will be to examine whether there are similarities between our 'sticky' Crohn's disease *E. coli* and two fully characterised UPEC's for which there are complete gene maps. We will search for genes related to disease causation and in particular for those genes related to cell adherence and entry using a method called suppression-subtractive hybridisation (SSH) which assesses similarities of genetic material between bacteria of the same type. This technique has been successfully used, by us and others, to detect unique genomic sequences in a number of bacterial pathogens, including *E. coli*. Any putative adhesion and invasion genes identified will then be selectively deleted in the Crohn's disease *E. coli* to confirm their function in assays of adherence and entry to bowel cells grown in the laboratory. We already have a number of these 'knockout' *E. coli* to test, some of our own and some from research colleagues in the USA who are working with *E. coli* strains isolated from Crohn's disease patients with small bowel disease. As an alternative approach, we also have constructed a mini gene library (~1000 genes) prepared from one of our known highly 'sticky' Crohn's *E. coli* isolates. Each separate gene sequence from library has been inserted inside another harmless 'non-adherent' *E. coli* so that we can test each one separately for adhesion properties.

These studies should greatly increase our understanding of this important group of bacteria and of their role in the cause of Crohn's disease, and will allow identification of novel therapeutic targets. An appropriate long term aim (which would lead on after this project) should be the development and testing of an *E. coli* vaccine. The current proposal would be an essential precursor to this.