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Regulation of cell number in the mammalian gastrointestinal tract: the importance of apoptosis

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SUMMARY

The regulation of cell number in adult tissues is determined by the balance of cell production and cell loss. In the gastrointestinal tract, where there are well defined zones of proliferation and migration of both epithelial cells and associated fibroblasts, it is widely held that cell loss occurs by shedding into the gut lumen. Since the evidence for this is not compelling, we investigated the distribution and amount of apoptosis in the normal mammalian gut. In the stomach, small intestine and colon of rodents and man, there is a small number of apoptotic bodies in the epithelium and in the immediate sub-epithelial connective tissue. Engulfment by adjacent epithelial cells and sub-epithelial macrophages accounts for the removal of apoptotic bodies. Apoptotic bodies are not randomly distributed but are found towards the distal end of the known cellular migration routes of both epithelial and mesenchymal cells. Furthermore, consideration of the absolute numbers of apoptotic bodies, their rapid clearance and the dimensions of the small intestinal villi and colonic crypts indicates that the cell loss in the normal murine intestine can largely be explained on the basis of the observed apoptosis. Despite being inconspicuous in histological material, apoptosis probably accounts for the bulk of cell loss in the gut and is a central feature of the regulation of cell number in adult tissues.

Key words: gut, apoptosis, cell number, villus, population control, epithelium, fibroblast

INTRODUCTION

Metazoa have solved three fundamental problems: how to differentiate the component cell types of their tissues; how to arrange these cell types into tissues; and how to regulate the number of any given cell type. This last issue is clearly of great importance since unrestrained cell production would be detrimental to the structure and function of an organism. In the past much attention has focused on how regulation of cell proliferation controls the production of new cells. However, examination of tissues from adult organisms demonstrates that the majority of cells are not dividing. Consequently the regulation of cell number must inevitably depend upon the control of cessation of cell proliferation. Moreover, since in many tissues cell production is a permanant occurrence, there must be well defined mechanisms for regulating cell loss (Ansari and Hall, 1992). In recent years the idea that apoptosis is a common mechanism for regulating cell loss has become well established (Kerr et al., 1972; Ellis et al., 1991; Raff, 1992). The notion that regulation of cell death is an important contributor to developmental processes is not new (Glücksmann, 1951; Saunders, 1966). However, it is now recognised that in many developmental processes apoptosis is a common event, accounting for extremely large cell losses (Oppenheim, 1991; Barres et al., 1992; Coles et al., 1993). The previous underestimation of its quantitative importance to dynamic population control is a consequence of the rapid clearance time of the end result of the process of programmed cell death, the apoptotic body. These structures, with highly characteristic morphology and sequence (Kerr et al., 1972), have a clearance time estimated to be 1-2 hours (Barres et al., 1992; Coles et al., 1993) and are consequently usually not a conspicuous histological feature in most developing or adult tissues.

The epithelium of the gastrointestinal tract has a highly stereotyped organisation with a continuous high level of cell proliferation (Leblond, 1964; Wright and Alison, 1984). The spatial organisation of the proliferative compartment is very well defined both in rodents and in man. Furthermore, there is a detailed understanding of the cell fluxes that leave the proliferative compartment of stomach, small and large intestine (Wright and Alison, 1984). Associated with the epithelium is a layer of fibroblasts that have been shown to have a similarly organised proliferative architecture, cell migration pattern and rate of movement (Pascal et al., 1968a,b; Marsh and Trier, 1974a,b; Parker et al., 1974). These epithelial and fibroblast fluxes must be associated with equivalent cell loss in order to maintain homeostasis. In the adult, mammalian gastrointestinal tract apoptosis has been noted in the proliferative compartments, and this increases in the context of genotoxic insult (Potten, 1977; Potten et al., 1990). Apoptosis is not widely recognised in the non-proliferative compartments of the normal gastrointestinal tract, although it has been recognised in pathological states (Walker et al., 1988; Lee, 1993) and a recent paper has described apoptosis in rat stomach and

duodenum (Benneddetti et al., 1990). In contrast, the massive cell loss that must occur in the gastrointestinal tract to balance the enormous cell production is reported, even in standard textbooks, to be entirely due to shedding from the superficial aspect of the gut (Warwick and Williams, 1973; Cotran et al., 1989; Junqueria et al., 1989; Morson and Dawson, 1979; Segal and Petras, 1992; Alberts et al., 1994). The evidence for this is not compelling (Messier and Leblond, 1960; David, 1967; Cheng and Leblond, 1974; Creamer et al., 1961; Tsubouchi and Leblond, 1979; Fereirra and Leblond, 1971; Yeomans and Trier, 1976) and the mechanism of fibroblast cell loss is unknown. The possible major contribution of apoptosis to these fluxes has not been previously recognised.

