epithelial cells accumulate molecular alterations by genetic and epigenetic mechanisms, such as regional hypermethylation as suggested by Chan *et al*[1] and Maekita *et al*.[2] Global DNA hypomethylation, cited by Dr Peyrin-Biroulet, might also influence tumour development by predisposing to the expression of genes involved in neoplastic growth or by inhibiting the chromosome condensation that leads to alterations in chromosome pairing and disjunction.[3] DNA hypomethylation has more often been associated with folate deficiency caused by alterations in the enzymes involved in folate metabolism, as seen when there is polymorphism in the gene that encodes methylene-tetrahydrofolate reductase.[4] Methionine is a precursor of S-adenosyl-methionine, the primary methyl donor for most biological methylation reactions, including that of DNA. As folate is involved in the remethylation of homocysteine to methionine, its deficiency leads to DNA hypomethylation and hyperhomocysteinaemia. Although the hyperhomocysteinaemia observed in the patients in our study was not due to folate deficiency but to cobalamin deficiency, it has been suggested that high concentrations of plasma homocysteine—independent of the cause—may increase the intracellular S-adenosyl-homocysteine (SAH) which inhibits DNA methyltransferases, leading also to global hypomethylation.[5] As hyperhomocysteinaemia also enhances the production of reactive oxygen species (ROS), it has been hypothesised that DNA hypomethylation mediated by SAH increases the vulnerability and sensitivity of DNA to homocysteine induced ROS.[5]

In the context of gastric carcinogenesis, however, bacterial and host factors have also to be considered. Among the *H pylori* virulence factors, CagA protein was recently identified as the major disease associated factor. Translocation of CagA into the host gastric epithelial cells through a specialised type IV secretion system encoded in the *cag* pathogenicity island is followed by CagA tyrosine phosphorylation which triggers abnormal intracellular signals. This abnormality deregulates cell growth, cell to cell contact, and cell migration, as well as enhancing epithelial cell turnover, which increases the risk of damaged cells acquiring precancerous genetic changes.[6 7] Factors linked to the host—such as genetics—might affect the immune response to the infection, which per se may contribute to the progression to gastric cancer. Among these, *IL1* gene cluster polymorphisms should be highlighted.[8] Using logistic analysis, we have demonstrated that both *cagA* positive status and *IL1RN* polymorphisms are independently associated with distal gastric carcinoma in the Brazilian population.[9]

Finally, although the Maastricht III consensus states that eradication of *H pylori* has the potential to reduce the risk of gastric cancer development and that the optimal time to eradicate the bacterium is before preneoplastic lesions are present, the results of our study do not allow us to answer the question posed by Dr Peyrin-Biroulet: ''Should we screen and treat *H pylori* positive patients for cobalamin deficiency to reduce the risk of gastric cancer?'' This is an unexplored area for future research, because it has not been established yet whether homocysteine is causally involved in gastric carcinogenesis or whether it is an indirect indicator of other involved mechanisms.

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References

1 **Chan AO**, Lam SK, Wong BC, *et al*. Promoter methylation of E-cadherin gene in gastric mucosa associated with Helicobacter pylori infection and in gastric cancer. *Gut* 2003;**52**:502–6.
2 **Maekita T**, Nakazawa K, Mihara M, *et al*. High levels of aberrant DNA methylation in Helicobacter pylori infected gastric mucosa and its possible association with gastric cancer risk. *Clin Cancer Res* 2006;**12**:989–95.
3 **Goelz SE**, Vogelstein B, Hamilton SR, *et al*. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985;**228**:187–94.
4 **Friso S**, Choi SW, Girelli D, *et al*. A common mutation in the 5,10-methylenetetrahydrofolate reductase genes affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA* 2002;**99**:5606–11.
5 **James SJ**, Melnyk S, Pogribna M, *et al*. Elevation in S-adenosylhomocysteine and DNA hypomethylation: potential epigenetic mechanism for homocysteine-related pathology. *J Nutr* 2002;**132**:2361–6S.
6 **Stein M**, Bagnoli F, Halenbeck R, *et al*. C-Src/Lyn kinases activate Helicobacter pylori CagA through tyrosine phosphorylation of the EPIYA motifs. *Mol Microbiol* 2002;**43**:971–80.
7 **Hatakeyama M**, Higashi H. Helicobacter pylori CagA: a new paradigm for bacterial carcinogenesis. *Cancer Sci* 2005;**96**:835–43.
8 **El-Omar EM**, Carrington M, Chow WH, *et al*. Interleukin-1 polymorphism associated with increased risk of gastric cancer. *Nature* 2000;**404**:398–402.
9 **Rocha GA**, Guerra JB, Rocha AMC, *et al*. IL1RN polymorphic gene and cagA-positive status independently increase the risk of noncardia gastric carcinoma. *Int J Cancer* 2005;**115**:678–83.

