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αB-Crystallin Expression in Celiac Disease – A Preliminary Study

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Aim. To investigate immunohistochemically αB-crystallin expression and distribution in biopsy sections of the small intestine of patients with celiac disease, and the relationship, if there is any, between the expression of this heat shock protein and the degree of mucosal atrophy.

Methods. Biopsy specimens of 22 subjects (12 celiac patients and 10 "normal" individuals) were investigated by immunoperoxidase screening procedures using monoclonal antibodies to detect the presence of αB-crystallin. Sections from each case were stained with hematoxylin and eosin to assess mucosal morphology.

Results. The epithelial cells of the small intestine stained more intensively for αB-crystallin in patients with celiac disease than in "normal" patients. The quantity and degree of intensity of immunohistochemically demonstrable αB-crystallin and its intracellular distribution in the duodenal mucosa of the patients with celiac disease were closely related to the degree of villus atrophy. There was a strong correlation between the number of patients with celiac disease and percentage staining score of αB-crystallin per total score (Pearson's $r=0.754$, $p=0.123$). The mean percentage score per total score was 20% among the patients with celiac disease. On the other hand, there was a weak negative correlation between the number of normal controls and percentage score per total score (Pearson's $r=-0.126$, $p=0.437$). The mean percentage score per total score was 4.6% among the normal controls. αB-crystallin stained the supra-nuclear region of the enterocytes with a 75% sensitivity and 71% specificity.

Conclusion. αB-crystallin, a stress response protein, is expressed in the small intestinal mucosa of patients with celiac disease, and the degree of mucosal atrophy compares significantly with the intensity of expression of αB-crystallin. We therefore conclude that an inappropriate stress response involving this heat-shock protein within the mucosa itself may be crucial as an initiating event in the architectural derangement of the mucosal damage associated with celiac disease.

Key words: *autoimmune diseases; autophagocytosis; atrophy; celiac disease; crystallins; heat shock proteins; intestinal mucosa*

Celiac disease is described under many synonyms, including adult celiac syndrome, non-tropical sprue, gluten-induced enteropathy, etc. It has been defined as "the condition in which there is an abnormal jejunal mucosa that responds morphologically to treatment with gluten free diet" (1). The disease is associated with intestinal lesion that causes architectural derangement of the mucosa in the form of villous atrophy, increased crypt length, and increased volume of the lamina propria (2). The disease, like most malabsorption disorders, is characterized by severe steatorrhea, muscular weakness, failure to thrive, loss of weight, ataxia, generalized hypotonia, and abdominal distension with the passage of soft, bulky, offensive stools (3,4). It commonly begins in childhood, but sometimes it is not evident before adolescence or early adult life. The disease is believed to be caused by (enterotoxic) α-gliadin, a protein component of cereals like wheat, barley, rye, and so forth (5).

A structural protein αB-crystallin is a major component of the eye lens, but it is also found in many extralenticular tissues. αB-crystallin is a highly conserved 20 kDa heat shock protein capable of aggregating with homologous proteins, including αA-crystallin and the small heat shock protein HSP28, to form large heteromeric complexes (6). αB-crystallin has recently been identified as the single immunodominant myelin autoantigen in multiple sclerosis (7). The functions of αB-crystallin in cells in different tissues are still unknown. Immunohistochemical studies, however, have indicated that its expression seems to be restricted to highly specialized cells, and that its distribution correlates well with high levels of oxidative function (8). Studies have also indicated that the pattern of αB-crystallin expression in reactive astrocytes seems to be consistent with the striking increase in oxidative enzymes in reactive astrocytes (9). It is possible that αB-crystallin is an enzyme or a cofactor for oxidative enzymes (10). The probable role of this heat shock protein in the etiology of celiac disease has not been revealed yet. We attempted to investigate the cause and the extent of mucosal damage

associated with celiac disease in relationship to the expression of αB-crystallin.

Material and Methods

Biopsy specimens of small intestine were taken from 22 subjects (12 celiac patients and 10 "normal" individuals). The biopsy sections on glass microscope slides were supplied by the Histopathology Department, Queens Medical Centre, Nottingham, UK. The 10 "normal" biopsies were taken from patients with symptoms related to gastrointestinal tract, but with normal duodenal biopsy histology. Twelve patients who entered the study had a confirmed diagnosis of celiac disease and had previously been followed up for at least five years at the University hospital. Procedures for the collection of specimens were done in accordance with the Helsinki declaration (11).

