Wheat deficient in gliadins: promising tool for treatment of coeliac disease

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Abstract

The toxicity of two varieties of bread wheat, one poor in alpha and beta gliadins and the other poor in alpha, beta, gamma, and omega gliadins, has been tested. The peptic-tryptic digest of these wheats was assessed using coeliac mucosa in an in vitro organ culture system. A significantly lower toxicity was found in respect of bread wheat containing all gliadin fractions. These results suggest new opportunities for the treatment of coeliac disease.

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Coeliac disease is a gluten induced enteropathy and its treatment is based on lifelong withdrawal of gluten from the diet.[1] Although strict adherence to a gluten free diet is mandatory for both intestinal mucosal recovery and prevention of complicating conditions such as lymphoma,[2] refractory sprue,[3] hyposplenism,[4] dietary compliance has been shown to be poor in most patients.[5–12] This may be caused by inadvertent gluten consumption, to social and educational problems, but also to the unpleasant taste of some gluten free foods.[13] New strategies to improve adherence to the diet are therefore necessary, and one might be that of breeding varieties of wheat lacking the toxic components of gluten – that is, gliadins.[14] Gliadins are an heterogeneous class of proteins whose genetic aspects have been clarified. Loci encoding gliadin components designated Gli-A1, Gli-B1, Gli-D1 (Gli-1 loci), Gli-A2, Gli-B2, and Gli-D2 (Gli-2 loci), are located at the end of the short arm of the chromosomes of groups 1 and 6 of wheat.[15] Gli-1 loci correspond to a family of associated genes that encode for omega and gamma gliadins; Gli-2 loci encode for alpha and beta components. Electrophoretic analyses have shown the existence, in durum and bread wheat, of genotypes lacking an entire cluster of gliadin components controlled by genes at a given complex locus.[16] Crosses between these lines have also resulted in production of lines lacking gliadin components controlled by two or more complex loci.[17] We have tested the toxicity of lines of bread wheats lacking all the Gli-A2 encoded gliadin components and a line lacking simultaneously Gli-D1 and Gli-A2 controlled gliadin components, using an in vitro model of coeliac disease.[18]

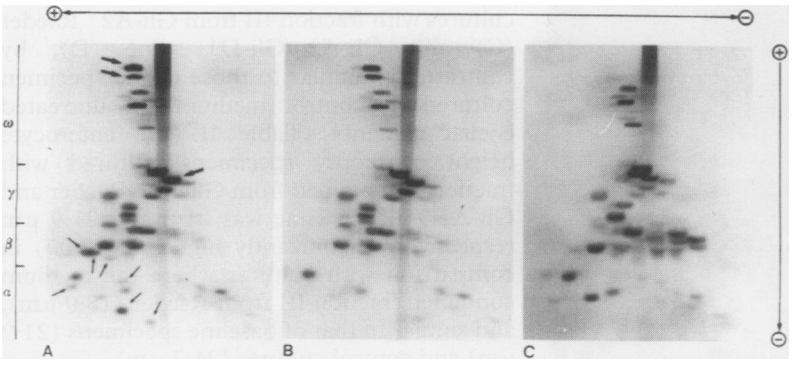

Figure 1: Two dimensional electrophoretic pattern of gliadins from cultivar Raeder (A), its variant lacking the Gli-A2 encoded gliadin components (B), and a line lacking both the Gli-A2 and the Gli-D1 encoded gliadin components (C). In the gliadin pattern of cultivar Raeder the components encoded by Gli-A2 and Gli-D1 loci are shown by small and large arrows, respectively.

Methods

PATIENTS

Ten adult patients with untreated coeliac disease, 11 with coeliac disease receiving a strict gluten free diet (mean (SD) diet duration 23 (16) months), and 10 subjects who had an intestinal biopsy that excluded any intestinal disease took part in the study. In all subjects multiple biopsy specimens were obtained from the distal duodenum during upper gastrointestinal endoscopy and for each case two specimens were processed for conventional histological examination and the remaining ones put in organ culture as explained below. Histological examination showed flat mucosa in all untreated coeliac patients, partial flat mucosa in eight and normal mucosa in three treated coeliac patients, and normal mucosa in the biopsied controls.

ORGAN CULTURE

The biopsy specimens were delivered in ice cold culture medium (composition: 6 ml Trowell's T8 medium, 2 ml NCTC 135 medium, 0·2 ml glutamine 200 mM (Flow Laboratories, Irvine, Scotland), 0·1 ml 1 M HEPES buffer (Gibco BRL, Life Technologies, Paisley, UK), 1000 U penicillin, 1000 U streptomycin (Sigma Chemical, St Louis, USA), and 1·5 ml fetal calf serum (Wellcome Research, Beckenham, UK).

