The role of intestinal glycosylation in determining individual responses to foods in inflammatory and neoplastic bowel diseases

JONATHAN RHODES

Department of Medicine, University of Liverpool, UK

Abstract
Purpose: To illustrate the hypothesis that alterations in mucosal glycosylation, particularly O-glycosylation, may result in altered interaction with carbohydrate-binding proteins (lectins) in the diet.

Design & methods: A summary of recent literature focusing on work by the author’s group demonstrating in vitro and in vivo lectin–epithelial interactions, particularly in the colonic epithelium.

Results: Similar alterations in O-glycosylation occur in the colonic epithelium in inflammatory diseases and in cancer. They include shortening of O-glycans and increased expression of onco-fetal carbohydrate antigens. Peanut lectin, which selectively binds the TF antigen, is shown to survive transit through the intestine and to cause significantly increased epithelial proliferation. Other lectins inhibit proliferation, e.g. edible mushroom lectin which becomes internalised and blocks nuclear-localising-sequence-dependent nuclear protein import.

Conclusions: Intestinal epithelial glycosylation is commonly altered in inflammation and cancer and we are only just beginning to understand the implications that this may have for interaction with carbohydrate-binding proteins that could originate from the diet, the gut microbiota or the host.

Key words: Colon cancer, lectins, glycobiology, inflammatory bowel disease, E. coli

Introduction

The mammalian intestine is coated with sugar—not sucrose of course—but the complex glycans that are present on secreted and cell surface glycoconjugates. In the colon there is a continuous coat of mucus made up largely of the secreted mucin, MUC2, that is heavily glycosylated. In the small intestine the mucus coat, although also dependent on secreted MUC2, is much patchier, probably to allow nutrient absorption, but in both the small and large intestine the luminal surface of the epithelial cells is covered with a ‘fuzzy coat’—the glycocalyx—that itself overlies the microvillous brush border [1]. This contains membrane-anchored glycoproteins that include members of the CEA (carcinoembryonic antigen) cell adhesion molecule family. The epithelial cell surface membrane itself also has many
transmembrane glycoproteins, e.g. MUC1, that are also heavily glycosylated, as well as glycolipids. This means that there is considerable potential for interaction between carbohydrate-binding proteins, so-called lectins that may be of dietary or microbial origin, and the secreted and cell membrane molecules that line the intestine. Many lectins of plant origin are tightly globular molecules that resist digestion in the mammalian intestine so have the potential to be biologically active even in the colon.

Most of the secreted and cell membrane glycoproteins are heavily glycosylated as a consequence of exuberant O-glycosylation, a process that takes place in the Golgi apparatus of epithelial cells. We have been interested in the alterations in this glycosylation that can be seen in inflammatory and neoplastic intestinal diseases. Similar glycosylation changes occur in inflammatory and neoplastic colonic diseases [2]. They are complex but include increased expression of oncofetal carbohydrate antigens. One of the commonest such antigens to be over-expressed is the Thomsen-Friedenreich (TF) antigen (galactose\(\beta\)1,3 Nacetylgalactosamine\(\alpha\)), a disaccharide that is O-linked to serine or threonine in glycoproteins and which is usually obscured by subsequent sialylation in the normal adult intestine [3]. The mechanism for its increased expression is unclear. The simplest explanation would be an alteration in the activities of the Golgi glycosyltransferases but there is little correlation between the glycosylation changes seen and the activity of these enzymes [4]. Alternatives include changes in molecular chaperones that are essential for normal glycosyltransferase function [5] or disorganisation of the Golgi. The latter has been reported in colon cancer [6] and can be induced in cell lines by agents such as bafilomycin that poison the Golgi membrane proton pump [7]. In the inflammatory bowel diseases, Crohn’s disease and ulcerative colitis, mucosal glycosylation changes can be seen even in unaffected identical twins and correlate with surface epithelial NF\(\kappa\)B activation, a subtle marker of inflammation that seems to precede histological inflammation [8].

Because of the common over-expression of the TF antigen in colonic disease we have explored particularly the effects of dietary lectins that have specific binding for TF. We have shown for example that ingestion of peanuts, which contain a TF-binding lectin, causes rectal mucosal proliferation in those individuals who express its receptor in their colonic epithelium [9] (Figure 1). Moreover, the lectin can even be identified in the peripheral blood after peanut ingestion [10]. Whether this matters is debatable but there is some evidence from case-control studies that increased peanut ingestion is associated with increased risk for colorectal cancer [11]. Peanut is, of course, a legume and all legumes tend to have a high content of lectins. The reliance on legumes as a protein source might possibly explain the probably normal (i.e. not reduced) risk for colorectal cancer in vegetarians [12]. To assess this further we conducted a case-control study of diet and colon cancer which supported the hypothesis that leafy green vegetables may be protective, possibly as a consequence of the relatively high content of galactose in their non-starch polysaccharides. In this study high consumption of legumes again associated with an increased risk for colon cancer [11] (Figure 2).

