Gut 1995; **36:** 321–323

Gut

Leading article

Intestinal proliferation in coeliac disease: looking into the crypt

Intestinal epithelial cell proliferation

In healthy subjects, the intestinal epithelium is in a dynamic state of equilibrium and cell proliferation is typified by high epithelial birth rates, which are restricted to the crypts of Lieberkühn.[1] This continuous cell division process is essential to replace damaged or lost epithelial cells, the number of which exceeds 10^{11} cells per day under normal 'steady state' conditions.[2] In adults, the transit period for epithelial cells on the villus is about three to five days.[3 4] This is reduced further in the proximal small intestine as a substantial number of cells are lost before reaching the conventional cell extrusion zone at the villus tip. The longterm potential for the intestinal crypts to manufacture and replace lost epithelial cells is achieved by an as yet undefined number of pluripotent stem cells that remain anchored within the lower crypt cell positions.[5] This stem cell population probably gives rise to a large number of undifferentiated crypt cells that actively proliferate as they transit through the lower two thirds of the crypt, a region termed as the proliferation compartment.[1 2 6] Cells then gradually decycle and differentiate in the upper third of the crypt (maturation compartment).

Intestinal proliferation in disease states

In certain disease states the balance between epithelial cell loss and replacement is severely disrupted.[6 7] Consequently, attempts have been made to discover if adaptive crypt proliferative responses exist in response to tissue damage. It is now clear that intestinal crypts are rapidly able to modify the cellular output or crypt cell production rate (CCPR) in response to various forms of damage, for example, food allergy,[8] graft versus host disease,[9] T cell activation,[10] resection,[11] and infection.[12]

Coeliac disease is a common genetically linked disorder of the proximal small intestine[13] and is characterised morphologically by severe crypt hyperplastic flat mucosa with a noticeable leucocytic infiltrate of the lamina propria and epithelium.[14] Mitotic figures are increased in number in the crypts and there is a greatly increased epithelial cell loss into the intestinal lumen. Two opposing kinetic views have been proposed in an attempt to explain the changed morphology that is characteristic of gluten sensitive enteropathy. Firstly, the considerable crypt hyperplasia has been described as an increased proliferative response needed to accumulate a greatly increased mass of proliferating and maturing cells.[15] Secondly, the mucosal derangement has been attributed to an impaired proliferative response resulting in a slower output of cells from the crypts.[16] This second view has now been largely discredited as both indirect and direct evidence exists for enhanced epithelial proliferative states in coeliac disease.

Indirect evidence for increased epithelial cell proliferation rates in coeliac disease was provided by the finding of both increased DNA content in gut washings[17] and also significantly raised pyrimidine precursor enzyme concentrations in mucosal scrapings.[18] These findings were largely ascribed to epithelial cells and no serious attempts were made to exclude contaminant leucocyte populations, which represent a substantial proportion of the cells within the intestinal mucosa and lumen. Direct evidence for increased crypt cell proliferation rates in coeliac disease has been shown by quantitative studies of DNA synthesising (S phase) and mitotic (M phase) epithelial cells in histological sections. Trier and Browning[15] used the nucleotide analogue tritiated thymidine, a low range beta particle emitter, to record S phase cells using autoradiography. These in vitro studies showed a 2·8-fold increase in DNA synthesising epithelial cells in the crypts from coeliac disease patients. Additional time course studies showed substantially raised epithelial cell migration rates in coeliac mucosa, reducing the normal epithelial life span of three to five days to about 24 hours. Furthermore, an increased number of mitotic figures has been recorded in the intestinal crypts from patients with coeliac disease.[19–23] These studies show that the hyperplastic crypts seen in this disorder, consistently possess a greater cell abundance and mitotic index (I_m) when compared with histologically normal mucosa. Additional in vivo metaphase arrest studies, using vincristine sulphate, showed that cell cycle times (the duration from one cell division to the next) was roughly halved in coeliac disease.[21] Thus, our present understanding of the intestinal hyperproliferative response in coeliac disease shows that two main adaptive mechanisms are responsible for replacing damaged surface epithelial cells comprising a three dimensional (3-D) expansion of the proliferation compartment, and a reduction in the cell cycle time.

Interpretation of existing kinetic data

The in vitro tritiated thymidine studies performed by Trier and Browning showed that significantly more S phase cells

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are present in coeliac crypts.[15] These data were, however, not presented as a percentage labelling index (I_s) curve, which would have permitted calculation of the average crypt labelling index, growth fraction, and cell cycle duration in this disorder. In addition, measurement of epithelial cell migration rates on villi as an indicator of crypt cell proliferation rates is misleading as it assumes that the crypt:villus ratio remains constant in both health and disease. Counting mitotic cells in longitudinal sections is hampered by the two dimensional (2-D) limitations of such an approach.[24] Accurate measurements of I_m are especially difficult using this method as mitotic cells are centripetally positioned within crypts, a feature that must be corrected, for example, by using mathematical constants such as Tannock's correction factor.[25] These problems can probably be overcome by assuming that crypts are cylindrical and remain so during disease states. Values for CCPR in coeliac disease have been obtained using the Feulgen stain in crypt squashes.[26 27] Although this technique provides accurate measurements for CCPR in different disease states, it provides little information regarding adaptive crypt responses as it cannot measure the crypt growth fraction and, consequently, accurately measure the crypt I_m.

