Dietary Lectins and the Possible Mechanisms Whereby They Induce Intestinal Injury

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Diarrhea of infancy and early childhood has many causes, including viral and bacterial infection. In some of these conditions the intestine may demonstrate few if any histological changes (e.g., cholera) or may show profound alterations (e.g., Salmonella, reovirus, etc.). One of the more common reactions of the intestine to various insults is the development of the “flat villus” pattern (Table 1). In general, this type of reaction is characterized by increased crypt mitotic activity and some tendency for “less mature” villus cells. The presence of an increased density of immature villus cells facing the intestinal lumen may have two important pathophysiological effects that may contribute to a slower recovery or to the establishment of a chronic condition. One effect is the likelihood that the less mature villus cells would have less efficient absorbing and brush border hydrolytic activities; the second, acquired lactose intolerance, is used as an explanation for an increased sensitivity to milk. This second effect presumably reflects the fact that the less mature villus cell would have a different plasma membrane structure (1), particularly in regard to the composition of the oligosaccharide chains of the constituent glycoproteins and glycolipids. These structural alterations in cell surface carbohydrate may make the cell more susceptible to the effects of dietary lectins, substances present in a normal diet that bind glycoproteins. Nachbar and Oppenheim (1a) have recently enumerated the dietary lectins uncovered to date (Table 2). In this chapter we discuss the evidence for lectin-induced intestinal injury and four interrelated mechanisms that may explain it.

THESIS 1: BINDING

Food lectins, in a manner similar to bacterial toxins, require an available cell membrane receptor site before cytotoxicity ensues.

Perhaps we should have reworded the above hypothesis differently, since lectins were originally defined by their binding to specific carbohydrates, and bacterial toxins were only later shown to similarly do so. Cholera toxin has been particularly well studied. Its “nontoxic” subunit B is necessary for binding
TABLE 1. Some causes of a flat villus lesion

Gluten-sensitive enteropathy (celiac disease)
Dermatitis herpetiformis
Tropical sprue
Infectious enteritides
  Bacterial
  Viral
GIardiasis
Kwashiorkor
Eosinophilic gastroenteritis
Immunodeficiency syndromes
Collagenous or unclassified sprue
Bacterial overgrowth
Cow’s milk protein sensitivity
Soy protein enteropathy
Neomycin and other drug enteropathies
Radiation

to an intestinal cell before the toxic subunit A can enter (2). The binding subunit of cholera toxin binds most effectively to a glycolipid, GM₁ (Fig. 1). However, it is the sialic acid (NANA) and galactose (Gal) residues that are required for this binding (3). Furthermore, Richards et al. (4) have demonstrated that cholera toxin acts as a lectin in that it can induce agglutination of erythrocytes.

We were one of the first to provide evidence that plant lectins can bind and agglutinate isolated intestinal epithelial cells (5). Rat intestinal cells were prepared as isolated cells by a method described by Stern (6) and tested for agglutination in the presence of concanavalin A (Con A), a lectin derived from the jack bean, or wheat germ agglutinin (WGA). Only small intestinal cells, as contrasted with gastric and colonic cells, possessed a significant number of binding sites to demonstrate agglutination with WGA. This was later confirmed by Etzler and Branstrator (7) using immunofluorescent techniques. When human intestinal cells were tested, the most remarkable finding was the marked agglutination of human fetal intestinal cells by Con A (5) (Table 3). These agglutination reactions were inhibited by the specific sugars that characterize the competing site on the cell surface. The marked agglutination of fetal intestinal cells suggested that the less developed intestinal cell had different cell membrane glycoproteins, ones

TABLE 2. Vegetable lectins that agglutinate human erythrocytes

<table>
<thead>
<tr>
<th>Food source</th>
<th>Sugar inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tomato</td>
<td>N-Acetylgalactosamine</td>
</tr>
<tr>
<td>Potato</td>
<td>N-Acetylgalactosamine</td>
</tr>
<tr>
<td>Stringbeans</td>
<td>N-Acetylgalactosamine</td>
</tr>
<tr>
<td>Green peas</td>
<td>α-Methylmannoside</td>
</tr>
<tr>
<td>Soybean</td>
<td>N-Acetylgalactosamine</td>
</tr>
<tr>
<td>Lentils</td>
<td>α-Methylmannoside</td>
</tr>
</tbody>
</table>