We have investigated the mechanisms by which TF-binding dietary lectins affect colonic epithelial proliferation and this has yielded some surprising results. Firstly, different lectins with similar binding affinities may have markedly different effects. Peanut and amaranth lectins stimulate proliferation whereas the lectins in common edible mushrooms and jackfruit inhibit proliferation, moreover the mechanisms by which they do this are very different. Peanut lectin binds to high molecular weight splice variants of the cell surface molecule CD44 that are overexpressed in neoplastic and inflammatory bowel disease and activates the MAPkinase signalling pathway [13]. It has its pro-proliferative effect without
any need for internalisation within the epithelial cell. The lectin from edible mushrooms, conversely, blocks cell-proliferation without cytotoxicity and does this by blocking one form of nuclear protein import (nuclear-localising-sequence-dependent) after the lectin has been internalised within the epithelial cell [14,15] (Figure 3). This lectin is much less robust than peanut lectin to heat or digestion however and it is uncertain whether these effects are relevant in vivo. Investigation of the anti-proliferative effect of another TF-binding lectin present in jackfruit (jacalin) has led to intriguing information about the mechanism by which MAPkinase pathways can be regulated within the cell. The lectin suppresses MAPkinase activation as a consequence of activation of an intracellular phosphatase [16]. It is quite likely that these dietary TF-binding lectins, all of which bind to glycans terminated by galactose, may be mimicking the effects of members of the naturally occurring galectin (galactose-binding lectin) family. Work into the functional effects of members of this family is still in its infancy but they are already looking likely to be very important in cancer cell biology [17]. We have recently shown that interaction between one member of the family, galectin3, and the TF disaccharide expressed on the transmembrane mucin, MUC1, on the membrane of colon cancer cells, results in polarisation of MUC1 towards one side of the cell thus revealing adhesion molecules which stick the cancer cell to the endothelium, a process that is probably very important in the development of cancer metastasis [18].

The altered mucosal glycosylation seen in inflammatory and neoplastic colonic disease also has the potential to cause altered mucosal recruitment of bacteria. We have shown that there is indeed altered mucosal recruitment, notably an increase in mucosa-associated adhesive E. coli, in Crohn’s disease and colon cancer [19] and this is supported by other studies [20–23]. The mechanisms for this recruitment are still being explored but we have shown that complex soluble oligosaccharides can inhibit this bacterial–epithelial interaction in vitro. Soluble plantain fibre has proved particularly effective and we are about to set up a controlled trial of plantain fibre in treatment of Crohn’s disease. We are also exploring the
Figure 2 (a and b). Odds ratios for colorectal cancer according to dietary factors; a case-control study of 6 months pre-illness food intake using a food-frequency questionnaire in patients with colorectal cancer and controls matched for age, sex and postcode. A weak positive association is shown with regular peanut consumption but, more importantly, the protective effect of fruit and vegetable fibre (but not cereal fibre) is shown to be statistically associated with its galactose content. This supports the hypothesis that high-galactose plant fibre might be particularly protective against colon cancer, perhaps by inhibition of lectin–carbohydrate interactions. A high intake of legumes, which tend to have a high lectin content, is shown in this study to associate with increased risk for colorectal cancer and might explain the relatively normal (rather than reduced) cancer risk in vegetarians. Broccoli was protective, as in other studies, but this is likely to be the result of other protective factors such as isothiocyanates. (NSP=non-starch polysaccharide.) From Evans et al., Gastroenterology 2002.

### a

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<thead>
<tr>
<th>Intake</th>
<th>Odds Ratio for colorectal cancer</th>
<th>Highest vs lowest quartile</th>
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<tbody>
<tr>
<td></td>
<td>All sites</td>
<td>Left-sided and rectal</td>
</tr>
<tr>
<td><strong>Regular peanut consumption</strong></td>
<td>1.37 (1.01, 1.85)</td>
<td>1.33 (0.95, 1.88)</td>
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<tr>
<td><strong>Total non-starch polysaccharide</strong></td>
<td>1.10 (0.75,1.60)</td>
<td>0.97 (0.60, 1.57)</td>
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<tr>
<td><strong>Fruit &amp; vegetable NSP</strong></td>
<td>1.01 (0.67,1.52)</td>
<td>1.32 (0.83, 2.11)</td>
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<tr>
<td><strong>Cereal NSP</strong></td>
<td>1.24 (0.84,1.83)</td>
<td>1.32 (0.85, 2.03)</td>
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Evans et al, Gastroenterology 2002

### b

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<td></td>
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<td>Left-sided and rectal</td>
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<tr>
<td><strong>NSP galactose</strong></td>
<td>0.67 (0.47, 0.95)</td>
<td>0.80 (0.53, 1.18)</td>
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<tr>
<td><strong>Non-legume green veg</strong></td>
<td>0.54 (0.35, 0.81)</td>
<td>0.63 (0.40, 1.01)</td>
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<tr>
<td><strong>Legumes</strong></td>
<td>1.61 (1.08, 2.39)</td>
<td>1.42 (0.94, 2.13)</td>
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<tr>
<td><strong>Broccoli</strong></td>
<td>0.67 (0.45, 1..00)</td>
<td><strong>0.61 (0.39, 0.96)</strong></td>
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Evans et al, Gastroenterology 2002
possibility that the mucosa-associated E. coli may be involved in colon cancer pathogenesis. If this proves to be the case this could then provide novel explanations for the substantial interactions between diet and colon cancer risk and then provide a much more scientific basis to inform dietary advice aimed at colon cancer prevention.

Conflict of interest statement:
The author, together with the University of Liverpool, has a patent application pending for the use of soluble plantain fibre in the treatment of Crohn’s disease.

References