In the present study we have performed a detailed morphological analysis of apoptosis in the gastrointestinal tract. We report that apoptosis does indeed occur in the non-proliferative compartments of all anatomical regions of the rodent and human gastro-intestinal tract, both in the epithelium and in the immediate sub-epithelial fibroblast layer. Furthermore, the amount of cell loss by apoptosis is not randomly distributed and closely approximates to the total cell loss required to balance cell production in the murine small intestine. The results provide further evidence that the extent of normal cell death and its functional significance are greatly underestimated and suggests a fundamental mechanism for regulating epithelial population size by apoptosis.

MATERIALS AND METHODS

Histology and immunohistochemistry

Mouse or rat small intestine and colon was removed, opened longitudinally and immediately immersed in 10% buffered formalin. In addition, macroscopically normal human small and large intestine was removed at time of laparotomy or endoscopy. In other experiments fixation was delayed or fixation of un-opened gut was performed. After 24 hours material was processed to paraffin wax and 3 μm sections prepared on silane-coated slides. After dewaxing and taking to water, sections were stained with haemotoxylin and eosin or used in immunohistological assays. The presence of intra-epithelial lymphocytes was demonstrated by the use of specific anti-lymphoid antibodies including anti-CD45 (Dako UK Ltd) using the peroxidase-ABC method (Dako UK Ltd). After immunostaining sections were lightly counterstained with haematoxylin, dehydrated and mounted in DPX. Negative controls included omission of the primary antibody and relevant immunohistological controls for the detection systems.

In situ end labelling

In situ end labelling (ISEL) was performed as previously described (Ansari et al., 1993). In brief, paraffin sections (3 μm) were dewaxed, taken through alcohol and air-dried. Sections were permeabilised with proteinase K at 10 μg/ml in TE buffer. Slides were then washed in distilled water, rinsed in 70%, 90% and 95% alcohol and air-dried. In situ end labelling was then carried out using 60 μl labelling mix, containing 0.01 mM each of biotin-14-dATP (Gibco/BRL), dCTP, dGTP and dTTP in 50 mM Tris-HCl (pH 7.5), 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 0.005% BSA (Fraction V, Sigma), and Klenow fragment of DNA polymerase I (FPLC*pure*^{TM}; Pharmacia). Sections were incubated at 37°C for 2 hours. The reaction was terminated by washing sections in distilled water three times. Biotin-labelled dNTPs were visualised with avidin-biotin complex (Dako UK Ltd) for 30 minutes at room temperature. Sections were washed in PBS three times (5 minutes each) and developed in diaminobenzidine-hydrogen peroxide. Sections were lightly counterstained with haematoxylin and mounted in resin. Negative controls included omission of the polymerase from the labelling mixture and relevant immunohistological controls for the detection systems. The staining patterns of ISEL-treated sections were compared with serial sections stained with haematoxylin and eosin.

Quantitation of apoptosis

Assessment of the number of apoptotic bodies was made on 3 μm haemotoxylin- and eosin-stained sections from the murine small intestine 25%, 50% and 75% from the ligament of Treitze, and in the transverse colon. In the small intestine material attention was focused on the villi, where a minimum of 200 full-length villus profiles in which the full length of the connective tissue core was identified were examined from each of three Balb/c mice. The distribution of apoptotic bodies was expressed in quintiles from the top of the villus. In the colon of three Balb/c mice, the distribution of apoptotic bodies was defined in 200 full-length, well orientated, crypts and expressed per quintile of the crypt. Apoptotic bodies were also identified in plastic-embedded sections from the jejunum of a Sprague Dawley rat. In human material apoptotic bodies were assessed in histopathologically normal stomach, small intestine and colon. In all cases the morphology of apoptotic bodies was critically assessed in order to exclude the confounding effect of intra-epithelial lymphocytes. The morphological characteristics of apoptosis were identified using standard criteria, including chromatin condensation, separation of the cell from adjacent enterocytes and formation of distinct membrane-bound vesicles, so called 'apoptotic bodies'. When clusters of more than one apoptotic body were seen, within a diameter of one cell in that tissue, these were taken as fragments of one cell, and counted as one apoptotic cell (Coles et al., 1993). Intra-epithelial lymphocytes were distinguished by their morphology and larger size. In any situation where a clear distinction could not be made between a lymphocyte and an apoptotic body, it was not scored.

RESULTS

Apoptotic bodies are present in the small and large intestinal epithelium and in adjacent lamina propria

Examination of both paraffin and thin resin sections of stomach, small intestine and colon from both rodents and man indicate that apoptotic bodies are present in the epithelium and in the immediate sub-epithelial connective tissue, although they are inconspicuous and uncommon (Fig. 1). In the stomach epithelium, they are most prevalent towards the base of the gastric glands and also at the most superficial aspects of the gastric mucosa. Occasional apoptotic bodies can also be seen at both these sites in the lamina propria close to the basement membrane. Some apoptotic bodies appear to be free in the extracellular space while others are seen within adjacent epithelial cells. In addition, those apoptotic bodies beneath the basement membrane appear to be within macrophages.