Labeled-avidin-biotin (LAB) reagents (Sigma, London, UK) of high analytical grade were used, including anti-αB-crystallin antibody (primary antibody), swine anti-rabbit antibody conjugated to biotin enzyme (secondary antibody), streptavidin C-3 enzyme label, hematoxylin stain, and hemocyanin conjugated to αB-crystallin peptide. Labeled slides were dewaxed in xylene and washed twice with 90% methanol to remove endogenous tissue hydrogen peroxide. Slides were carefully dried with tissue paper and incubated with 2-3 drops of normal swine serum in tris-buffered saline (TBS) in dilution 1:5 for 20 min to block non-specific binding to the tissue. After several washings in TBS, slides were incubated with anti-αB-crystallin antibody (primary antibody) diluted in TBS (1:20) for 30 min. Two to three drops of swine anti-rabbit antibody covalently attached to biotin enzyme (secondary antibody) diluted in TBS were added to slides and incubated for 30 min. Then a drop of enzyme-labeled streptavidin C-3 was added, and the incubation continued for 30 more min, when followed incubation in DAB (di-aminobenzidine tetrahydrochloride in TBS) solution containing H_2O_2. Slides were then washed under tap water, transferred into 0.5% $CuSO_4$ solution for 10 min, and counterstained in hematoxylin and blued in Scotts solution. They were differentiated in acid alcohol, dehydrated in absolute alcohol, cleaned in xylene, and finally mounted and observed under the microscope. The optimal antibody dilution was performed by antibody serial dilution titers.

The method using LAB was repeated in the control group; 3 drops of hemocyanin-conjugated αB-crystallin were added and incubated overnight. The solution was spun down for 10 min to obtain a pellet of αB-crystallin-antibody complex. The supernatant was used for the positive control slides. The principle behind this is that anti-αB-crystallin antibodies will bind the *Fab* fragment to hemocyanin-conjugated αB-crystallin peptide. Therefore, as the binding site would have already been occupied, any binding in the tissue will not be attributed to specific αB-crystallin binding. It is expected therefore that such treated slides will give no staining to αB-crystallin, and any incidental staining will be due to non-specific binding of the primary antibody in the *Fc* fragment.

The association of variables was analyzed by the Pearson's product-moment correlation (one-tailed). The statistical analysis was performed by the SPSS statistical package.

Results

All cases of celiac disease examined showed moderate to severe villus atrophy, with increased number of intra-epithelial lymphocytes, increased number of chronic inflammatory cells in the lamina propria, and crypt hyperplasia. The "normal" biopsies

Table 1. Microscopic and αB-crystallin staining description in celiac disease and normal subjects

Patients with celiac disease			Patients with no celiac disease		
age (years)	microscopic description	αB-crystallin staining	age (years)	microscopic description	αB-crystallin staining
		Women			
32	Villus atrophy with crypt hyperplasia	Weak staining of the sub-nuclear region	17	Normal villus pattern of the duodenal mucosa	Weak enterocytes staining
39	Partial villus atrophy	Moderate staining of the enterocytes	38	Normal duodenal mucosa	No staining
67	Subtotal villus atrophy, increased chronic inflammatory cells	Strong supranuclear staining of the enterocytes	40	Normal duodenal mucosa	No staining
22	Flattened villi and dense infiltrate of chronic inflammatory cells	Moderate enterocytes staining	32	Normal duodenal mucosa	No staining
27	Complete villus atrophy with crypt hyperplasia	Moderate enterocytes staining	73	Normal duodenal mucosa	Partial staining of enterocytes
47	Villus atrophy with moderate crypt hyperplasia	Strong staining of the supra-nuclear region of the duodenal epithelial cells	35	Normal duodenal mucosa	No staining
43	Complete villus atrophy with increased chronic inflammatory cells	Strong staining of the supra-nuclear region of the duodenal epithelial cells			
		Men			
17	Subtotal villus atrophy	Weak staining of the enterocytes	32	Normal duodenal mucosa	Weak enterocytes staining
39	Subtotal villus atrophy with increased chronic inflammation	Strong supra-nuclear staining of the enterocytes	87	Normal duodenal mucosa	No staining
44	Section shows a fibro-epithelial skin tag	No staining	67	Normal duodenal mucosa	No staining
40	Loss of villi with abnormal surface mucosa infiltrated by lymphocytes	Weak staining of the enterocytes	71	Normal villi	Weak enterocyte staining
52	Sub-total villus atrophy	Weak enterocytes staining			

Table 2. Summary of immunohistochemical staining scores of patients with and patients without celiac disease

Patients with celiac disease					Patients without celiac disease				
staining description	score	No. of patients	total score	% score per total score	staining description	score	No. of patients	total score	% score per total score
None	0	1	0	0	none	0	6	0	0
Weak	+1	4	+4	14.81	weak	+1	3	+3	11.10
Moderate	+2	3	+6	22.20	moderate	+2	1	+2	7.40
Strong	+3	4	+12	44.44	strong	+3	0	0	0