Each specimen was mounted villous surface uppermost on the wire grid of a sterile organ culture dish (Falcon Plastics, Los Angeles, California), and the central well was filled with roughly 1 ml of culture medium, with and without the addition of antigen as explained later, so as to touch the under surface of the

TABLE I *Treated coeliac patients: enterocyte height (μm)*

Patient No	Baseline	Culture time 24 hours			
		Control	With Raeder	With Gli-A2⁻ Raeder	With Gli-A2⁻/Gli-D1⁻ line
1	30·7	28·9	22·3	ND	31·0
2	32·6	31·9	28·8	33·2	ND
3	31·7	27·2	22·3	28·0	25·7
4	31·6	30·1	18·1	25·9	26·9
5	32·7	26·3	21·9	28·0	ND
6	32·2	30·4	26·7	34·1	ND
7	29·4	27·0	25·0	ND	27·2
8	31·3	29·1	25·6	ND	27·9
9	32·8	30·1	20·7	31·4	27·6
10	31·3	26·7	24·3	ND	28·0
11	33·7	33·8	25·5	37·7	27·7
Mean (SD)	31·8 (1·2)	29·2 (2·3)	23·7 (3·0)	31·2 (4·1)	27·7 (1·5)

ND=not done.

specimen. The outer well of the culture dish contained NaCl solution. The dishes were placed in a sterile anaerobic jar, which was gassed with 95% oxygen/5% carbon dioxide for 30 minutes, then sealed and kept in an incubator at 37°C for 24 hours.

ANTIGENS

Seeds lacking Gli-A2 encoded gliadin components (Gli-A2⁻) were found by analysing single kernels of the soft bread wheat cultivar Raeder using a two dimensional electrophoretic technique.[19] Gli-A2⁻ seeds (deficient in alpha and beta gliadins) were detected along with seeds possessing a normal gliadin pattern. Analyses were carried out on half seeds preserving the embryo part, which was grown to obtain increased amount of Gli-A2⁻ Raeder. Production of the Gli-A2⁻/Gli-D1⁻ line (deficient in alpha, beta, gamma, and omega gliadins) has been described elsewhere.[17]

Specimens from all patients and controls were cultured in control medium – that is, medium without any added antigen – and in antigen containing media – that is, with the addition of 1 mg/ml of fraction III[20] obtained from cultivar Raeder, Gli-A2⁻ Raeder, and Gli-A2⁻/Gli-D1⁻ line (Fig 1), respectively.

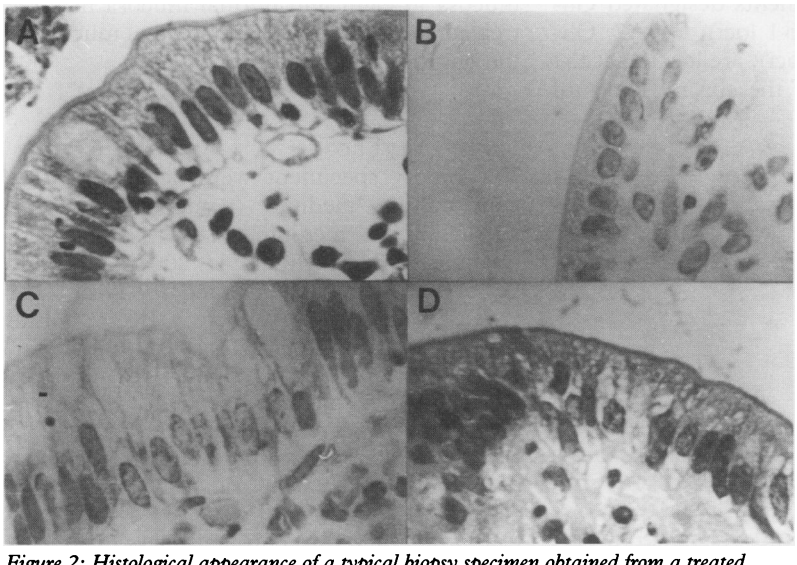

Figure 2: Histological appearance of a typical biopsy specimen obtained from a treated patient after culture with and without antigen. After culture in control medium (A), after culture with Raeder fraction III (B), after culture with fraction III from Gli-A2⁻ Raeder (C), after culture with fraction III from Gli-A2⁻/Gli-D1⁻ line (D).

