Leading article

Peptides and gastrointestinal mucosal integrity

The gastrointestinal epithelium plays a vital part as a barrier against luminal acid, proteolytic enzymes, and ingested noxious agents. This barrier function is dependent on constant renewal of the epithelium and, in the stomach and colon, on the presence of an overlying adherent mucus gel. The epithelium of the gastrointestinal tract is one of the most rapidly proliferating tissues in the body with a dynamic equilibrium existing in the rates of cell production, migration, and surface shedding. When a mucosal injury occurs, it is generally rapidly repaired by a combination of increased cell migration and proliferation.

Current research implicates a variety of peptides as central to the maintenance and restoration of gastrointestinal mucosal integrity. It has been difficult to understand, however, the function of these individual peptides in the overall control of mucosal integrity and repair because the site of production, mechanism of action, and change in concentration in areas of mucosal injury varies according to the peptide examined. It is therefore useful to categorise these peptides according to their function in vivo:

Mucosal integrity peptides – such as transforming growth factor α and pancreatic secretory trypsin inhibitor, which are constitutively expressed in the mucosa throughout the gastrointestinal tract and which function to maintain normal mucosal integrity.

Luminal surveillance peptides – such as epidermal growth factor, which is continuously secreted into the lumen but is probably only of major importance in the stimulation of mucosal repair following a breach in the mucosa.

Rapid response peptides – such as spasmolytic polypeptide, a member of the trefoil peptide family, whose production is rapidly upregulated at sites of damage and is probably of particular importance in the early stages of mucosal repair.

Transforming growth factor α as a mucosal integrity peptide

Transforming growth factor α (TGFα) is made in the mucosa throughout the gastrointestinal tract[1] and is synthesised as a 160 amino acid precursor molecule that spans the cell membrane. Subsequent exposure of the external domains to specific proteases releases a soluble 50 amino acid form. The biological function of the membrane bound form is unclear, but might function in a paracrine manner to stimulate the common epidermal growth factor (EGF)/TGFα receptor on adjacent cells. TGFα is trophic to a variety of cell lines in vitro and to the intestine of rats when given systemically.[2] In addition, administration of TGFα decreases the amount of gastric damage that occurs when rats are exposed to ulcerogens such as ethanol or stress.[3] Taken together, these studies suggest that the function of TGFα within the mucosa is to maintain normal epithelial integrity. It should be noted, however, that TGFα immunoreactivity is predominantly located in the upper, non-proliferative, regions of the glands, which suggests its major actions may be on cell differentiation and migration rather than on cell division.

Pancreatic secretory trypsin inhibitor (PSTI) as a mucosal integrity peptide

PSTI is a 56 amino acid protein that potently inhibits trypsin and other serine proteases. It was originally isolated from the pancreas but has subsequently been identified in mucus producing cells throughout the gastrointestinal tract (Fig 1). It is the only protease inhibitor known to be secreted into the intestinal lumen.[4] PSTI provides an interesting contrast with TGFα as a mucosal integrity peptide in that its predominant role is probably to prevent excessive digestion of gastrointestinal mucus. The mucus layer forms a continuous viscoelastic gel in the stomach and colon. This probably functions to lubricate the passage of food, to maintain the surface of gastric epithelial cells at a neutral pH (in combination with bicarbonate secretion), and to act as a barrier to the passage of bacteria in the