Modified from Nachbar and Oppenheim (1), with permission.
that provided more binding sites for Con A. With rather simple logic, we felt that the less mature crypt cell should also exhibit more Con A binding sites. This was more difficult to confirm, but Podolsky and Weiser (8) using Con-A-derivatized nylon fibers, appeared to select cells from a mixture of crypt and villus cells that exhibited a high rate of $^3$H-thymidine incorporation.

At that time we also considered the possibility that gluten or gliadin, extracts of flour that contain the toxic factor that instigates relapses in celiac disease, may also be lectins for intestinal epithelial cells. Douglas (9) had demonstrated that a glycopeptide derived from gluten bound to intestinal cells of patients with celiac disease but not to cells of patients without celiac disease. It was not clear whether this glycopeptide possessed any agglutinating or cytotoxic properties. Unpublished data from our laboratory suggested that gluten may be a lectin. Furthermore, we were also able to demonstrate that the intestinal epithelial cell surface of patients with celiac disease had more sites for galactose incorporation, i.e., more available N-acetylglucosamine residues (10).

### TABLE 3. Agglutination of human intestinal epithelial cells by wheat germ agglutinin (WGA) and concanavalin A (Con A)

<table>
<thead>
<tr>
<th>Tissue source</th>
<th>Time (min)</th>
<th>WGA</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult small intestine</td>
<td>5</td>
<td>+</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>++</td>
<td>(-)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>++++</td>
<td>(-)</td>
</tr>
<tr>
<td>Fetal intestine</td>
<td>5</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>+</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>+</td>
<td>++++</td>
</tr>
</tbody>
</table>
On the basis of these data and many assumptions, we developed a hypothesis for a lectin-like gluten-induced injury in celiac disease (11) that requires binding to cell membrane receptors in villus cells of celiac patients, receptors that are not as readily available (or reduced) in normal patients. The nature of these receptors is unknown, but it was postulated that a less mature villus cell or immature crypt cell might normally possess these receptors, so that with chronic damage to the villus cell by gluten, and the attempt to replenish the injured mucosa by increasing cell turnover, more immature cells would be available for binding gluten. In this manner, an increasing cycle of greater susceptibility to gluten was envisioned (5).

Recently Kottgen et al. (12) demonstrated that the toxic fraction of gluten binds to oligomannosyl glycoproteins and not to the complex-type of glycoprotein. They also showed that rat intestinal crypt cell brush border membranes possessed more of this type glycoprotein than do villus cell brush border membranes. These workers, however, did not demonstrate that this specific gluten:glycoprotein binding could be demonstrated in patients or if such binding would lead to cytotoxic changes directly rather than by an immune-mediated process (13). We postulate that direct cytotoxicity by plant lectins, including gluten, is possible if they bind to the cell membrane, but the mechanism of cytotoxicity needs to be defined.

**THESIS 2: MEMBRANE PERTURBATION**

Food lectins that can induce cytotoxicity do so by virtue of the nature of their binding to cell plasma membrane, whereby they alter membrane structure and function.

This is in contrast to the concept of how most bacterial toxins work. With bacterial toxins there is a binding protomer and a toxic protomer, as in cholera and *E. coli* heat-labile enterotoxins. Entrance of the toxic protomer into the cell is required for toxicity since its toxicity is dependent on enzyme activities associated with the toxic fragment. In the case of cholera and *E. coli* heat-labile enterotoxin, these enzyme activities appear to be NAD glycohydrolase and ADP-ribosyltransferase activities; these two enzymes in combination activate adenylate cyclase through an NAD-dependent ADP-ribosylation of a component of the cyclase system (14). Thus, bacterial toxins appear to be made up of binding and toxic molecules, whereas plant lectins have only one protomer. It should be noted that there are exceptions to this generalization. For example, ricin, a very potent toxic lectin from castor beans, has two chains, a B chain, which binds to cell surfaces, and an A chain, which must enter the cell and inactivate ribosomes and thus protein synthesis (15).