The identification of apoptotic bodies in both the small intestine and colon is complicated by the presence of many intra-epithelial lymphocytes whose staining characteristics and size could lead to misinterpretation. Furthermore should the lymphocytes undergo apoptosis they would confound any assessment of epithelial apoptosis. Consequently, we examined preparations of human small intestine and colon in which lymphocytes were marked with anti-CD45 antibodies. In no case were the apoptotic bodies seen to have a rim of CD45 immunoreactivity, indicating that they are unlikely to be of lymphoid derivation (Fig. 2). The possibility that lymphocytes undergoing apoptosis lose CD45 immunoreactivity can be

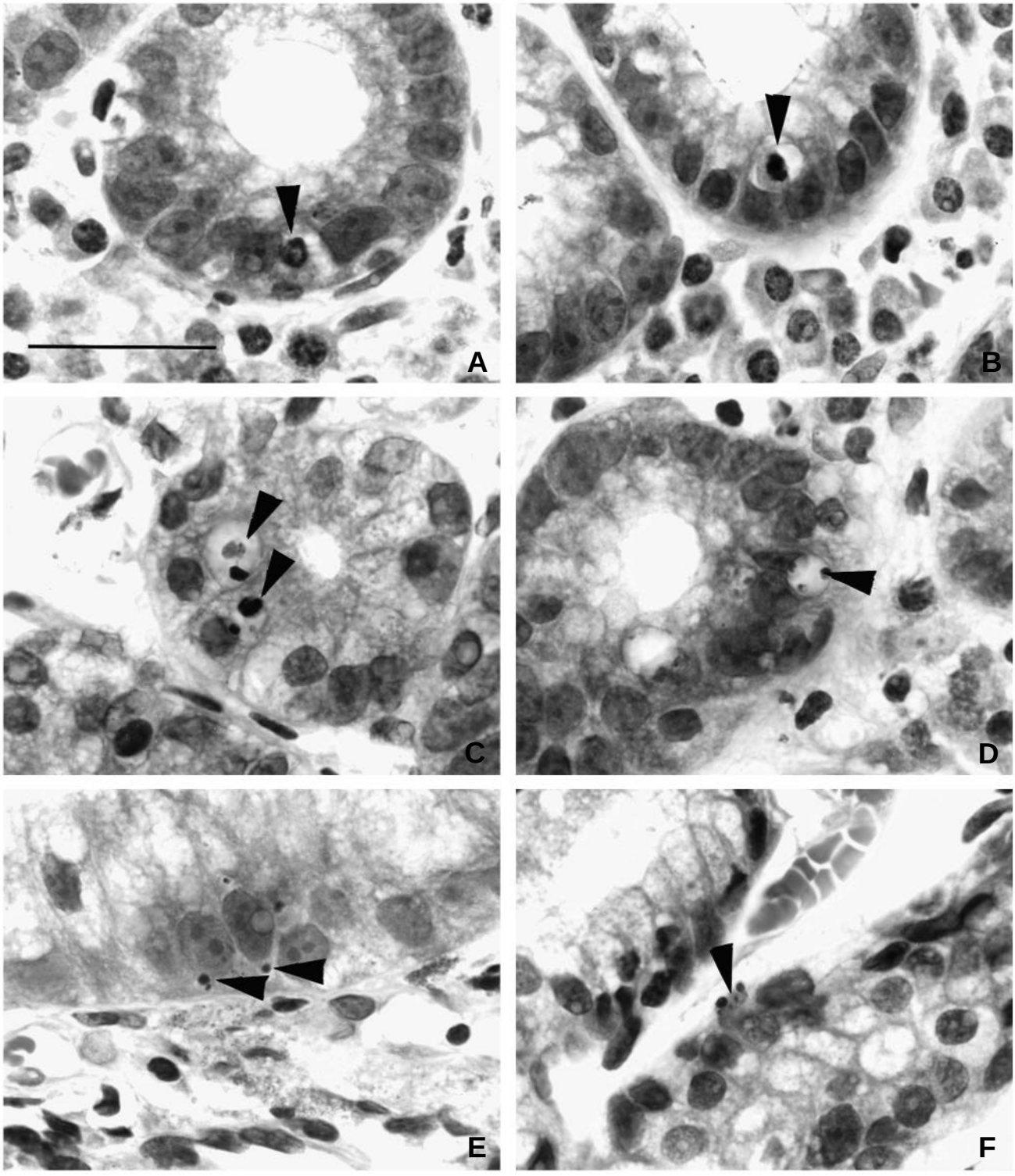

Fig. 1. The full range of morphological features of apoptosis are seen in the gastrointestinal tract (arrowheads). A colonic epithelial cell with chromatin condensation (A) with subsequent further nuclear condensation and shrinkage of the cytoplasm (B). Later stages of the process involve the formation of apoptotic bodies and their engulfment by adjacent epithelial cells as seen in the stomach (C), colon (D) and small intestinal villus (E). Apoptotic bodies may also be identified in the immediately sub-epithelial connective tissue (F). Bar, 50 μm.

excluded, since examination of CD45 expression in lymphocyte apoptosis as seen in germinal centres indicates that this CD45 expression is retained (not shown).