Biofilms in the normal human large bowel: fact rather than fiction

Studies from a variety of scientific fields point at the importance of biofilms in the gut. For example, Jeffrey Gordon and colleagues,[1] evaluating data from immunologists, environmental engineers and glycobiologists, proposed that ''symbionts inhabiting the polysaccharide-rich mucus gel layer overlying the gut epithelium constitute a biofilm-like community and that retention in such a matrix benefits the host by promoting functions served by the microbiota, including digestion of luminal contents and fortification of host defenses.'' Evaluating our own immunological data, data from microbiologists and the medical literature, we independently came to the same conclusions.[2]

Direct observations of biofilms in the normal gut were lacking as recently as five years ago, probably because preservation of the epithelial glycocalyx, like the preservation of other glycocalyx structures, is technically challenging, as has long been known.[3–5] In fact, we have found that manipulations as seemingly innocuous as washing with saline can disrupt biofilms from normal bowel tissue.[6] With this in mind, we devised an approach that reproducibly preserves biofilms in the normal, unprepped (without flushing of the luminal contents) bowel,[6] and examined fresh, unprepped human appendixes that had been removed from recipients during kidney–pancreas transplant procedures. Our laboratory has subsequently recapitulated our previously published observations using a fresh, normal, unprepped human appendix from a deceased organ donor (fig 1). To date, biofilms have been observed in the normal proximal (not distal) large bowel of mice,[7] rats,[6] baboons[6] and humans.[6] Thus, although enteric biofilms are likely in a steady state of shedding and regrowth, and although the percentage of epithelium covered with biofilms is unknown, there is no doubt that biofilms are indeed present in the normal bowel.

In a recent article (*Gut* 2007;**56**:343–50) Alexander Swidsinski and colleagues flatly dispute our findings of biofilms in the human appendix, referring to our work as ''fiction''. The investigators, following a 6-hour fixation procedure with a non-aqueous solvent, find no biofilms in the human appendix and conclude that biofilms do not exist in the normal human bowel. We do not dispute or doubt the observations made by Swidsinski *et al*., since their results confirm what we have already demonstrated: biofilms in the normal colon are not stable using common preservation techniques.[6]

Of interest is the fact that Swidsinki's laboratory has been successful at preserving biofilms in biopsies of the diseased bowel of humans,[8 9] in the normal bowel of laboratory mice,[7] but not in the normal human appendix (*Gut* 2007;**56**:343–50). A likely explanation for these observations is that, for technical reasons, some tissues were more effectively preserved than others. The idea that the typically thick-walled appendix is particularly difficult to preserve is supported by the fact that Swidsinski and colleagues used a 6-hour fixation procedure for the appendix, but only a 2-hour procedure for the biopsy samples. Another potential explanation for the observations made by Swidsinski's laboratory is that biofilms in the diseased human bowel may be more resilient than biofilms in the normal human gut. Indeed, biofilm formation in the normal bowel is probably supported by the immune system,[10–12] and since inflammatory bowel disease is associated with an enhanced immune response, it is not surprising that biofilm formation in the diseased state would be profoundly increased.

Finally, we would point out that it is not overly surprising to observe biofilms in the normal colons of humans since similar observations have been made in the normal colons of laboratory animals as diverse as mice[7] (observations from Swidsinski's own laboratory) and baboons[6] (observations from our laboratory).