The question remains, for those toxic plant lectins made up of only one protomer, how are cell injury and death produced? One answer may come from an observation of Li and Kornfeld (16). They studied the cytotoxicity of WGA on various cells in tissue culture. At concentrations close to the LD₅₀, they noted an inhibition of α-aminoisobutyric acid and cycloleucine transport but
no alteration in the transport of 2-deoxy-D-glucose and an enhanced uptake of colchicine. They took these observations to be evidence that WGA altered plasma membranes in such a manner as to impair a number of transport systems.

Histologic evidence in support of lectin perturbation of the plasma membranes of intestinal cells was recently presented by Lorenzsonn and Olsen (17). They studied the effect of orally administered Con A and WGA on rat intestines and observed marked effects on epithelial cell plasma membranes, particularly brush borders. These changes could be seen within 1 hr of administration, were specific for each lectin, i.e., dependent on sugar-specific binding to cell membrane, and in the early stages were similar to changes seen with antibody–plasma membrane antigen (antisucrase:sucrase) complex formation. These changes consisted of extensive shedding of plasmalemma as membrane vesicles, increased cell exfoliation, and shortened villi. At high lectin concentrations, where cell exfoliation and shortened villi were most prominent, there also appeared to be evidence for increased endocytosis of membrane.

Thus, according to theses 1 and 2, plant lectins toxic to a cell must bind and perturb membrane structure, resulting in altered cell function leading to cell injury. Host cell factors obviously become very important.

**THESIS 3: HOST GLYCOPROTEINS**

Host inheritable determinants of cell membrane glycoprotein and glycolipid structure may define the “avidity” of the cell membrane receptor sites of lectin binding and, hence, toxicity.

Cell membrane glycoproteins are synthesized by a rather complex process that is intimately dependent on maintenance of cell structure and membrane turnover. We emphasize the following features of glycoprotein synthesis, which may bear on susceptibility to and mechanism of lectin cytotoxicity. (a) Most cell plasma membrane proteins are glycosylated. These are usually of the complex-type carbohydrate chains, but many glycoproteins have both the complex and the high-mannose structures mixed into one glycoprotein (Fig. 2). An occasional glycoprotein may also have O-linked carbohydrate chains. (b) Synthesis of glycoproteins and their modification occur along the route of membrane synthesis and involve a number of discrete steps (Fig. 2). The core sugars are first assembled onto a lipid intermediate. This core is then transferred en bloc to an asparagine of a glycoprotein (18). At this point or earlier some oligosaccharides are phosphorylated at a mannose site. This phosphate, if not removed, prevents further glycosylation into the complex type; it also affects the function of lysosomes (19).

These early steps occur in the RER and result in a glucosylated high-mannose structure. The glucose are then rapidly removed, leaving a high-mannose structure. In the SER, specific mannosidases remove most but not all mannose residues (down to three) (20). This is required before SER and Golgi glycosyltransferases can add galactose, N-acetylgalcosamine, sialic acid, and fucose to make the complex carbohydrate structure typically associated with serum and
membrane glycoproteins. (c) These newly formed membrane glycoproteins may be inserted into the plasma membrane, where, if they are enzymes, they may be activated (21), or where they may function as growth receptors or receptors for endocytosis (22). (d) There is good evidence that membrane receptors, many of which (if not all) are glycoproteins, are reutilized by being put back on the plasma membrane. This pathway probably involves GERL, an acronym emphasizing the functional interrelationship of Golgi, endoplasmic reticulum, and lysosomes (23) (Fig. 3).

