Commentary

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Cell death – where is thy sting?

Apoptosis or programmed cell death has become a hot topic in the past few years. This occurs when cells turn on a regulated self destruction programme at the end of their functional life, and is distinguished from necrosis: cell death caused by extrinsic factors, such as ischaemia or toxic attack. Apoptosis has been used to attempt to explain a large number of cell processes, such as tumorogenesis (lack of apoptosis) and embryogenesis (tissue remodelling).

The apoptotic process entails a defined set of morphological and biochemical features that are different from those seen with necrosis. These features include the following morphological changes: cytoplasmic blebs appear and the cell shrinks, the nucleus becomes shrunken with margination of chromatin and fragments, and eventually the cell separates into intact, discrete membrane bound bodies that are rapidly phagocytosed. Additionally, the biochemical hallmark of apoptosis is DNA fragmentation that occurs at regular intervals, creating a ladder appearance on agarose gel electrophoresis.

The TUNEL technique used in the paper by Moss *et al* on page 811 takes advantage of the DNA fragmentation by labelling the nicked fragmented ends of the DNA and thereby quantitating the number of cells undergoing apoptosis. Moss and colleagues compare an apoptotic index, using the TUNEL technique, in the mucosa of untreated coeliac disease, treated coeliac disease, and normal small bowel. They found that the apoptotic index of the epithelial cells was increased in coeliac disease, when compared with normal mucosa, and decreased substantially (close to that of normal mucosa) after short-term treatment with a gluten free diet. Additionally, they analysed the epithelial cell proliferation of the same tissue with a cell cycle specific antibody (Mib-1 or Ki-67) and showed that cell proliferation is increased in coeliac disease mucosa and decreases after treatment, although not as quickly as compared with the apoptotic index.

Moss *et al* found that in normal small bowel mucosa, most (65%) of the apoptotic cells were found in the upper third of the villus column, the remainder were seen in the crypt base (21%) and between the villus tip and crypt base (14%). Other reports have also described the majority of apoptotic cells at the villus tips using the TUNEL technique,[1 2] however Merritt *et al*[3] only show apoptotic cells in the base of the crypts using a more stringent TUNEL technique. They state that the staining seen in the villus is 'the result of an excessive sensitivity of the preparations'. The TUNEL technique is difficult to use in the intestine and number of cells labelled is dependent on conditions used.[2 4] Analysis of tissue sections by light microscopy is inconclusive as well, with different authors supporting their findings with the TUNEL assay morphologically,[2 3 5] and the paper by Moss *et al.* Ultrastructural analysis would tend to support the view that apoptotic cells are primarily found on the end of the villus.[5 6] While the issue is not clear, the traditional maturation theory of small enterocytes, with proliferation of cells in the crypt, maturation along the villus, and finally cell loss at the villus tips would also support the expectation of greatest apoptotic activity at the villus tip.

The controversy regarding apoptotic activity in the normal small bowel should not change the finding of increased apoptotic activity in coeliac disease by Moss *et al.* Even if the TUNEL assay used is too sensitive as Merritt *et al*[4] state, comparison of an apopototic index using the same technique should still be valid (even with a 'increased baseline').

The most important variable in producing the final morphological and functional picture in coeliac disease is the cell mediated response to gluten. The understanding of apoptosis may provide an essential link to explain the profound epithelial abnormality in what is primarily an immunological disease.[7] Future studies might look at the signalling mechanisms involved with the increased apoptosis in coeliac disease. Apoptosis is a highly regulated process that is believed to be triggered by signalling through cytoplasmic receptors or by cell surface membrane. Numerous signalling substances such as glucocortioids,[8] ceramide,[9] monoclonal antibodies to specific cell surface molecules such as Fas[10] and lectins[11] have been implicated in triggering apoptosis.

Additional insight into the regulation of apoptosis and its role in the production of, or maintenance of mucosal injury might come from studying other disease models. The most frustrating of these clinically is refractory coeliac disease.[12] Although uncommon, these patients pose a chronic clinical challenge. Many of them have lesions indistinguishable from either untreated or partially treated coeliac disease. It would be of interest to see if their interaction between proliferative and apoptotic activity is unique in any regard in relation to the kinds of data in the findings of Moss and colleagues.

In a parallel vein presumed hypoproliferative or normoproliferative 'flat' lesions also provide a model to study apoptosis and its regulation. Examples include microvillus inclusion disease, and the lesions induced by chemotherapy, folate or B12 deficiency.[13] The mucosal lesions in these disorders often exhibit a 'flat' or patchily abnormal mucosa with only modest lamina propria inflammation and no apparent increase in mitotic figures in relation to comparably appearing lesions seen in coeliac disease.

Finally, one cannot help but wonder if the regulation of apoptosis were understood and altered, could it result in treatments to improve small bowel structure and function in diseases associated with either a hyper or hypoproliferative state?

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