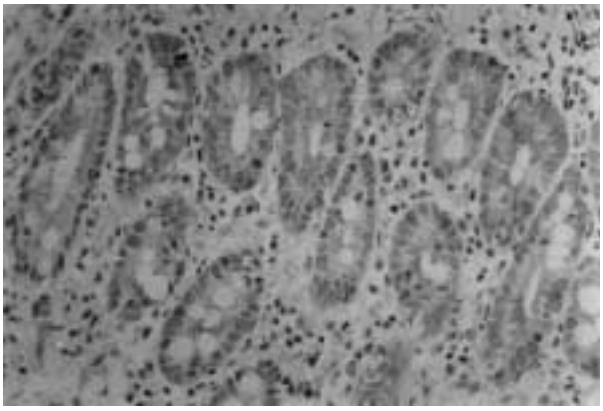

Figure 1. Strong αB-crystallin staining of the supra-nuclear region of the enterocyte in a celiac patient.

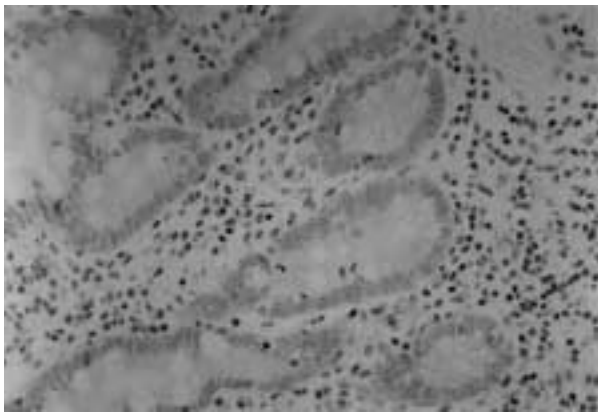

Figure 2. Absence of αB-crystallin in a normal control slide.

showed a normal duodenal mucosa architecture with no inflammatory change or pathology (Table 1).

The quantity and degree of intensity of immunohistochemically demonstrable αB-crystallin and its intracellular distribution in the duodenal mucosa of the patients with celiac disease as presented by scores were closely related to the degree of villus atrophy (Table 2). Pearson's correlation revealed a strong correlation of 0.754 between the number of celiac patients and percentage staining score per total score ($p = 0.123$). The mean percentage score per total score was 20% in the group of patients with celiac disease. On the other hand, Pearson's correlation showed that there was a weak negative correlation of –0.126 between the number of normal of controls and percentage score per total score ($p = 0.437$). The mean percentage score per total score was 4.6%.

αB-crystallin stained the supra-nuclear region of the enterocytes in all the slides of patients affected with celiac disease (Table 1, Fig. 1). Normal controls did not give any staining pattern (Fig. 2). αB-crystallin staining gave a 75% sensitivity and 71% specificity test.

Discussion

αB-crystallin has recently been shown to act as a chaperone, protecting other proteins from stress-induced damage (12). However, accumulating evidence indicates that celiac disease is a multifactorial disorder where several heritable factors in conjunction with environmental factors are involved in the disease development (13). The primary pathogenic trigger in celiac disease is still unknown (14). The presence of T-cell reactivity to αB-crystallin in patients with celiac disease as well as multiple sclerosis (MS) has been suggested and implicated in various etiological studies (7). αB-crystallin and other intracellular ubiquitous protein-dependent degradation underlies a multitude of biological processes, including the cell cycle, cell differentiation, and responses to stress (15). It has been shown that, in skeletal muscle, overall protein degradation involves a protein-proteasome system and that the property that leads to rapid ubiquitin-dependent degradation is the presence of a basic, acidic, or bulky hydrophobic residue at its N-terminus (16).

We studied the duodenal mucosal biopsies from 12 patients with celiac disease, using monoclonal antibodies to αB-crystallin, and 10 control biopsies from patients with symptoms related to gastrointestinal tract, but with normal duodenal biopsy histology. The quantity and degree of intensity of immunohistochemically demonstrable αB-crystallin and its intracellular distribution in the duodenal mucosa of the patients with celiac disease was closely related to the degree of villus atrophy.

The observed strong correlation coupled with the levels of sensitivity and specificity strongly suggested that αB-crystallin probably acted as a cross-reacting antigen in the mucosal damage possibly via a local autoimmune process, involving the activation of helper T cell against α-gliadin in individuals with celiac disease. If this is the case, then it is quite possible to aim therapies at the early stages of the disease at the selective down-regulation of the αB-crystallin-specific autoimmune responses, which will thus help in the management of celiac disease and malabsorption disorder for that matter.

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