

Elevated levels of serum antibodies to the lectin wheat germ agglutinin in celiac children lend support to the gluten-lectin theory of celiac disease

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Lectins recognize carbohydrate moieties of glycoproteins and glycolipids, and can elicit several biological effects, including cell agglutination, cell activation and mitogenesis. According to the gluten-lectin theory, celiac lesions represent a response to a toxic lectin, putatively wheat germ agglutinin (WGA). In this study we compared the serum antibody levels IgA, IgG and IgM to WGA and to gliadin in children under investigation for celiac disease (CD), as compared to reference children. We found that the levels of IgA and IgG to WGA as well as gliadin were significantly higher in celiac children on a gluten-containing diet, compared to children on gluten-free diet and reference children. These findings lend support to the concept that WGA is a biologically significant component of gluten. Since WGA can mimic the effects of epidermal growth factor (EGF) at the cellular level, we hypothesize that the crypt hyperplasia seen in celiac children could be due to a mitogenic response induced by WGA.

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Although the clinical picture of celiac disease (CD) has been well known for more than a century, it is not exactly known how the ingestion of gluten causes disease. At least four major theories have been implicated: an enzyme deficiency causing incomplete digestion of gluten, an abnormal glycosylation facilitating a lectin-like interaction between gluten and the enterocytes, a genetic constitution predisposing for an abnormal immune response or environmental factors, such as infection with type 12 adenovirus (1). During the last few years, the genetic model has attracted much interest and the basis for CD has been described as a cell-mediated immune reaction to gluten peptides (2–4). However, some animal studies have also put emphasis on the gluten-lectin theory, originally proposed by Weiser & Douglas (5). A lectin is a carbohydrate-binding protein or glycoprotein of non-immune origin, which can agglutinate cells and/or precipitate glycoconjugates (6), but also elicit a number of other biological responses. Each lectin has its own sugar specificity, and the reaction can be inhibited by the corre-

sponding carbohydrate (7). According to the gluten-lectin theory, gluten acts as a lectin and causes cytotoxicity after binding to the carbohydrate moieties of the enterocyte surface membrane.

The lectin activity of gluten has been identified as wheat germ agglutinin, WGA (8), and elevated levels of antibodies to WGA have previously been reported in sera from 13 celiac patients (9). These antibodies did not cross-react with other gluten antigens (9). The best inhibitor of WGA binding has been shown to be chitobiose, *i.e.* poly-N-acetylglucosamine (7), and addition of this sugar has been used to prevent unspecific binding of WGA (8).

Animal studies have shown morphological changes of small intestinal mucosa after WGA exposure (10, 11). Other studies have focused on the interaction between WGA and the epidermal growth factor, EGF, which regulates the proliferative response of the intestinal epithelial cells of the crypts (12). The EGF receptor is a glycoprotein which is recognized by WGA, and WGA interaction initiates strong autophosphorylation of the receptor (13).

Thus, WGA might provide a clue also to the morphological aberrations seen in celiac disease such as crypt hyperplasia and shortening of the microvilli by mimicking the effects of EGF.

WGA may also directly cause an inflammatory response in the mucosa, since treatment with WGA causes oxidative metabolism and free radical formation in granulocytes (14). Indirectly, local immune complex formation between for instance IgG and WGA may elicit and/or enhance a similar response.

The aim of this study was to assess the levels of antibodies IgG, IgA and IgM towards WGA and gliadin in celiac children compared to reference children using an enzyme-linked immunosorbent assay (ELISA). The impact of gluten-free diet was also studied.

Methods

Patients

Four groups of patients were studied. Blood was taken on the same day as the intestinal biopsy from a total of 167 children, between the age of 7 months and 17.9 years. All biopsies were performed to exclude or verify a suspicion of CD, or to follow-up the effect of gluten-free diet or gluten challenge according to the ESPGAN (European Society for Gastroenterology and Nutrition) criteria (15), and were obtained by suction biopsy under fluoroscopic guidance at, or distally to the ligament of Treitz. The histopathology was classified according to the Alexander classification (16), where a normal intestinal mucosa is classified as grade I, and grades II–IV indicate increasing severity of the lesion with grade III–IV suggesting gluten intolerance.

