Expression of C-myc oncogene in coeliac disease

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SUMMARY A monoclonal antibody, produced by peptide immunisation was used to detect the distribution of p62^{c/myc} by immunohistology in normal and coeliac small intestinal mucosa. The effect of gluten in four treated coeliac patients was investigated by taking serial jejunal biopsy specimens for six hours after a 10 g oral gluten challenge. There was a progressive increase in p62^{c/myc} staining intensity in the villus enterocytes extending to the crypts, which accompanied the classical morphological changes occurring in the mucosa.

Coeliac disease is characterised by a small intestinal villus atrophy that improves on treatment with a gluten free diet. The rate of differentiation and control of the cell cycle of small intestinal enterocytes in this condition differs from that of normal controls,¹ although the pathogenesis is unknown.

Recombinant DNA techniques have permitted the identification of DNA sequences termed cellular oncogenes, including c-myc, which are the cellular homologues of genes first identified in retroviruses,² and which have vital regulating roles in the control of cell growth.³ Oncogene products can be detected by immunohistology, permitting comparisons of their quantity and distribution,⁴ which may elucidate their role in the development of cellular pathology.

A monoclonal antibody to the c-myc gene product p62^{c/myc}, constructed by peptide immunisation techniques,^{5,6} was used to assess changes in the p62^{c/myc} content of the small bowel mucosa from non-coeliac controls, treated and untreated coeliac patients, and four treated coeliac patients subjected to a gluten challenge.

Patients and methods

Jejunal biopsy specimens taken from controls and treated and untreated coeliac patients as part of their diagnostic management, were fixed in 10% formalin and processed to 5 µm sections.

The diagnosis of coeliac disease was made from the

presence of the classical changes in the morphology of peroral small intestinal jejunal biopsy specimens. Jejunal subtotal villus atrophy, which improved on treatment with a gluten free diet and recurred following a gluten challenge, was considered to be diagnostic of coeliac disease.

PATIENTS

Controls

Five subjects had a jejunal biopsy for diagnostic purposes; jejunal mucosal morphology was normal with no evidence of coeliac disease. The diagnoses in these patients were irritable bowel syndrome (four cases) and unexplained short stature (one case).

Untreated coeliac patients

Five patients had untreated coeliac disease; jejunal biopsy specimens showed subtotal villus atrophy.

GLUTEN CHALLENGE

Four additional coeliac patients who had all received a gluten free diet for more than six months were given a gluten challenge after the passage of a Quinton multiple jejunal biopsy capsule, as previously described.^{7,8} The gluten challenge was provided by 10 g of gluten suspended in 150 ml of water and ingested over five minutes.

MONOCLONAL ANTIBODY

The techniques of peptide synthesis, immunisation of mice, and screening procedures used for deriving Myc 1-6E10 have been described elsewhere.⁴ The purified antibody showed p62^{c/myc} in paraffin blocked fixed sections.⁴

Fig 1 Immunohistochemical staining pattern of monoclonal antibody to p62^{c/myc} on small intestinal mucosa from patient with coeliac disease taking gluten free diet.

STAINING TECHNIQUE

Antibody binding was detected by incubating the sections with a 1/500 dilution of the antibody and an immunoperoxidase staining technique⁹ using a 1/40 dilution of peroxidase labelled rabbit antimouse immunoglobulin (Dako Ltd, High Wycombe).

Results

NON-COELIAC CONTROLS

In all five subjects the overall villus architecture was entirely normal, and there was faintly positive cytoplasmic staining of the apical third of the enterocytes covering the villus tips without any nuclear staining. There was no staining of surface cells below the tips of the villi, nor of any crypt cells. Goblet cells, intraepithelial lymphocytes, and cells within the lamina propria were all uniformly negative.

TREATED COELIAC PATIENTS

The biopsy specimens from all five treated coeliac patients showed normal villus architecture, crypt length, and intraepithelial lymphocyte counts. The

staining pattern of p62^{c/myc} was identical with that seen in the controls in that there was faintly positive cytoplasmic staining only of the apical third of enterocytes on the villus tips (fig 1).

UNTREATED COELIAC PATIENTS

In all five untreated coeliac patients there was total villus atrophy with crypt hyperplasia. All the enterocytes, both in the crypts and on the surface, showed positive nuclear staining for p62^{c/myc}. In addition, there was strongly positive cytoplasmic staining (maximal in the vicinity of the Golgi apparatus) of surface enterocytes. The cytoplasmic staining extended to cells in the mouths of crypts, but all the deeper cells in the proliferative and maturation compartments were unstained. There was no staining of intraepithelial lymphocytes nor of goblet cells. There was, however, moderate nuclear and cytoplasmic staining of all the connective tissue and inflammatory cells of the upper third of the lamina propria. The appearances were similar to those in the treated coeliac patients five to six hours after a gluten challenge (figs 2 a and b).

Fig 2 (a and b) Immunohistochemical staining pattern of monoclonal antibody to p62^{c/myc} on small intestinal mucosa from patient with treated coeliac disease five hours after oral gluten challenge.

