Lectins, colitis and colon cancer

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Understanding the pathogenesis of inflammatory bowel disease (IBD) is one of the biggest challenges in gastroenterology. Although no individual discovery has provided a 'helicobacter-like' leap in understanding, we can build a surprisingly clear picture by bringing together some of the known facts.

Inflammatory bowel disease: a spectrum of disease that results from interacting genetic and environmental factors

An individual who has an identical twin with Crohn's disease has approximately 50% risk of developing the disease, whilst an individual who has an identical twin with ulcerative colitis has approximately 10% risk of developing ulcerative colitis1. Genetic studies based on affected sibling pair analysis have identified several loci which contain as yet unidentified IBD genes. One of the best substantiated loci, on chromosome 16, contains a gene which confers a risk specifically for Crohn's disease2, but other loci contain genes which confer a risk for both Crohn's disease and ulcerative colitis3. The genetic overlap between these two conditions comes as no surprise because it has long been known that there are 'mixed disease' families which include some members with Crohn's disease and others with ulcerative colitis. Smoking status has a powerful effect on the disease phenotype within these mixed-disease families, with non-smokers tending to develop ulcerative colitis and smokers developing Crohn's disease4. It is therefore a statement of fact rather than a hypothesis that Crohn's disease and ulcerative colitis represent a spectrum of IBD that results from interaction between genetic and environmental factors. The twin studies suggest that genetic and environmental factors may be equally important for Crohn's disease, but for ulcerative colitis environmental factors are more important than genetic.

Explaining the phenotypic differences between Crohn's disease and ulcerative colitis: effects of diet and phagocyte function

Why is Crohn's disease histology typified by granulomas and a relative lack of goblet cell depletion? Why does Crohn's disease, but not ulcerative colitis, respond to enteral feeding as sole therapy?

There are two conditions, chronic granulomatous disease5 and glycogen storage disease type 1b, in which defects in phagocytic cell function result in intestinal disease that closely mimics Crohn's disease. In the case of X-linked chronic granulomatous disease, the defect has been characterised at the molecular level. There is a mutation in an NADPH oxidase gene, a consequence of which is that the phagocytic cells (neutrophils and macrophages) are unable to mount a respiratory burst and are therefore unable to kill phagocytosed bacteria. Segal, whose team was the first to characterise this defect7, showed over 20 years ago that patients with Crohn's disease have defective chemotaxis of peripheral blood neutrophils into perspex chambers containing their own sera placed over forearm skin abrasions8. In subsequent studies, we and others have shown that the neutrophils function normally in vitro but that in vivo there seem to be circulating inhibitors of neutrophil function9. Recently, it has been shown that at least two-thirds of Crohn's disease patients have circulating antibodies to an antigen present in baker's yeast, Saccharomyces cerevisiae: anti-Saccharomyces cerevisiae antibody (ASCA). The epitope for this antibody is oligomannan, a mannose polymer10 present in many bacterial cell walls including, but not exclusively, mycobacteria, and which can itself inhibit neutrophil function11. Oligomannan shed by intramucosal bacteria is therefore a plausible candidate for a tissue inhibitor of neutrophil function. Inhibition of the function of neutrophils and macrophages that have been recruited to the mucosa could then result in failure to clear intramucosal bacteria, resulting in a chronic granulomatous reaction which penetrates deep into the mucosa. We have preliminary evidence which suggests that the goblet cell depletion seen in ulcerative colitis is neutrophil driven, so the lack of goblet cell depletion that typifies Crohn's disease could also reflect defective neutrophil function at the mucosal level. If this general hypothesis is correct, the treatment of Crohn's disease might be better targeted towards stimulation of neutrophil function rather than towards suppression of lymphocyte function. Granulocyte colony stimulating factor (GCSF) has been shown to be effective at treating the Crohn's-like bowel disease that affects individuals with chronic granulomatous disease5 or

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glycogen storage disease type 1b4, and an impressive response to GCSF therapy has recently been reported in a case of fistulating Crohn's disease12. The GCSF therapy not only results in increased neutrophil numbers but also stimulates their function.