MORPHOMETRY

Just after 24 hours in culture, biopsy specimens were removed, fixed in 10% formol, dehydrated, embedded in paraffin wax, and serially sectioned.

Changes in enterocyte height during culture were measured according to Howdle *et al*[21] by an observer unaware of the culture conditions and used as a marker of toxicity. The measured enterocytes were on the lateral upper third of the villi or, when villi were absent, on the surface of the mucosa far from the openings of the crypts. At least 20 enterocytes were measured in each section and the mean enterocyte height was calculated for each section.

STATISTICS

The results were evaluated by the Student's *t* test for paired data.

Results

Table I gives results obtained in treated coeliac patients. Mean enterocyte height was 31·8 μm before culture and 29·2 μm after culture in control medium; this difference is significant ($p<0·01$), is in keeping with previous findings,[21] and reflects the in vitro environment. The mean enterocyte height of specimens cultured in the presence of fraction III from Raeder wheat was 23·7 μm, significantly lower in comparison with baseline specimens and specimens cultured in control medium ($p<0·001$ for both comparisons). The mean enterocyte height of specimens cultured with fraction III obtained from Gli-A2⁻ Raeder and the Gli-A2⁻/Gli-D1⁻ line was 31·2 and 27·7 μm, respectively, similar to that of specimens cultured in the absence of any antigen and significantly higher ($p<0·001$ and $p<0·005$, respectively) than in specimens cultured with fraction III from cultivar Raeder.

Figure 2 shows the histological appearance of a typical biopsy specimen obtained from a treated patient after culture with and without antigen. After culture in control medium (A) the epithelial layer is regular, enterocytes are tall with polar nuclei. In specimens cultured with fraction III from Raeder (B) there is obvious morphological deterioration, and in particular the enterocytes are irregular and of strikingly reduced height. The enterocytes of cultures with fraction III from Gli-A2⁻ Raeder (C) and Gli-A2⁻/Gli-D1⁻ line (D), by contrast, are similar to those of the specimen cultured in control medium. In untreated coeliac patients (Table II) the enterocyte height of biopsy specimens cultured with fraction III obtained from Gli-A2⁻ Raeder and Gli-A2⁻/Gli-D1⁻ line was 21·8 and 21·9 μm respectively, significantly higher ($p<0·05$) in comparison with cultures where the medium contained fraction III from Raeder (18·9 μm), and similar to that of baseline specimens (21·9 μm) and control cultures (21·7 μm).

In biopsied controls (Table III) a significant variation of enterocyte height was seen comparing baseline specimens to those cultured with control medium (33·1 *v* 30·0 μm,

TABLE II *Untreated coeliac patients: enterocyte height (μm)*

Patient No	Baseline	Culture time 24 hours			
		Control	With Raeder	With Gli-A2⁻ Raeder	With Gli-A2⁻/Gli-D1⁻ line
1	21·9	25·4	19·6	25·2	ND
2	24·1	24·9	23·0	21·9	24·0
3	19·0	21·2	20·3	ND	21·9
4	21·5	22·4	18·0	21·8	25·7
5	20·8	23·1	20·5	22·8	ND
6	25·6	21·0	ND	24·3	18·8
7	26·3	21·9	ND	22·0	21·9
8	22·3	ND	19·0	23·2	20·8
9	19·9	19·2	17·1	18·0	ND
10	17·7	15·9	13·8	17·1	20·2
Mean (SD)	21·9 (2·8)	21·7 (2·9)	18·9 (2·7)	21·8 (2·7)	21·9 (2·3)

ND=not done.

$p<0·001$). Cultures in the presence of any of the three antigens tested did not differ significantly from those with control medium.

Discussion

Gliadins are the components of wheat gluten that are toxic to coeliac mucosa.[22] On the basis of electrophoretic mobility four gliadin fractions have been recognised and named alpha, beta, gamma, and omega, respectively.[23] Although toxicity has been reported for all the four gliadin components,[24 25] the evidence favours the view of a decreasing toxicity going from alpha to omega gliadins.[26 27] Treatment of coeliac disease is based on lifelong withdrawal of gluten containing foods. Unfortunately, compliance to gluten free diet has been shown to be poor in many patients,[5–12] also because of the poor palatability and high costs of some commercially available gluten deprived foods. These problems underline the need for wheat varieties that are naturally poor of gliadin components.