While great care is required for the confident identification of apoptotic bodies we are able to define their distribution in both small and large intestine (Fig. 3). It was found that

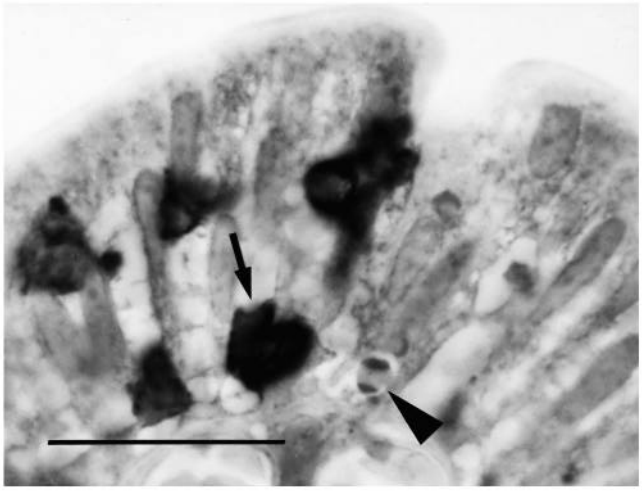

Fig. 2. The apoptotic bodies are not lymphoid in origin. CD45 expression is a marker of lymphocyte phenotype (arrow) and the visible apoptotic body is not expressing this antigen (arrowhead). Bar, 50 μm.

apoptotic bodies could be identified within the epithelium of the majority (but not all) small intestinal villi (overall mean of 0.905 per villus), and that they were more frequent towards the top of the villus at all the anatomical sites examined. Similarly, apoptotic bodies could be identified in the lamina propria close to the basement membrane (overall mean of 0.213 per villus). Again they tended to be present towards the top of the villus. Apoptotic bodies were frequently present in the proliferative compartment of the small intestinal crypt as has been previously described (Potten, 1977; Merritt et al., 1994). In the colon, apoptotic bodies were to be found towards the top of many (but not all) crypts, both in the epithelium (overall mean of 0.74) and in the lamina propria (overall mean 0.245).

Delayed fixation alters the staining with the ISEL method

Fixation effect was studied on ISEL with particular reference to apoptosis in the small intestine. Segments of the mouse gut were fixed in formalin at varing periods after removal. All tissues showed occasional cells labelled at the base of the crypts with the morphology of apoptotic cells. Tissues that had been opened longitudinally and fixed immediately, showed ISEL labelling in very occasional cells towards the tips of the villi. These labelled nuclei were consistently morphologically abnormal, being pyknotic and small. In tissues that were left for 15 minutes and then fixed, there was a dramatic increase in the number of ISEL-positive cells (Fig. 4). The number of ISEL-positive cells increased with increasing delay of fixation and also appeared further down the villus. Tissue that was fixed after a delay and without prior opening showed the highest amount of labelling. The labelled cells showed various morphologies from normal to highly distorted shapes, but did not show any morphological features of apoptosis. In histological material from poorly fixed specimens, especially if lumenal contents were present, sloughed cells were apparent, many with the features of apoptotic cells.