The group classification was:

- a) At presentation (group AP). In the first biopsy, 40 children turned out to have a mucosa which suggested CD (Alexander grade III–IV).
- b) After gluten-free diet (group GFD). Thirty-seven samples were obtained from children who had been on a gluten-free diet (GFD) for at least 6 months, and all the mucosal specimens were classified as normal.
- c) After gluten challenge (group GC). Thirty-two samples were obtained from children after gluten challenge for a median time of 4 months (range 2–104 months), and the histopathologic judgment of these specimens was Alexander grade III or IV.
- d) Reference children (group R). In 58 children who were admitted because of signs and symptoms compatible with CD, e.g. poor weight gain and/or diarrhoea, the mucosal specimens were classified as normal or nearly normal. They were considered as not having CD, and they will be referred to as reference children in this study.

Further follow-up of the children in the groups AP and GFD was performed after the completion of the present study and all children were classified as celiacs according to the original ESPGAN criteria (15).

ELISA analysis

We employed a slight modification (17) of the original ELISA procedure. After heat inactivation of the serum samples for 30 min at 56°C, they were run in triplicates, and the median value was used for calculation. The sera were diluted 1:25, and analyzed in 96-well micro-titer plates from Haegar Plastics (Oslo, Norway).

Gliadin antibody analyses. Gliadin (G 3375) was obtained from Sigma Chemical Co (St. Louis, MO). Conjugated antisera were horse-radish peroxidase (HRP)-labelled antihuman immunoglobulins from Dakopatts AB (Stockholm, Sweden), viz. anti-IgG (P 214), anti-IgA (P 216) and anti-IgM (P 215). As a positive control in each plate we used serum from a volunteer with measurable levels of all antibody classes analyzed. Negative controls were obtained from sera without detectable antibody levels, and PBS (phosphate-buffered saline). The absorbance, i.e. optical density (O.D.) was read at 492 nm with a Titertec Multiscan reader (Eflab Oy, Helsinki, Finland). The O.D. values were assumed to reflect concentrations of antibodies in arbitrary units, AU.

WGA antibody analyses. The lectin antigen was obtained from Boehringer Mannheim GmbH (Mannheim, Germany) and diluted to 25 µg/ml in PBS. The samples were run as described for anti-gliadin analysis. Three samples from each patient were also run after addition of 10 mM N-acetylglucosamine (Sigma Chemical Co., St. Louis, MO) to ascertain that the ELISA assay did not reflect solely binding of the antibody as a glycoprotein to the surface-associated lectin. Results are shown without subtraction of the background, which was $\approx .2$ AU/ml for anti-IgG and anti-IgM and $\approx .05$ for anti-IgA.

Ethics and statistics

Informed consent was given by the parents of the participating children, and the study was approved by the Human Research Ethics Committee at the Faculty of Health Sciences, Linköping University. For evaluation of differences between the groups, the Mann-Whitney U-test was used. Correlation analysis was performed by paired coefficients of correlation. In the Figures, the mean and standard error of the mean (SEM) values are depicted.

Table 1. Correlation between anti-gliadin and anti-WGA antibodies measured in the same blood sample ($n = 167$), with and without addition of 10 mM N-acetylglucosamine

Antibody	Correlation	
	without sugar	with sugar
IgA	0.64	0.67
IgG	0.70	0.74
IgM	0.10	0.32

Results

Effect of sugar addition

The addition of 10 mM N-acetylglucosamine decreased the antibody levels with 5.1% for IgG-anti-WGA and 4.7% for IgA-anti-WGA. This indicates that only a minor part of the ELISA-values might reflect lectin to antibody-carbohydrate interaction. For IgM-anti-WGA, the addition of sugar increased the apparent antibody level by 28%. Although this difference seems significant, the basal values of IgM-anti-WGA were in all instances low and close to the background.

The coefficient of correlation between analyses of the same sample with and without sugar addition was .98 for IgG-anti-WGA, .99 for IgA-anti-WGA and .96 for IgM-anti-WGA.

However, to avoid even low but unwanted interference of lectin-carbohydrate interaction, only absorbance values obtained with 10 mM N-acetylglucosamine addition are shown in the figures.

Correlation between levels of antibodies to WGA and gliadin

In order to be able to evaluate the clinical impact of anti-WGA antibodies, a measurement of anti-gliadin antibodies was run in parallel (Table 1). There was a reasonable good correlation for IgG and IgA antibodies, but less so for IgM (Table 1).