Table Detection of p62^{c/myc} in jejunal biopsy specimens after gluten challenge

Case No	Time												
	0	½	1	1½	2	2½	3	3½	4	4½	5	5½	6
1	±	±	+	+	+	+	+	++	++	++	++	++	++
2	±	±	+	+	+	+	++	++	++	++	++	++	++
3	—	±	+	+	+	+	++	++	++	++	++	++	++
4	±	±	±	+	+	+	++	++	++	++	++	++	++

GLUTEN CHALLENGE

The biopsy specimens taken before gluten challenge in these four patients were similar to those of the controls and the treated coeliac group in that the overall villus morphology was normal, there was no crypt hyperplasia, and the intraepithelial lymphocyte count was low. The staining pattern of p62^{c/myc} was also identical.

After gluten challenge progressive deterioration of villus architecture began at two hours, and by six hours there was partial or subtotal villus atrophy in all four patients (table). The biopsy specimens obtained at half an hour and one hour after the challenge showed p62^{c/myc} staining of a few enterocyte nuclei at villus tips, in addition to the faint cytoplasmic staining seen before the challenge. By three hours there was noticeable shortening of villi and lengthening of crypts. There was now positive staining of all the cytoplasm, not just the luminal third, of all enterocytes in the upper third of the (shortened) villi. Nuclei were stained as in the earlier specimens, and from three to six hours this staining became more intense and extended to affect all enterocytes, including those in the crypts. As the villi became stunted the cytoplasmic staining of enterocytes extended into (not from) the crypt mouths. By six hours all four patients showed similar morphological changes and staining patterns to those seen in the untreated coeliac patients (figs 2 a and b).

CONTROLS

Several controls were used for the histological assessment of expression of p62^{c/myc} by the antibody to this peptide. As a specificity control sections were incubated with the monoclonal antibody Myc 6E10, which had been blocked by the peptide to which it had been raised (100 µl Myc 1 6E10, µg peptide D at 4°C overnight). No staining was present on sections incubated with the blocked antibody. As a further control sections were incubated with a monoclonal antibody (X63) of the same subclass and concentration as Myc 1 6E10. No staining was present in any of the sections incubated.

Discussion

The presence of low amounts of the c-myc oncogene product in maturing jejunal enterocytes from control subjects and treated coeliac patients suggests that the c-myc oncogene may participate in small intestinal cellular differentiation. In untreated coeliac disease the presence of increased amounts of the c-myc oncogene product both in the nucleus and cytoplasm of enterocytes of the upper third of the stunted villi suggests that the c-myc oncogene may participate in the premature senescence and desquamation of these cells. A gluten challenge in four treated coeliac patients induced an abnormal staining pattern within two hours of the challenge when the villus morphology was deteriorating. This suggests that the c-myc oncogene may participate in the premature death of these small intestinal villus enterocytes and therefore the pathogenesis of this disease.

One of the typical features of untreated coeliac disease is small intestinal villus atrophy, in which the crypts are elongated and increased in diameter. The crypts contain enterocytes with an increased mitotic rate in the lower third compared with that of controls.¹⁰

The molecular mechanisms entailed in the control of cell proliferation and differentiation of the small bowel have been difficult to study. Advances in genetic engineering and monoclonal antibody technology have enabled antibodies to be raised against the protein products of oncogenes. These techniques permit an investigation of the molecular basis for the disruption of control mechanisms in several diseases.

Gastrointestinal cell renewal was examined with the rat small bowel mucosa as a model. This showed that as well as a stem cell component, most cell production takes place in the proliferative compartment at the crypt base, from which the immature cells migrate up into the maturation zone. As the cells enter this area they rapidly lose their capacity to divide and undergo differentiation, resulting in their emergence on to the villi. After a lifespan of three to five days in normal subjects the cells senesce and desquamate into

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the small bowel lumen.¹¹ The careful regulation of cell division, maturation, and shedding must entail coordinated gene expression for it to continue with such precision.

Use of a metaphase arrest technique has shown that the cell cycle time (Tc) and mitotic duration (TM) in the normal small bowel mucosa is about 50 hours and one hour, respectively.¹² In coeliac disease there is an increase in the mitotic index due to an increased rate of cell division. Not only is there a change in the cell cycle time, but the cells fail to differentiate into mature cells.¹³ Cell loss in coeliac disease, as measured by DNA from the washings from perfused segments of small bowel, shows a six-fold increase above normal.¹⁴ There is a concomitant increase in cell production in untreated disease, due to a higher number of proliferating cells and a doubling of the rate of cell division.

The c-myc gene and its product p62^{c/myc} have been implicated in the control of cell cycle activation and differentiation. The activation or expression of p62^{c/myc}, probably in combination with other oncogene products, is in part responsible for the increase in the rate of cell proliferation, decrease in cell cycle time, and abnormal differentiation. The true nature of the regulating mechanism, whereby gluten challenge results in an increase in the rate of cell loss, remains unknown. It is unlikely that such mechanisms operate only in coeliac disease, and other insults that result in a compensatory proliferation may produce similar changes. An oncogene product cascade system may have a role. It is known that p62^{c/myc} can partly replace the dependence of cells on other stimulating and growth mitogens such as platelet derived growth factor, epidermal growth factor, insulin, or steroids.¹⁵

The c-myc protein product is a nuclear associated protein and has a role in the control of the cell cycle and differentiation, though its exact function remains to be determined. It is probable that the insult produced by gluten results in a number of oncogene products being over or inappropriately expressed. Overproduction or abnormal expression of p62^{c/myc} may play a part in the failure of the dividing cells in the small bowel mucosa to attain a fully differentiated state as well as preventing dividing cells from re-entering Go, and therefore it may participate in the pathogenesis of coeliac disease.

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