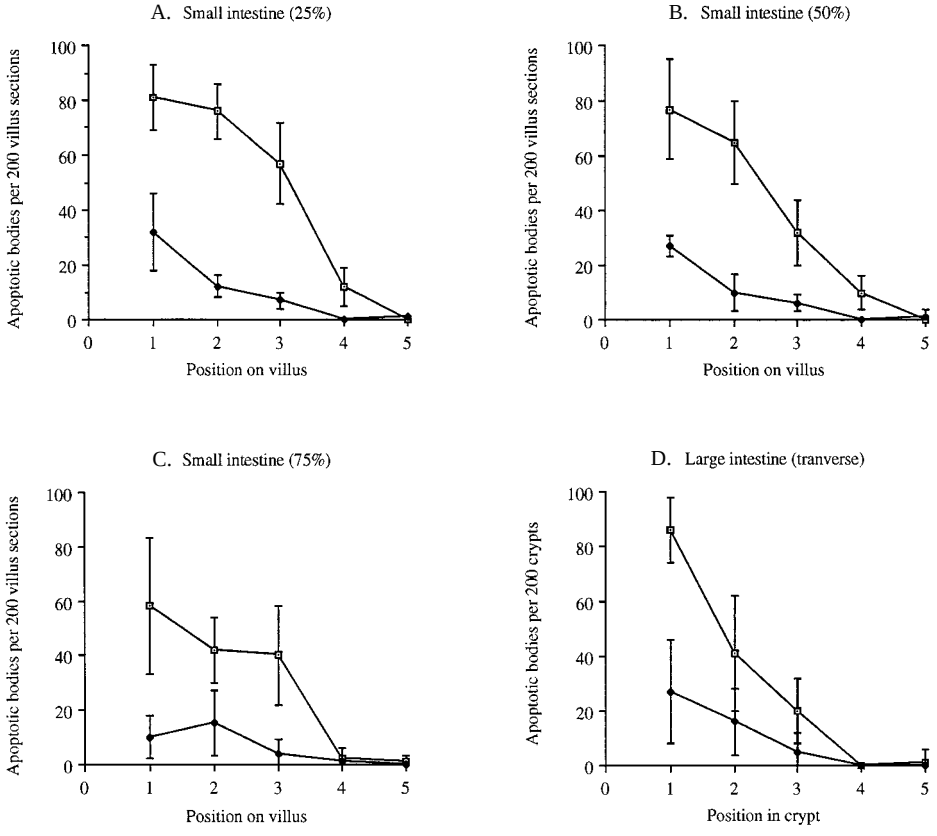

Fig. 3. (A-D) The distribution of apoptotic bodies in the epithelium (□) and sub-epithelial tissues (◆) of the gastrointestinal tract. Quantitation was performed as described in the text and the distribution of apoptotic bodies expressed in relation to position on the small intestinal villus in the colonic crypt (1 top quintile, 5 bottom quintile). Each point represents the mean of three animals (± s.d.).