Antibody levels and differences between the groups

To assess the variation and possible clinical significance of antibodies to WGA in children being under investigation for CD, groups of children were studied at presentation (AP), after gluten-free diet (GFD), and after challenge with gluten (GC), and compared with reference children (R).

The levels of anti-WGA and anti-gliadin antibodies are shown in Fig. 1 for IgG and Fig. 2 for IgA. There is a similar variation between the groups with regard to IgG and IgA antibodies to either antigen (gliadin or WGA). IgM antibodies to gliadin and WGA did not differ significantly between any of the groups.

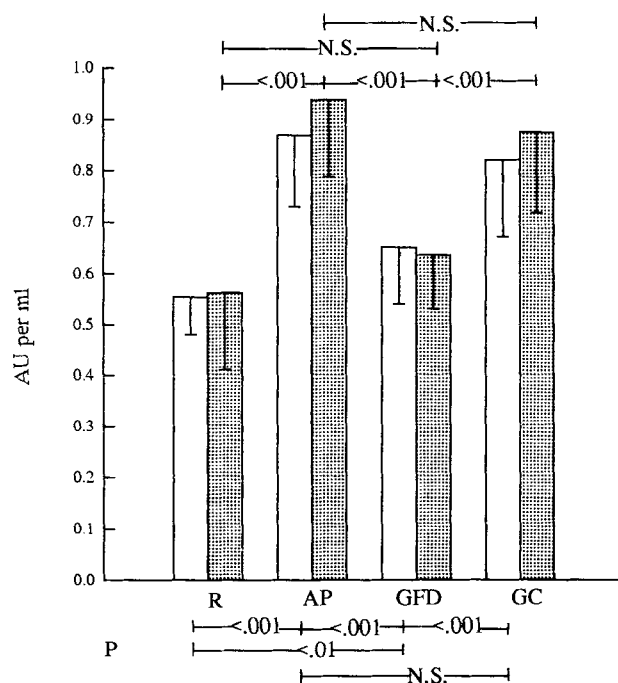


Fig. 1. Serum antibody IgG levels, mean values $\pm$ SEM, in arbitrary units, AU per ml, to wheat germ agglutinin, WGA; (open bars) and gliadin (closed bars). The WGA measurements were performed after addition of 10 mM N-acetylglucosamine. The groups were: R (reference, non-celiac children; $n = 58$), AP (celiac children at presentation; $n = 40$), GFD (celiac children on gluten-free diet; $n = 37$), GC (celiac children after gluten challenge; $n = 32$). P indicates statistically significant differences between the groups connected with lines: for gliadin antibodies on top of the figure and for WGA antibodies at the bottom.

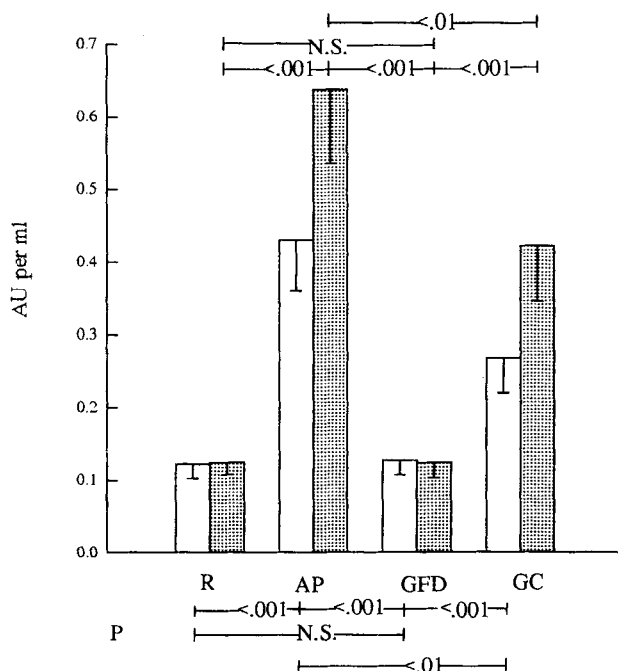


Fig. 2. Serum antibody IgA levels, mean values $\pm$ SEM, in arbitrary units, AU per ml, to wheat germ agglutinin, WGA; (open bars) and gliadin (closed bars). The WGA measurements were performed after addition of 10 mM N-acetylglucosamine. For the naming of the groups, please see text in Fig. 1.