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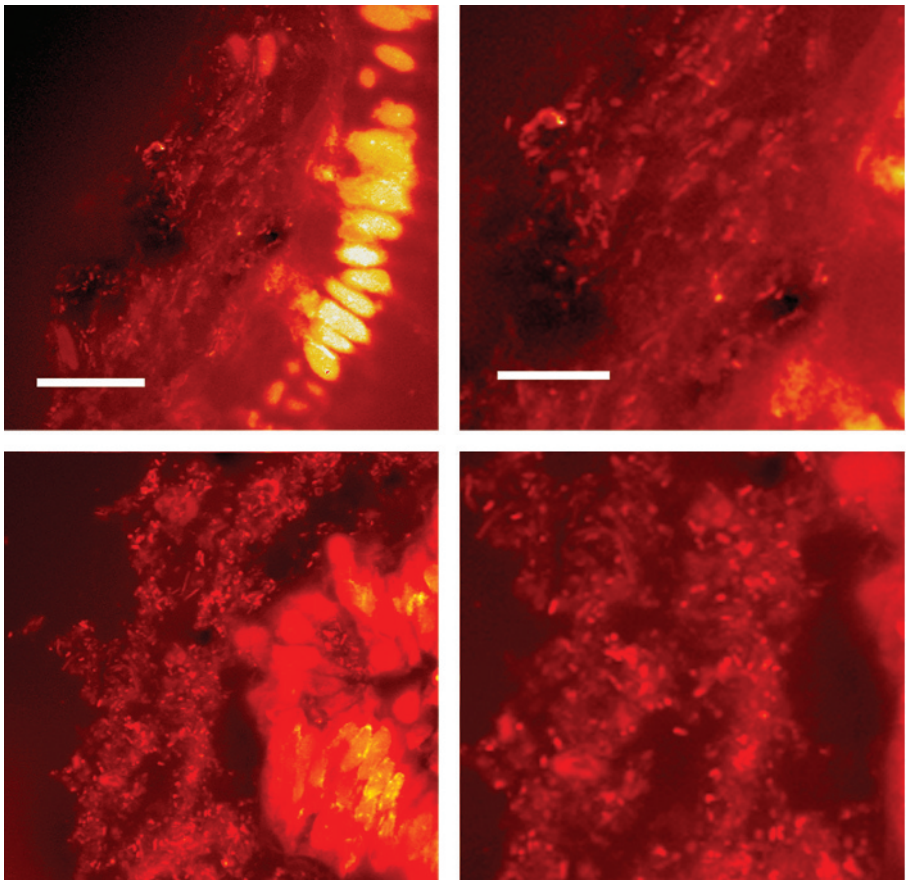

Figure 1 Biofilms adjacent to epithelium in a normal human appendix obtained from a deceased organ donor were observed using a confocal laser microscope following flash freezing, cryosectioning and rapid staining of the tissue with acridine orange as previously described.[6] Two representative sections are shown, and images on the right show an enlarged section of the images on the left. Photos were taken of the areas at the border between the epithelium and the lumen. The smaller fluorescent spots are bacteria within the mucus layer stained with acridine orange, and the larger brightly stained areas are the nuclei of the epithelial cells that also stain with acridine orange. The bars = 30 μm (panels on left) and 15 μm (panels on right).