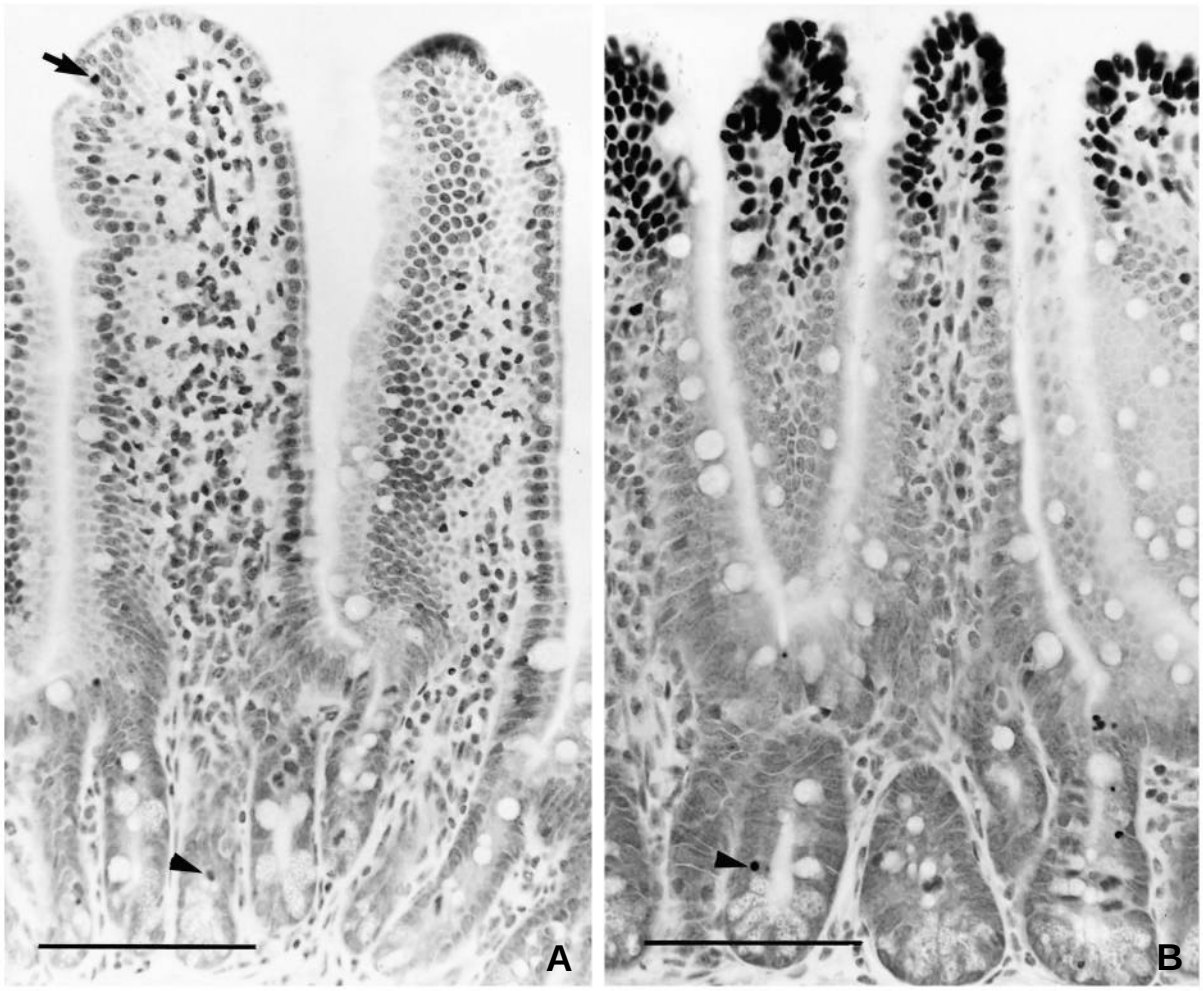

Fig. 4. Optimally prepared and fixed small intestine shows only very occasional detectable ISEL labelling (arrow, A), although if fixation is delayed the most superficial enterocytes show strong labelling (B). In both (A) and (B), ISEL-labelled apoptotic bodies are present in crypts (arrowhead). Bar, 200 μm.

DISCUSSION

The lining of the gastrointestinal tract is replaced every two to three days in rodents and other mammals (Wright and Alison, 1984). This massive turnover is maintained by a spatially constrained cell production and well characterised directional fluxes. After exiting the proliferative compartment the intestinal epithelial cells differentiate, mature and become functional, with important roles in absorption, secretion and the maintenance of a selectively permeable barrier estimated to cover between 15 and 21 m^2 in man (Davenport, 1977). Clearly, the cell production must be exactly balanced by cell loss in order to maintain homeostasis. While a great deal is known about cell production in the gut, much less is understood about cell loss (Potten and Hendry, 1983; Wright and Alison, 1984; Potten and Loeffler, 1990). The mechanism of cell loss is widely held to be some form of exfoliation from the superficial portion of the small intestinal villus, the top of the colonic crypt and inter-crypt epithelium and the most superficial aspect of the stomach. This view is based upon cytological analysis of small intestinal washings, where 85% of the exfoliated cells were epithelial (Pink et al., 1970), estimation of DNA loss (Croft et al., 1968), direct observation of canine small intestine delivered into a laparotomy wound and pinned to a cork board (Creamer et al., 1961), and direct inference from the known high turnover rates in the gastrointestinal tract (Messier and Leblond, 1960; Cheng and Leblond, 1974; Tsubouchi and Leblond, 1979; Fereirra and Leblond, 1971; Yeomans and Trier, 1976). Critical examination of these studies suggests that the evidence is not compelling, and the studies do not provide any indication of the quantitative importance of epithelial shedding. Furthermore, the frequently cited electron micrographic study by David (1967) actually shows a pyknotic cell leaving the intact epithelium and it is argued that 'under normal conditions the desquamation takes place *after the components of the cell degenerate and the whole cell shrinks with the development of pyknosis* without the continuity of the epithelial covering being broken' (our italics). Finally, epithelial shedding (sic) is seen in poorly preserved specimens.

Cell loss by exfoliation is thus not a compelling explanation for the massive physiological loss of epithelial cells from the superficial aspect of the gastro-intestinal tract. Furthermore, it

is difficult to believe that the loss of epithelial cells from the downward fluxes that exist in the gut can be by this mechanism. In the stomach and small intestine there is a continual downward migration of cells from the putative stem cell zones. In addition the proximal colon, at least in the rat, also has a bi-directional flux with a downward component (Sato and Ahnen, 1992). What is the fate of these cells? A similar problem is faced when considering the fate of the pericryptal fibroblast sheet. This poorly understood population of cells has a proliferative architecture similar to that of the gastro-intestinal epithelium and indeed its migration rates are approximately identical. Consequently the production and subsequent flux of these cells must be exactly balanced by cell loss. Loss by shedding is difficult to accept, since such alterations in the integrity of the mucosal lining would be osmotically, nutritionally and microbiologically disadvantageous for the organism. Loss by further migration of the fibroblasts to deep sites in the mucosa (perhaps via the central portion of the villus) seems improbable and does not resolve the issue. While discontinuities of the epithelial lining of the gut can be seen in pathological states and in poorly prepared specimens, the identification of sloughing epithelial and or fibroblast cells is not encountered. What then can be the explanation for the cell loss seen in the gut?