Crohn's disease can be treated by enteral feeding and avoiding normal food, with a response similar to that achievable by high-dose corticosteroids13; moreover, unlike corticosteroid therapy, it is associated with endoscopic evidence of healing of ileal ulceration14. Ulcerative colitis, however, does not respond to bowel rest combined with intravenous feeding15. One explanation for this difference – possibly the most likely – is that ulcerative colitis does not affect the small intestine. Mixed results have been reported for the effects of dietary therapy in colonic Crohn's disease, but most such cases have at least some microscopic inflammation in the small intestine. It seems reasonable to assume that there are factors in the normal modern diet harmful for Crohn's disease. There is some evidence incriminating long-chain triglycerides16,17. There is a striking inverse correlation between the response rates achieved in published trials of whole protein or peptide based enteral feeds and the proportion of calories given as long-chain fat. Also, pigs with an anti-peristaltic reversed ileal loop developed ileal inflammation when fed a high fat diet but not when fed a low fat diet18. An alternative hypothesis has recently been suggested based on evidence that titanium oxide, used in the food industry as a white colouring agent, might provoke granulomatous inflammation, possibly by acting as a hapten19. Further studies are needed to identify a reasonably broad diet that can be used to keep patients with Crohn's disease in remission; the current 50% relapse rate six months after returning to normal food discoursages regular use of enteral feeding as a first-line therapeutic option.

Epithelial glycosylation as a possible link between inflammatory bowel disease and colon cancer

Glycosylation changes are similar in Crohn's disease, ulcerative colitis, colon cancer and colon adenomas, affecting both epithelial cell surface glycoproteins and secreted mucins20. They include approximately 50% shortening in the length of mucin O-linked oligosaccharides21, increased sialylation22, particularly of peripheral blood group structures, reduced sulphation23,24, and increased expression of oncofetal carbohydrate structures such as galactose β1, 3 N-acetylgalactosamine α-, the Thomsen-Friedenreich antigen (Galβ1,3GalNAcα-) which is overexpressed in the mucosa in colon cancer and inflammatory bowel disease. Normal colonic mucosa is negative using this staining technique, although more sensitive techniques can show low level expression of peanut lectin receptor in normal mucosa.

Fig 1. Colonic mucosal biopsy samples: (a) colon cancer, (b) ulcerative colitis. Staining with peroxidase-conjugated peanut lectin identifies the Thomsen-Friedenreich blood group oncofetal antigen (Galβ1,3GalNAcα-) which is overexpressed in the mucosa in colon cancer and inflammatory bowel disease. Normal colonic mucosa is negative using this staining technique, although more sensitive techniques can show low level expression of peanut lectin receptor in normal mucosa.

minded by the expression of a gene encoding for a fucosyl transferase.

There is some epidemiological evidence to support parallel inheritance of IBD and colon cancer. When ulcerative colitis patients are compared with age-matched controls, the relative risk for colorectal cancer remains approximately constant over time, although patients who develop colitis at an earlier age show a greater relative risk25, possibly explained by greater inherited 'dose'. If colitis were the cause of the cancer, the age-corrected relative risk would have been expected to rise progressively with time. There are several possible explanations why these studies failed to show such a rise. One mundane explanation is that this is an artefact due to patients being removed from colon cancer risk by colectomy. Alternatively, the approximately 11-fold increased colon cancer risk in patients with extensive colitis might be co-inherited with the risk for colitis. In keeping with this, there is no significant increase in risk for rectal cancer in patients with persistent proctitis26, suggesting
that chronic inflammation alone is not sufficient to cause the increased cancer risk. Further studies are needed to determine whether the non-colitic relatives of patients with IBD have increased risk for colorectal cancer.

Glycosylation abnormalities in IBD are likely to be functionally important whether or not they are inherited or acquired. The altered glycosylation of epithelial cell surface glycoproteins gives them the potential to recruit lectins of microbial or dietary origin that would otherwise pass through the intestinal lumen without interacting with the mucosa. In support of this hypothesis, we have shown that peanut lectin, which binds selectively to the Thomsen-Friedenreich carbohydrate antigen that is overexpressed in colon cancer and IBD, stimulates proliferation in cell lines and mucosal explants. Amongst patients with colonic symptoms who nobly ate 100 g of raw peanuts per day for five days prior to flexible sigmoidoscopy, there was a 40% increase in rectal mucosal proliferation in those who expressed the lectin receptor in their rectal mucosa, whereas those who did not express the lectin receptor showed no significant increase in rectal mucosal proliferation (Fig 2). To our surprise, another Thomsen-Friedenreich binding lectin, present in the edible mushroom Agaricus bisporus, showed the opposite effect, causing marked inhibition of proliferation in a wide range of cell lines without any significant cytotoxicity. In further studies to elucidate the mechanism of this unusual effect it was found that the mushroom lectin becomes internalised, concentrated in the perinuclear region, and inhibits nuclear localising sequence-dependent nuclear protein import (Fig 3). The lectin is much less resistant to heat or digestion than peanut lectin, so this may be of little relevance in vivo, but it demonstrates the functional significance of cell surface and intracellular glycosylation. It also suggests that the lectin might be a powerful tool in helping to elucidate mechanisms of nuclear protein import. Its intracellular ligands might, for example, represent the so far undiscovered intracellular ligands for the family of naturally occurring galactose-binding lectins (galectins), whose expression is related to cancer invasion and metastasis.