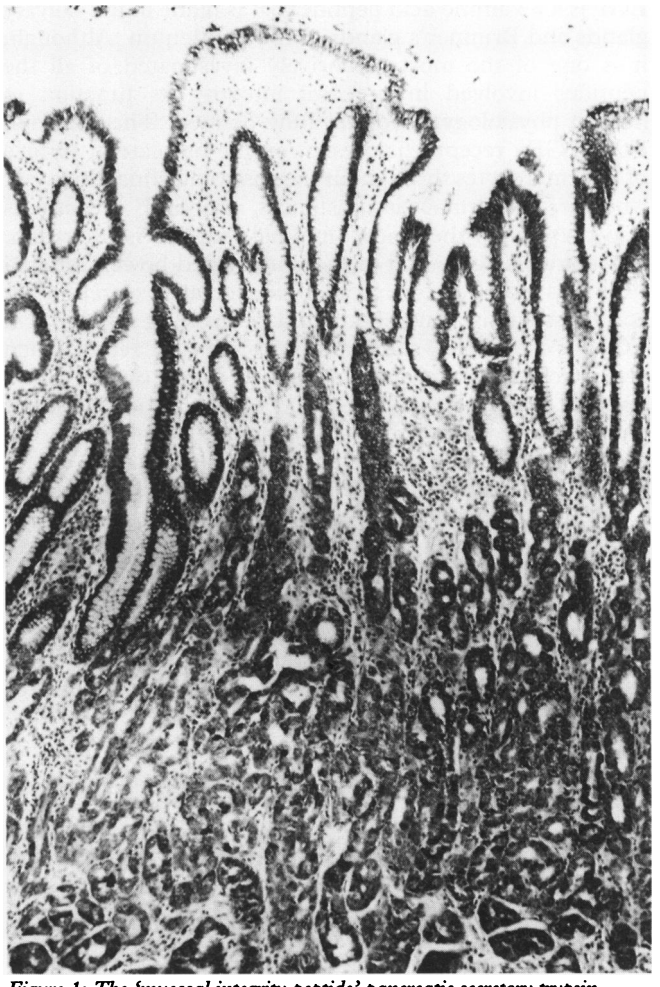

Figure 1: The 'mucosal integrity peptide' pancreatic secretory trypsin inhibitor (PSTI) is secreted by mucus producing cells throughout the gastrointestinal tract. In the gastric body, PSTI immunoreactivity is localised to the surface, foveolar, and mucous neck cells. Patients with atrophic gastritis or gastric ulcer, or both, have a 70% reduction in tissue activity of PSTI.[5] This reduced anti-protease activity may explain the abnormalities of mucus found in these patients. Figure reproduced from reference 5 with the permission of Gastroenterology.

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colon. Under normal circumstances, the mucus layer is in a state of dynamic equilibrium with continuous luminal loss from shearing and proteolysis being balanced by new production from the mucus producing cells. The mucus layer is abnormal and structurally weaker in the stomachs of patients with atrophic gastritis or gastric ulcer, or both, and in the colon of patients with colitis. These changes probably reflect an imbalance in protease/antiprotease activity acting on the mucus gel. In patients with gastric ulcer this imbalance results from reduced antiprotease (PSTI) production,[5] possibly in combination with increased proteolysis resulting from duodeno-gastric reflux. A similar imbalance occurs in the colon of patients with colitis resulting from reduced colonic PSTI production in combination with increased luminal protease activity, probably of bacterial origin.[6 7] The function of PSTI in gastrointestinal mucus can therefore be considered analogous to α_1 antitrypsin in respiratory mucus as a deficiency of α_1 antitrypsin results in lung disease. In addition to its antiprotease activity, PSTI has been shown to stimulate growth of several cell lines[8] possibly acting via the EGF receptor.[9] This suggests that PSTI may be important in directly maintaining mucosal integrity in a similar way to TGFα as well as by its mucus protecting role.

EGF as a luminal surveillance peptide

EGF is a 53 amino acid peptide that is made in the salivary glands and Brunner's glands of the duodenum. Although, it is one of the most extensively investigated of all the peptides involved in mucosal integrity its function in human physiology is not yet fully defined. The common EGF/TGFα receptor is present on the basolateral surface of the enterocytes throughout the gastrointestinal tract, but whether, in normal circumstances, the EGF receptor is also present on the apical (luminal) surface is uncertain. Most studies agree that in the undamaged bowel, luminal EGF has no effect on proliferation or acid secretion. In contrast there is a large body of data showing that luminal EGF is capable of stimulating growth and repair when given to the damaged bowel of both rats[10 11] and humans.[12] Endogenous EGF seems to play an important part in the healing of chronic gastric ulceration as removal of the submandibular glands of rats significantly delays healing.[13] Additional studies are clearly required to discover if luminal EGF has any effects in the undamaged intestine of humans, particularly in view of our recent finding that EGF is susceptible to proteolytic digestion in both the stomach and small intestine.[14 15] Most groups would agree, however, that the predominant function of EGF is to act as a 'luminal surveillance peptide', which is readily available to stimulate repair at sites of gastrointestinal damage.

Spasmolytic polypeptide as a rapid-response peptide

Spasmolytic polypeptide is a member of the 'trefoil peptide' family, which derived their name from their unusual cysteine-rich 'three leaf' structure. Three members of this family have been identified in humans; pS2, spasmolytic polypeptide, and intestinal trefoil factor.[16] The production of all three trefoil peptides is considerably upregulated at sites of gastrointestinal damage in conditions such as peptic ulceration (Fig 2) and inflammatory bowel disease. There is evidence that trefoil peptides participate in mucosal repair: when a mucosal injury occurs, one of the earliest repair processes is the migration of surviving cells from the edge of the damaged region over the denuded area tore-establish epithelial continuity, a process termed 'epithelial restitution'.[17] Restitution is not dependent on cell division as restitution occurs within the first few hours after an injury but the mitotic rate does not increase until much later; one to two days after the injury. Recent studies suggest that trefoil peptides, particularly spasmolytic polypeptide, play a key part in this process of restitution: the production of spasmolytic polypeptide is considerably upregulated within 30 minutes of injury in animal models of gastric damage[18] and exogenous human spasmolytic polypeptide increases cell migration in in vitro models of cell wounding[19 20] and also acts as a cytoprotective agent in rats treated with the ulcerogenic agent indomethacin.[19]

