Growth stimulating activity associated with an altered cell renewal pattern in the small intestine

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SUMMARY A growth stimulating activity has been recently identified in the rat proximal intestine during the transient stages of adaptation after small bowel resection. This study shows that the growth stimulating activity is associated with all the cells of the crypt-villus axis. At the same time the thymidine kinase activity is detectable in all the cells implying a more extensive proliferative zone during the transient stages of adaptation. The presence of the growth stimulating activity along with the more extensive proliferative zone suggests that the activity modulates the proliferation capacity of intestinal epithelial cells.

The epithelial cells of the small intestine are a renewing cell population characterised by segregation of the proliferative zone in the crypts and a mature differentiated zone in the villus cells. These cells have a rapid turnover and an equilibrium exists between cells produced in the crypt and cells lost at the villus tip. This equilibrium is altered by a small bowel resection. After resection there is an increase in cell proliferation in the remnant small intestine and a resultant adaptive hyperplasia when a new steady state is reached.¹ A growth stimulating activity has been identified from the rat proximal intestine after a 50 % small bowel resection, and this could play a role in the observed adaptive response.² This activity was detectable in resected animals 48 h, 72 h, 96 h but not at eight days after resection when presumably a new steady state is reached. Normal animals fed *ad libitum* do not show this activity. In this study the distribution of the stimulatory activity along the crypt-villus axis of male Sprague-Dawley rats was examined four days after small bowel resection to ascertain whether it is associated with proliferating cells or with differentiated villus cells.

Methods

SURGERY

Proximal small intestine was obtained from 150 g male Sprague-Dawley rats (MacDonald Farm, St-Anne de Bellevue, Que) after a 50 % small bowel

resection, carried out as previously described.² The animals were fed orally with a regular diet.

CELL ISOLATION

Four days after surgery the resected animals were killed by decapitation and the proximal small intestine removed for experimentation. The intestine was rinsed with saline, one end tied off with 4-0 surgical thread and everted. The everted intestine was rinsed in $\text{Ca}^{++}/\text{Mg}^{++}$ free Dulbecco's phosphate buffered saline containing aprotinin (10 U/ml), cut into approximately 5 cm pieces, and transferred to an Erlenmeyer flask containing $\text{Ca}^{++}/\text{Mg}^{++}$ free Dulbecco's phosphate buffered saline (2.5 ml/5 cm intestine), 1.5 mM ethylenediamine tetraacetate, 0.5 mM dithiothreitol, and aprotinin (10 U/ml). The tissue was incubated for different times at 37 °C under gentle shaking (110 rpm) in a New Brunswick water bath. Thus, sequential fractions of isolated cells were obtained. The different cell fractions were centrifuged at 1000 rpm for three minutes and the pellet washed twice with $\text{Ca}^{++}/\text{Mg}^{++}$ free Dulbecco's phosphate buffered saline containing aprotinin.³

PREPARATION OF THE GROWTH STIMULATING FRACTION

The growth stimulating fraction was prepared from the sequential fractions as described previously.² The cells were suspended in 2 × volumes of water, immersed in a boiling water bath for five minutes and homogenised. The supernatant was discarded and the precipitate extracted with cold 0.1 N HCl. The extract

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was centrifuged at 9000 g for 40 min and the supernatant kept. A further purification of the extract, by binding to CM-Sephadex C-25 at pH 4.8 then eluting with 0.01 N HCl, 1 M NaCl,⁴ was used in these experiments. The protein content was determined by measuring the absorbance at 275 nm and using $E_{275} = 6.0$ for bovine serum albumin.⁵

IN VITRO ASSAY OF DNA SYNTHESIS BY THE GROWTH STIMULATING FRACTION

The stimulation of DNA synthesis was quantified by measuring the rate of incorporation of thymidine into DNA of mouse intestinal explants maintained in organ culture as previously described.² One unit of stimulation is defined as the stimulation index above control per μg protein in the growth stimulating fraction.

ENZYME ASSAY

Thymidine kinase in the cell fractions was measured according to the method described by Klemperer and Haynes⁶ except that the product was isolated using DEAE impregnated paper discs (Whatman DE-81) according to the method of Breitman.⁷ Enzyme activity is the cpm/thymidine monophosphate/g protein/min. Alkaline phosphatase was determined according to the method of Eicholz.⁸ The specific activity is the micromoles p-nitrophenol/g protein/min. The protein content in the cell fractions was determined by the method of Lowry.⁹ The percentage of cells isolated in each successive cell fraction was determined by the protein content in the given fraction.

Results

The results shown in Fig. 1a indicate that the growth stimulating activity is distributed throughout the crypt villus axis. At the same time, thymidine kinase activity is detectable in all the fractions implying that all the cells along the crypt villus axis are proliferating (Fig. 1b). A comparison of normal animals with four day resected animals shows that at any given point the thymidine kinase activity is at least four-fold greater than in the controls, whereas, the alkaline phosphatase distribution is not markedly different.

The growth stimulating activity is known to be present in the proximal small intestine for a limited time, four days, after a 50% small bowel resection.² The results in Fig. 2 show that the levels of thymidine kinase are raised throughout the crypt villus axis for the same time period, two and four days post-resection. When the activity is diminished at eight days a new steady state is reached and the thymidine kinase distribution (Fig. 2) changes to resemble that of the intestine from normal animals.

Fig. 1 Distribution of (a) growth stimulating activity and (b) marker enzymes (●—● thymidine kinase, x—x alkaline phosphatase) along the crypt villus axis of the proximal small intestine after a small bowel resection, at four days. The open Δ show the alkaline phosphatase activity from normal animals. One unit of stimulation is defined as the stimulation index above control/ μg protein of growth stimulation fraction tested with the assay. The thymidine kinase activity is the cpm thymidine monophosphate/g protein/min. Alkaline phosphatase activity is the p-nitrophenol μmol produced/g protein/min.

Discussion

In the intestine it is generally accepted that proliferation takes place in a discrete crypt cell compartment and as the cells migrate up towards the lumen they differentiate and lose their capacity to proliferate. Immediately after a resection there is a rapid increase in proliferation¹⁰ before a new steady state is established with an increase in crypt depth and villus height. This new steady state is reached within eight days after a 50% jejunal resection in the rat. The growth stimulating activity is also present during this period of transition² and the results shown here suggest that it is associated with a more extensive proliferative zone identified by thymidine kinase

Fig. 2 Distribution of thymidine kinase activity in normal animals (Δ) and resected animals 2 ($\times$), 6 ($\blacksquare$), and 8 ($\circ$) days postresection. Normal animals were fed *ad libitum*. The same portion of intestine was taken from the two and eight days animals as described above for the four day postresection animals. A similar portion was studied in the normal animals.

activity. When the activity is no longer present (at eight days after resection) distribution of the thymidine kinase activity resembles that of normal animals.¹¹

The intestinal epithelium manifests a basic pattern of cell proliferation and then differentiation for cell renewal. Both proliferation and differentiation occur in discrete zones. During the transient stages of postresectional adaptation the proliferative zone is more expansive and is at the same time associated with the presence of the growth stimulating activity. When the new steady state is reached the stimulating activity is no longer detectable and the discrete proliferating (crypt) and differentiating (villus) compartment are again observed. Thus it appears that the change from the steady state of the normal animal to the postresectional steady state takes place *via* an intermediate phase in which all the cells are capable of proliferating. The association of the growth stimula-

ting activity with all the cells along the crypt villus axis during this intermediate phase strongly implies that it plays a role in this transition.

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