Thus, cell surface membrane glycoprotein differences expressed by HLA typing may indicate genetically determined differences in oligosaccharide sequences making up this glycoprotein. The association of certain HLA types with celiac disease may reflect altered immune status but also reflects a different glycoprotein structure on the cell surface (24). This difference in glycoprotein structure could be a determinant of the avidity of lectin binding to cell surfaces. Very minor changes may be significant. For example, loss of sialic acid on the hepatocyte membrane receptor for serum glycoproteins prevents binding and endocytosis of these glycoproteins (25).

Certain cells in tissue culture have been shown to be resistant to lectin cytotoxicity because of changes in glycoprotein structure (26,27). One, a mouse
FIG. 3. Intestinal plasma membrane synthesis: interrelationships of endocytosis and recycling.

melanoma cell, demonstrated an increase in a fucosyltransferase (26). Another, a mouse lymphoma cell line, was deficient in one of the glucosidases necessary for removing the glucose from the original core carbohydrate (27). Failure to phosphorylate a mannose in the glycoprotein synthesis pathway may alter lysosomal structure (19) and function. It is conceivable that lectins too tightly bound to glycoprotein receptors may trigger endocytosis but lead to failure of lysosomes to degrade certain lectins. This may produce cell malfunction, perhaps by clogging the GERL pathway.

THESIS 4: DIFFERENTIATION AND GLYCOPROTEINS

Incomplete differentiation of intestinal cells may result in altered cell membrane glycoprotein structures with increased "avidity" to cell membrane receptor sites for certain lectins and, hence, persistence of cytotoxicity, still greater number of immature cells, and worsening clinical conditions.

This latter thesis is concerned with chronicity and may pertain either to a genetic disorder, which celiac disease may represent, or to an infectious insult to the intestinal mucosa. Our data showing increased Con A binding sites on rat crypt cells (8) and of Con A agglutination of human fetal cells (5) would support this thesis, as would the findings of Kottgen et al. (12) that rat crypt cell brush border glycoproteins bind gluten to a much greater extent than do glycoproteins from villus brush border. DeRitis et al. (28) have also shown that the toxic fraction of a protease digest of gliadin specifically inhibited development and morphogenesis of small intestines from 17- and 18-day-old rat fetuses maintained in organ culture but had no effect on the intestine from a 21-day-old fetus.
Structural alterations of intestinal cell surface membrane glycoproteins based on their state of differentiation are supported by our work, which showed a gradient of glycoprotein synthesis from crypt to villus (1). Work by Quaroni et al. (29) has confirmed a gradient of glycoprotein differences between villus and crypt. They showed, however, that the major differences in patterns of radioactively labeled membrane glycoproteins were found when the luminal plasma membranes of villus cells, i.e., brush border membranes, were compared to those of crypt cells. Only minor differences were detected in comparing lateral-basal membranes of villus and crypt cells.

SUMMARY

Four theses have been presented that suggest possible mechanisms whereby dietary lectins might add to or initiate intestinal cell injury. Lectins are viewed to be similar to the binding protomers of bacterial enterotoxins in that there must be a specific receptor on the cell surface to which the lectin binds. The character of this receptor is that of a glycoprotein in which the specificity and avidity of binding are determined by the oligosaccharide chain of the membrane glycoprotein. This structure is, in part, genetically determined in a way similar to HLA; in fact, some lectin receptors may be HLA antigens. Cytotoxicity by lectins is probably a result of membrane perturbation with alterations in membrane function such as transport, but an enzyme associated with the lectin and internalized into the cell interior with effects on protein synthesis, etc., may also be a possibility for some lectins. Other mechanisms of cytotoxicity are suggested. Lectin injury may complicate or be complicated by bacterial or viral infections of the intestine. Any insult to the intestine may stimulate intestinal cell turnover with production of slightly less differentiated villus cells; these cells may be more susceptible to dietary lectin binding and cytotoxicity, and a cycle of increasing disease and chronicity may be established. Inversely, lectin injury may make the intestinal cell more susceptible to bacterial or enterotoxin binding, resulting in bacterial proliferation with increased enterotoxin production. These concepts should be considered in evaluating the pathophysiology of persistent infantile diarrhea.

REFERENCES