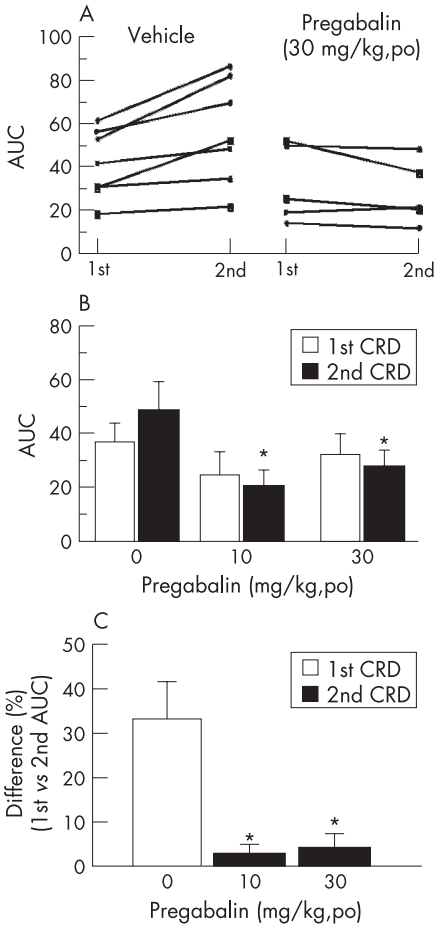

Figure 1 Oral pregabalin decreased the area under the curve of contraction (AUC) of the abdominal electromyogram to two successive tonic colorectal distensions in rats. (A) Individual rat's response to the first and second colorectal distension (60 mm Hg, 10 minutes each and a 10 minute interval) one hour after administration of vehicle (water) or pregabalin (30 mg/kg orally). (B) Group mean of the AUC in rats given vehicle or pregabalin 10 or 30 mg/kg. (C) Per cent difference in AUC between the second and first colorectal distensions in vehicle and pregabalin (10 and 30 mg/kg) treated rats. Values are mean, error bars = SEM. Differences within and between groups were analysed using one way analysis of variance (ANOVA) or a two way repeated measures ANOVA (one factor repetition). *$p<0.05$ *vs* the corresponding vehicle treated rats. CRD, colorectal distension; po, oral administration.

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References

1 **Sonnenburg JL**, Angenent LT, Gordon JI. Getting a grip on things: how do communities of bacterial symbionts become established in our intestine? *Nat Immunol* 2004;**5**:569–73.
2 **Everett ML**, Palestrant D, Miller SE, *et al*. Immune exclusion and immune inclusion: a new model of host-bacterial interactions in the gut. *Clinical and Applied Immunology Reviews* 2004;**5**:321–32.
3 **Luft JH**. The structure and properties of the cell surface coat. *Int Rev Cytol* 1976;**45**:291–382.
4 **Allen A**, Pearson JP. The gastrointestinal adherent mucous gel barrier. *Methods Mol Biol* 2000;**125**:57–64.
5 **Fassel TA**, Edmiston Jr CE. Bacterial biofilms: strategies for preparing glycocalyx for electron microscopy. *Methods Enzymol* 1999;**310**:194–203.
6 **Palestrant D**, Holzknecht ZE, Collins BH, *et al*. Microbial biofilms in the gut: visualization by electron microscopy and by acridine orange staining. *Ultrastruct Pathol* 2004;**28**:23–7.
7 **Swidsinski A**, Loening-Baucke V, Lochs H, *et al*. Spatial organization of bacterial flora in normal and inflamed intestine: a fluorescence in situ hybridization study in mice. *World J Gastroenterol* 2005;**11**:1131–40.
8 **Swidsinski A**, Ladhoff A, Pernthaler A, *et al*. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002;**122**:44–54.
9 **Swidsinski A**, Weber J, Loening-Baucke V, *et al*. Spatial organization and composition of the mucosal flora in patients with inflammatory bowel disease. *J Clin Microbiol* 2005;**43**:3380–9.
10 **Bollinger RR**, Everett ML, Palestrant D, *et al*. Human secretory immunoglobulin A may contribute to biofilm formation in the gut. *Immunology* 2003;**109**:580–7.
11 **Orndorff PE**, Devapali A, Palestrant S, *et al*. Immunoglobulin-mediated agglutination and biofilm formation by Escherichia coli K-12 requires the type 1 pilus fiber. *Infect Immun* 2004;**72**:1929–38.
12 **Bollinger RB**, Everett ML, Wahl S, *et al*. Secretory IgA and mucin-mediated biofilm formation by environmental strains of Escherichia coli: role of type 1 pili. *Mol Immunol* 2005;**43**:378–87.

Pregabalin decreases visceral pain and prevents spinal neuronal activation in rats

We read the recent article by Houghton *et al* (*Gut* 2007, Apr 19 [Epub ahead of print]), reporting that pregabalin, a new generation of $\alpha_2\delta$ ligand, increased sensory thresholds to normal levels in 26 patients with irritable bowel syndrome (IBS) and baseline rectal hypersensitivity, in a randomised double blind, placebo controlled, parallel group study. The authors concluded that $\alpha_2\delta$ ligands are worthy of further physiological and clinical investigations for diseases affecting gut sensory function. Experimental studies to date indicate that pregabalin prevents colorectal allodynia and hyperalgesia in rats exposed to intracolonic trinitrobenzene-sulphonic acid[1] or septic shock.[2] Visceral hyperalgesia and symptoms in IBS are, however, characterised by the absence of overt colonic damage or mucosal abnormality. In the study we describe here, pregabalin given orally in a rat non-inflammatory model