Discussion

The "gluten-lectin theory" for how gluten exerts its effect on the small intestine has gained considerable support from animal studies. After intraluminal administration of the lectins concanavalin A or WGA in the rat intestine, extensive changes of enterocyte height, villus length and microvillus organization have been noted (18). It was also shown that the lectin WGA can cause mucosal membrane permeability changes, and achieve disarrangement of the cytoskeleton and shortening of microvilli, resembling the early changes of CD (10, 11, 19, 20). Combined feeding of rats with gliadin and swainsonine, a mannosidase inhibitor, disturbed the enterocytic maturation, presumably caused by increased binding of gliadin to enterocytic glycoproteins (21). Alternatively, the carbohydrate composition of for instance the EGF receptor (12) might have been disturbed and therefore also the cell proliferation. Several studies (13, 22, 23) have shown that EGF induces cross-linking and auto-phosphorylation of the receptor by activation of tyrosinkinase activity, and WGA interaction can also cause auto-phosphorylation of the receptor (13). Furthermore, feeding rats lectins from the red kidney bean caused disruption of the microvilli (24). Incubation of extracts of a plant lectin, *Phaseolus vulgaris*, in pig jejunal mucosa in organ culture caused a reduction of the mean enterocyte height, a reduction in the villus: crypt ratio and shortening of the microvilli (25). Similar results have been found in *in vitro* studies of cell lines of human origin. Incubation of WGA to the cell line Intestine 407 caused depolymerization of the filamentous (F-)actin structure of the cells (20). This effect was probably mediated by release of Ca^{2+} from intracellular stores, due to the formation of inositol-tri-phosphate (IP3) (19). Incidentally, a protective effect of mannan and oligomers of N-acetylglucosamine has been shown against the toxic effect of wheat flour and other cereals in a system using the cell line K 562(S) (26). Moreover, polyamines such as spermin and spermidine also displayed a protecting effect on this cell line against the toxic effect of gliadin peptides, albeit cell agglutination induced by the lectins WGA and concanavalin A was not prevented by the polyamines (27). Also cultured human small-intestinal mucosa from celiac patients with active disease was shielded by mannan and oligomers of N-acetyl-glycosamine (26). In studies of cultured mucosal specimens from both celiac and reference children, a positive staining for WGA was noted in celiac children with active disease, while no staining was observed in control mucosae (28). This may indicate that more immature crypt cells, forming the major epithelium of the

celiac mucosa, were more rich in WGA-binding glycoconjugates.

In concordance with these studies indicating a role of the lectin WGA in CD, we have found very high levels of serum antibodies IgA and IgG to WGA in celiac children on a gluten-containing diet. We also observed that the WGA-antibodies decreased after institution of a gluten-free diet. This decrease may have at least two explanations. First, avoidance of exposure to a food allergen will cause a reduction of the corresponding antibody level (29). Second, the healing of the gut will give a decreased total uptake of intact antigen, since the antibody levels to other foods, unrestricted in the gluten-free diet, has been shown to decrease significantly after institution of the gluten-free diet (30). However, after gluten challenge which caused a relapse of the gut lesion, the antibodies to WGA rose significantly, as was previously observed for gliadin antibodies (30), but not for other food antigens, *viz.* betalactoglobulin and ovalbumin (30). Thus, the levels of antibodies towards WGA and gliadin showed similar reactions to gluten exposition and withdrawal, but the coefficient of correlation between them was not 100%, substantiating previous reports that the WGA antibodies do not crossreact with gluten antigens (9). The effect of addition of the sugar to inhibit unspecific binding was minor (approximately 5%).

The observed elevated levels of antibodies towards WGA in parallel with antibodies to gliadin in celiac children at presentation and during gluten challenge in this study may imply that the lectin WGA can have a causative role in the development of CD, as suggested by the results obtained in organ culture studies from several species. It may also reflect that there is an exaggerated response in celiac children to WGA in gluten-containing foods during phases of active disease with a structurally and functionally diseased small intestinal mucosa. Since WGA can mimic the effects of EGF (12) it is tempting to hypothesize that the crypt cell hyperplasia seen in CD is a mitogenic response. Further, WGA can directly stimulate an oxidative burst in granulocytes (14) or possibly indirectly via IgG-WGA immune complex formation in the mucosa do so. Therefore, several of the ingredients in a local inflammatory process are also present.

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