Apoptosis is well known to occur within the proliferative compartment of the small intestine at a low spontaneous rate that is increased after genotoxic insults in a p53-dependent manner (Potten 1977; Potten et al., 1990; Merritt et al., 1994). In the colon there is also a low spontaneous rate of apoptosis in the lower parts of the crypt and this is also increased after genotoxic insult (Pottten et al., 1992; Merritt et al., 1994). Apoptosis is not generally recognised elsewhere in the gut epithelium or in the lamina propria (including in the villus core), neither is its quantitative importance apparent. Recently, Lee (1993) reported that apoptosis could be indentified in a range of drug-induced pathologies in the colon. As part of these studies he quantitated the amount of apoptosis in the normal human colonic mucosa and observed 0.94±0.5 (s.e.) apoptoses per colonic crypt. Similar figures (0.62±0.15 (s.d.)) were reported by Walker et al. (1988), who also noted apoptosis in the lamina propria (0.84 ± 0.14 (s.d.)). In the small intestine we have only been able to find a single previous report of apoptosis in villi. Benneddetti et al. (1990) described the occurrence of apoptotic bodies in the duodenal villi (3±1 (s.d.)) and stomach (1.4±1.1 (s.d.)) of young rats.

The data presented in this paper indicate that apoptosis occurs in the non-proliferative compartments of the gut in a spatially regulated manner. The evidence that it is indeed apoptosis that we are assessing comes from the detailed morphological analysis of both wax- and resin-embedded material, coupled with the evidence from use of the in situ end-labelling method. The absence of CD45 immunoreactivity associated with the apoptotic bodies strongly suggests that lymphocyte apoptosis is not the major contributor to this cell death. The amount of apoptosis, as judged by apoptotic bodies, seen in rodent and human small intestine was similar and comparable to the previous reports. Apoptosis was also seen in the lamina propria, immediately beneath the epithelium and thus may account for fibroblast cell loss. The distribution of apoptosis in both epithelium and lamina propria was not uniform and quantitatively increased with position up the villus or along the colonic crypt. This suggests that apoptosis occurs with increasing frequency with increasing distance from the proliferative compartment. In keeping with this, the distribution in the human stomach was biphasic with a small second peak in the gland base, consistent with the known bi-directional flux in this tissue. No identifiable second peak was seen in the small intestine, presumably since such a peak would be small and difficult to resolve from the level of apoptosis seen in the proliferative compartment. It should also be noted that the observed amount of apoptosis as assessed by numbers of apoptotic bodies may be an underestimate of the true value because of the difficulty of distinguishing with absolute confidence between apoptotic bodies and intra-epithelial lymphocytes. Even using the ISEL technique to identify apoptotic bodies provides an underestimate (Ansari et al., 1993).

If apoptosis occurs in the gastrointestinal tract, how significant is it to the maintenance of cell number in this tissue? As stated above the data presented here should be considered as a conservative estimate of the amount of apoptosis. Since in the mouse about 1200 epithelial cells migrate onto a villus per day (Potten and Hendry, 1983), then an equal number must be lost. Only an average of 1 apoptotic body is seen in the villus epithelium per murine villus section. However, this section is only 3 μm in thickness while a villus is between 120 and 150 μm (138±15 μm) in diameter, thus there will be, at any given instant, about 40 to 50 apoptotic bodies on a villus. These structures are cleared very rapidly, and possibly in less than one hour (Barres et al., 1992; Coles et al., 1993), thus in a 24 hour period there may be 960 to 1200 apoptotic bodies per villus: approximately equal to the villus influx. The amount of apoptosis in the villus epithelium differs between different parts of the small intestine, reflecting the differences in net villus influx previously reported (Wright and Alison, 1984). Similar calculations are possible in the transverse colon where between 2.7 and 8.8 cells are produced per crypt per hour (depending on method employed; Wright and Alison, 1984) and where we observe 0.74±0.15 apoptotic bodies per murine crypt section. Since a crypt is 50±5 μm in diameter there are about eight apoptotic bodies per colonic crypt. The observable apoptosis can, under normal conditions, therefore explain much (and possibly all) the cell loss in the murine small intestine and colon. That some loss of cells (or fragments thereof) occurs by shedding in vivo cannot, however, be denied (Creamer et al., 1991; David, 1967; Croft et al., 1968), since even in closed experimental systems (Del Buono et al., 1992) some cellular mass accumulates in the lumen. What is not clear is the origin of this nor its quantitative significance in terms of epithelial homeostasis, although it may be quantitatively significant (Croft and Cotton, 1973). It would seem to us that the numerical analysis presented above provides compelling support for apoptosis having a central role in the regulation of cell number. The clearance of apoptotic bodies appears to occur by engulfment by adjacent epithelial cells in many cases, although macrophages may be involved in the removal of subepithelial apoptosis and some shedding probably occurs (David, 1967).