Confocal microscopical measurements of intestinal proliferation

In an attempt to overcome these many limitations we have developed a novel confocal microscopical technique for accurate measurement of intestinal proliferation in children. This method utilises confocal microscopy to create high resolution 3-D images of intact propidium iodide stained crypts. The experimental methodology and results are described in this issue of *Gut* by means of non-invasive optical sectioning through intact microdissected crypt. The 3-D shape of individual crypts was accurately recorded.[28] The technique also provided a unique opportunity to count the total number of dividing cells identified in individual crypts and to map these relative to the crypt architecture. By recording the absolute mitotic frequency at each cell position within individual crypts, confocal microscopy was able to provide high resolution 3-D I_m distribution curves. We then applied this technique to critically test the adaptive proliferative responses described for coeliac disease.[22]

Proximal small intestinal crypts from children with a histologically normal mucosa or with active coeliac disease were examined using confocal microscopy. Proliferative measurements were also performed using conventional methods on histological sections. Confocal microscopy corroborated previous reports that coeliac crypts possess significantly more cells (3·8-fold) when compared with histologically normal structures. 3-D image reconstitution showed that these crypts were flask shaped and not cylindrical, which had been assumed previously for mathematical modelling purposes.[25] On average, control and coeliac disease patients possessed 33% and 20% more epithelial cells in the lower compared with the upper half of the crypt, respectively. 3-D I_m distribution curves for control and coeliac crypts were used to create conventional 2-D I_m curves and, therefore, permitted a direct comparison to longitudinal section data. These data show that considerable discrepancies exist between I_m measurements obtained using the two different methods. Confocal microscopy values for CCPR are consistently three to four-fold lower than those calculated using longitudinal sections, showing that Tannock's correction factor does not compensate effectively for this feature in sectioned material. In addition, assuming that the crypts are cylindrical profoundly changes the shape of the I_m distribution curves. Indeed, the shape of the I_m curve is highly dependent on the method used. As the growth fraction is mathematically derived from the I_m distribution curves[6 7] this changes the defined size of the proliferation and maturation compartments.

New insights into the intestinal proliferative response

The confocal microscopical studies have corroborated previous reports that showed an intestinal hyperproliferative response in coeliac disease in children. Indirect assessment of crypt cell populations using sectioned material provides data that consistently overestimate the CCPR and underestimate the crypt growth fraction in histologically normal and diseased mucosa. These errors arise as a direct consequence of the inherent problems of counting mitotic figures in 2-D so that existing mathematical constants must be revised to compensate for these limitations. The widely used Feulgen crypt squash technique provides an accurate method to calculate the CCPR, shown by the similar values obtained using confocal microscopy.[26 27] This technique, however, cannot provide data relating to the spatial positioning of mitoses within single crypts or generate accurate I_m distribution curves, which are needed to calculate the crypt growth fraction. The confocal microscopy I_m distribution curves extends our understanding of the adaptive crypt responses that operate in coeliac disease. These curves show that when the 3-D shape of the crypt is taken into consideration, a predominantly uniform mitotic activity is evident throughout a large portion of the crypt. Low values for cell division are evident near the crypt base and also towards the crypt-villus junction, as these represent sites where cells actively decycle to enter G_0 and terminal differentiation. Consequently, the rapid decline in I_m recorded in the vicinity of the crypt-villus junction contrasts with a slow cut off model for intestinal proliferation, as proposed originally by Cairne *et al.*[29]

In conclusion, the confocal microscopy data suggest that a 3-D expansion in girth and length of the intestinal crypts is the only adaptive crypt response that occurs in children with coeliac disease. Previously reported adaptive changes including crypt growth fraction or cell cycle duration were not confirmed as there was no significant shift in either control or coeliac disease I_m max or $max_{50\%}$ values in the I_m distribution curves that would reflect such changes. Consequently, we are now applying the confocal microscopy method to assess whether new mathematical constants can be developed that will permit accurate measurements of adaptive intestinal proliferative responses in disease states using the longitudinal section technique. This may be of particular importance in intestinal cancer where there are characteristic proliferative changes.[30–32] It is now possible to apply the confocal microscopy technique to such tissues to confirm whether the described changes are real or result from experimental artifact.

This work was supported by the Queen Elizabeth Hospital for Children Research Appeal Trust. The authors thank Dr Doug Winton (Department of Pathology, University of Cambridge) for helpful comments.

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