Previous experience with gliadin deficient wheat consisted of feeding experiments with flour derived from a wheat line lacking the Gli-A2 locus on chromosome 6A.[28] Bread wheats in which the 6A chromosomes were missing and compensated for by two extra chromosomes, either 6B or 6D (nulli-tetra wheats), were proposed as non-toxic in coeliac disease, but eventually they were found to be toxic and difficult to produce.[29] The wheats we have used lack the gliadin components encoded by the Gli-A2 complex locus as the nulli-tetra wheats,[28 29] but they differ from them because they do not have any increase in the amount of the alpha and beta gliadins coded for by the Gli-B2 or Gli-D2 genes carried by chromosomes 6B or 6D. These wheats have the normal set of chromosomes and because of this they are also easy to produce.

To assess whether these wheat lines were or were not toxic for coeliac patients we have used the organ culture technique.[18] This in vitro system permits direct challenge of specimens of intestinal mucosa with the putative toxic agent and has been used successfully in studies of gluten toxicity.[18 21 24] These experiments have shown that 1 mg/ml of a peptic-tryptic digest of wheat flour (fraction III)[20] is harmful to coeliac mucosa but not to normal mucosa.[21] In this study we have compared the toxicity of equal concentrations of fraction III from cultivar Raeder, that contains all gliadin fractions, to that of Gli-A2⁻ Raeder, which is considerably deficient in alpha and beta gliadins, and of the Gli-A2⁻/Gli-D1⁻ line, which is partially deficient in alpha, beta, gamma, and omega gliadins. We have used enterocyte height as a marker of toxicity[21] and by this morphometric method we have shown that the peptic-tryptic digest from cultivar Raeder is harmful to untreated and treated coeliac mucosa in vitro while these new varieties are not.

The toxicity of Raeder gliadin digest that we have seen in untreated coeliac mucosa confirms previous findings[18 21 24] while our results in treated mucosa are in keeping with those of Howdle *et al*[21 24] but in contrast with Falchuck *et al.*[18] There are two possible explanations for this discrepancy. Firstly, in our treated patients the baseline enterocyte height was lower than in controls suggesting that patients, although treated, had not reached full morphological remission. Secondly, different techniques have been used for the assessment of mucosal damage: morphometry by Howdle and by us, changes in brush border enzyme activity by Falchuck, which, however, have been shown to be unreliable for this purpose.[30]

As far as the wheat lines that we have produced and assayed are concerned, they are gliadin deficient but still contain gliadin components. The lack of toxicity that we have seen in vitro, then, may depend on the lower gliadin content of 1 mg/ml of fraction III obtained from Gli-A2⁻ Raeder and Gli-A2⁻/Gli-D1⁻ line in comparison with the same concentration of fraction III from cultivar Raeder. The in vitro toxicity of 1 mg/ml of fraction III has been shown to be comparable with that of 100 μg/ml alpha gliadin,[24] and our antigens were in fact mostly deficient in alpha gliadin. Therefore, confirming a previous study[24] we have shown that low concentrations of alpha gliadin do not have in vitro toxicity. It is possible, however, that using higher concentrations of fraction III from the tested wheat lines some toxicity might develop. Moreover, although the organ culture technique is widely accepted as an in vitro model of coeliac disease, it mirrors mucosal changes taking place within 24 hours of challenge with a given concentration of antigen. It cannot be excluded that daily exposure to

TABLE III *Biopsied controls: enterocyte height (μm)*

Patient No	Baseline	Culture time 24 hours			
		Control	With Raeder	With Gli-A2⁻ Raeder	With Gli-A2⁻/Gli-D1⁻ line
1	30·2	28·6	25·1	27·8	ND
2	32·8	34·0	31·4	32·6	ND
3	32·5	28·8	30·3	ND	27·9
4	33·1	27·7	ND	26·5	28·2
5	33·3	31·2	31·7	30·8	29·5
6	34·3	31·3	31·0	ND	31·6
7	37·2	29·7	32·2	ND	32·8
8	34·8	30·9	34·3	30·5	27·7
9	32·5	ND	30·5	31·4	ND
10	30·0	28·2	29·1	29·3	28·6
Mean (SD)	33·1 (2·1)	30·0 (2·0)	30·6 (2·5)	29·8 (2·1)	29·5 (2·0)

ND=not done.

flour obtained from the gliadin deficient wheats that we have tested in vitro may cause in vivo mucosal damage in coeliac patients.

In conclusion, we believe that our results open new perspectives in the treatment of coeliac disease. This approach, preliminary to careful in vivo feeding studies, seems particularly promising as we are already breeding further generations of wheat lines more and more deficient in toxic gliadins.

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