Gavrieli et al. (1992) reported that DNA strand breaks typical of apoptosis could be recognised along the entire length of the small intestinal villous epithelium using DNA end-labelling. They interpreted this to indicate apoptosis in the small intestine. Using a similar method we also found staining

of these cells (Ansari et al., 1993), but considered their results to reflect an artifact due to sub-optimal fixation since the cells did not show any of the morphological features of apoptosis. Our experimental data, and the recent observations of Polzar et al. (1994), support this idea, since the number, intensity and extent of labelling with the ISEL method increases with delayed fixation. Careful examination of optimally prepared material does, however, indicate the presence of ISEL-positive apoptotic bodies in numbers comparable to those seen both in the resin preparations and standard wax-embedded sections. How then can we interpret the changes reported by Gavrieli et al. (1992) and also the alterations associated with delayed fixation? It may be that the cells of the villus are destined to die but that their lifespan is extended by factor(s) delivered to the villus. After death (or removal from the intact animal) loss of viability proceeds and autolysis occurs. The ability to survive this stress is then inversely proportional to the probability of the cell dying by apoptosis. The villus tip cells being closer to apoptotic death are less resistant to autolysis than the lower villus cells, suggesting regulation of apoptotic process.

At present we can only speculate as to the mechanisms that regulate cell loss in the intestine, although it has been reported that levels of a candidate apoptotic endonuclease (DNase I) increase towards the tip of the villus (Polzar et al., 1994). The possibility that the regulation is intrinsic, perhaps due to decay of some critical molecule to below a triggering threshold, has been indirectly investigated. Using the intestinal fatty acid binding promoter to regulate expression of SV40 large T antigen, Hauft et al. (1992) induced a second wave of proliferation on the villus. If the initial level of the relevant molecule is defined at the end of the proliferative compartment then the re-inititiation of proliferation might be expected to increase villus length. In these experiments the resultant villi were not dis-proportionately long. While far from conclusive these data do not support an intrinsic regulation model. An alternative model would be extrinsic regulation, and spatially regulated expression of survival factors is an attractive notion. Spatially regulated expression of potential survival factors including TGFα, the EGF receptor and other peptide growth factors have been described. In addition, spatially regulated gradients of TGFα expression along the small intestinal crypt villus axis (Barnard et al., 1990) and colonic crypt (Avery et al., 1993) have been reported. Previous authors have reported alterations in cell adhesion molecules or substratum along the crypt villus axis, and used this to support the idea of cell loss by shedding. For example, Beaulieu (1992) has proposed that differential expression of β_1 integrins along the crypt villus axis facilitates cell loss. Probsteimer et al. (1990) have argued that cell shedding at the villus tip is a consequence of altered expression and function of J1/tenascin at the upper part of the villus. However, recent work relating to the possible role of adhesion molecules as components of the survival system cast a new perspective on these data. Frisch and Francis (1994) have proposed that interactions between matrix and cell are critical to regulation of apoptosis. Similar observations have come from the work of Streuli and colleagues who have shown that primary and secondary cultures of mammary epithelial cells are dependant on extra-cellular matrix for survival (S. Pullan, J. Wilson, J. Tilly, J. A. Hickman, C. Dive and C. H. Streuli, personal communication). It may also be relevant that signalling via adhesion molecules is now well established (Doherty and Walsh, 1994). Thus a model might be proposed where alterations in expression of adhesion molecules and/or other cell substratum interactions, including associated peptide growth/survival factors have an effect on reducing the probability of survival of cells on the crypt-villus axis. Such interactions might also act on the peri-crypt fibroblast sheath. Our inability to effectively culture gastrointestinal epithelial cells might reflect our poor understanding of these interactions and our inability to recreate optimal conditions for survival. Finally, it may be relevant that the product of the APC gene interacts with intra-cellular components of cell adhesion systems (Rubinfield et al., 1993; Su et al., 1993). Could the very mild phenotypic defect in non-lesional mucosa in familial adenomatous polyposis and the subsequent development of adenomata reflect alterations not of proliferation but of survival?

The model of cell number control in gastrointestinal epithelium in which apoptosis has a central role is more widely applicable. Many epithelia have a similar proliferative architecture in which cells stream from a proliferative compartment, along a given vector (or radius) and end at a particular anatomical site e.g. endometrium (Hopwood and Levison, 1976), liver (Zajicek et al., 1988; Bennedetti et al., 1988), adrenal (Zajicek et al., 1986), pancreas (Walker, 1987; Ansari et al., 1993), salivary gland (Zajicek et al., 1985), kidney (Zajicek and Arber, 1991), breast (Strange et al., 1992; Allan et al., 1992)). As with the gastrointestinal tract, the streams have a mesenchymal as well as an epithelial component. While the rate of cell production in different tissues is very variable, and in some it is very low, cell loss must continually occur in a regulated way to balance production. In all cases apoptosis has been reported at the distal end of the pathway. The data presented in this paper, together with these ideas, strongly suggest that apoptosis is a central component of cell number regulation in all epithelia.

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