The lectin/galactose hypothesis for colon cancer

These experiments with dietary lectins have established the possibility of significant interactions between dietary lectins and the intestinal mucosa, but it seems highly unlikely that much of the risk for colon cancer could be accounted for by...
differences in consumption of one or two specific dietary lectins. The common expression of terminal galactose, for example as part of the Thomsen-Friedenreich antigen, by mucosal glycoconjugates in colon cancer and precancerous conditions led us to propose a broader hypothesis. Noting that lectins tend to stimulate the proliferation of cells to which they bind (mushroom lectin being an exception), we speculated that galactose-binding lectins of dietary or microbial origin might tend to promote colon cancer, whereas galactose, present in dietary fibre, might bind the galactose-binding lectins in the intestinal lumen and thus competitively inhibit their effects (Fig 4). It is interesting to note that galactose is present in many vegetable and fruit fibres but almost absent from cereal fibre.

We therefore set up a case-control study of diet and colon cancer on Merseyside to test this hypothesis. A 160-item, previously validated, food frequency questionnaire was used to assess the pre-illness diet of colon cancer patients and matched controls. This study showed no significant protective effect for total cereal or vegetable fibre but demonstrated a significant protective effect for the galactose content of fibre. It also showed a protective effect for non-legume (and hence low lectin) vegetables and for brassicas, which are known to contain the cancer protective...

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**Table 1. The main pieces in the inflammatory bowel disease (IBD)/colon cancer jigsaw puzzle.**

- Crohn's disease and ulcerative colitis have common genetic factors
- Differences in their phenotypes seem to be related to environmental factors, particularly smoking (with smokers more likely to develop Crohn's disease and non-smokers more likely to develop ulcerative colitis)
- Crohn's disease, but not ulcerative colitis, responds to dietary therapy
- Crohn's disease is closely mimicked by inherited conditions in which neutrophils are defective (including chronic granulomatous disease and glycogen storage disease type 1b); in these conditions, the IBD responds to neutrophil stimulation by granulocyte colony stimulating factor
- Crohn's disease and ulcerative colitis are both associated with an increased risk of colon cancer
- Crohn's disease, ulcerative colitis, colon cancer and colon adenomas are all associated with similar alterations in mucosal glycosylation
- The alterations in glycosylation have functionally significant effects on the interaction between the colonic mucosa and intraluminal lectins

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**Fig. 4. The lectin-galactose hypothesis for the effect of diet on colon cancer risk.** Cell surface glycoproteins in premalignant colonic epithelium (eg in colonic polyps and inflammatory bowel disease) commonly express terminal galactose, which is usually concealed by sialylation or ester sulphation in normal human colon. This allows galactose-binding lectins of dietary or microbial origin to bind to the mucosa. These lectins are likely to have a mitogenic effect on the mucosal cells, but their binding – and hence their mitogenic effect – could be inhibited by galactose contained within certain fruit and vegetable (but not cereal) fibres (Gal = galactose; NAc = N-acetyl).

**Fig 5. A proposed explanation for how the inflammatory bowel disease and colon cancer 'jigsaw pieces' might fit together.**
isothiocyanates. This is further evidence to support the principle that the protective effects of diet against colon cancer are related to a few specific constituents rather than to a simple mechanical effect of fibre.

Inflammatory bowel disease and colon cancer: sticking the jigsaw puzzle pieces together

The main facts which constitute the IBD/cancer jigsaw puzzle can be summarised as shown in Table 1. How these jigsaw pieces should be stuck together is a matter for speculation, but the pieces are all statements of fact. The ultimate explanation for the pathogenesis of IBD and its related colon cancer risk will have to be able to account for them.

Figure 5 shows a possible model for linking these pieces together. More work is needed, not only to determine the correctness of this model but also to investigate further the enormous potential for biologically important interactions between the intestinal mucosa and intraluminal lectins of dietary or microbial origin.

The glycosylation changes found in IBD and colorectal cancer could turn out to be either genetic or acquired. In either case, they are functionally significant and present a plausible mechanism for the increased cancer risk by altering the functional relationship between the colonic epithelium and intraluminal lectins of dietary or microbial origin.

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