Lesser known peptides

There is considerable interest in the role of the family of peptides designated TGF β in the regulation of cell proliferation, differentiation, and cell migration. They are structurally distinct from TGFα and, in mammals, three isoforms have been identified designated TGF β1, 2, and 3. All three TGF β isoforms are potent inhibitors of proliferation in vitro and in vivo. Studies using rat intestine have shown all three forms are expressed in the intestinal epithelium in a vertical axis gradient such that the proteins are virtually undetectable in the proliferating crypts but are highly expressed at the villus tips (Beauchamp *et al*, conference proceedings, Colorado, 1994). This suggests that TGF β may be important in preventing proliferation of cells once they have left the crypts. TGF β is also a potent stimulant of cell migration and it is of interest that the

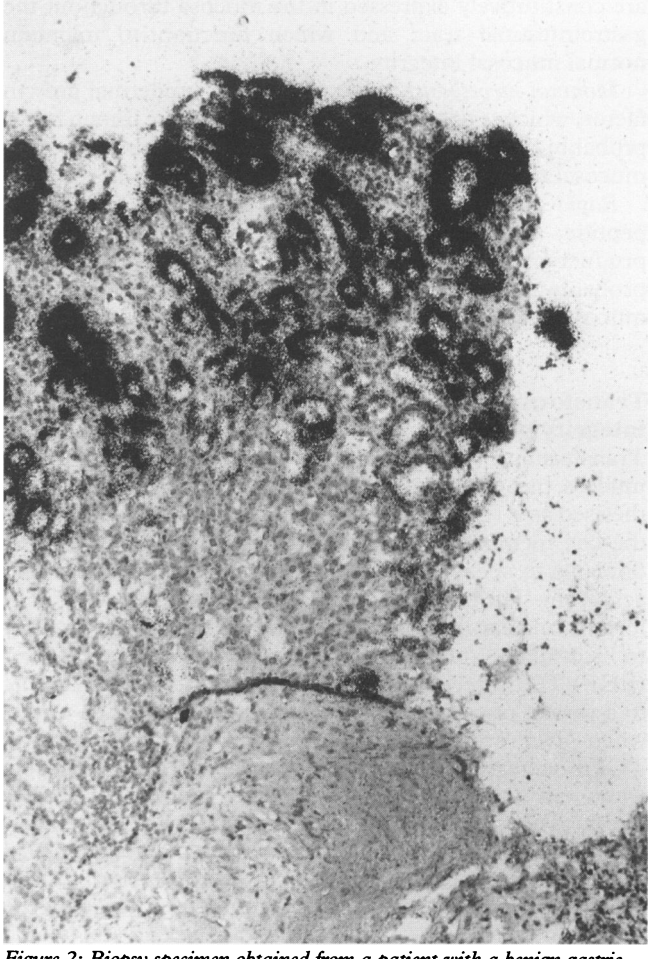

Figure 2: Biopsy specimen obtained from a patient with a benign gastric ulcer. In situ hybridisation studies show the presence of large amounts of the 'rapid response peptide' spasmolytic polypeptide mRNA at the ulcer edge. Animal studies suggest that this increase occurs within 30 minutes of an acute gastrointestinal injury.

stimulation of cell migration induced by TGFα and EGF, but not trefoil peptides, is dependent on the release of TGF β.[19-21]

Basic fibroblast growth factor (bFGF) is normally present in the human gastric mucosa and is a potent stimulant of blood vessel formation. Preliminary studies suggest that a recombinant (acid stable) form of bFGF stimulates ulcer healing in humans.[22] Although its overall importance in mucosal homeostasis has still to be defined, it may function as a 'mucosal integrity peptide.' This idea is supported by the recent finding that mucosal concentrations of bFGF in the stomach of patients with healed ulcers are lower than normal, possibly pointing to a weakened mucosal defence.[23]

Interrelations between peptides

This categorisation provides a useful framework for considering the role of these peptides in vivo, however, their functions are often interrelated, for example, expression of the trefoil peptide pS2 at sites of mucosal damage may be mediated by luminal EGF as the pS2 gene has an EGF responsive element.[24] It is therefore possible that the overexpression of pS2 in enterocytes surrounding areas of damage results from increased passage of EGF through the damaged mucosa to the basolaterally placed EGF receptor. A further example of this interrelation is that the 'mucosal integrity peptide' TGFα (and possibly PSTI) acts on the same receptor as the 'luminal surveillance peptide' EGF, the difference in categorisation reflects the presence of the mucus barrier and the location of the EGF receptor on the basolateral surface of the enterocyte rather than on intrinsic differences in the molecules themselves. The maintenance of mucosal homeostasis is thus dependent on multiple interrelated factors and when considering their function in vivo, they should be considered as part of a highly integrated system rather than as separate entities.

Conclusions

The role of peptides in mucosal defence and repair is both complex and poorly defined. The proposed classification should increase the understanding of their function in the overall maintenance of mucosal homeostasis and help in experimental design. It also emphasises that these peptides act in an integrated fashion and not in isolation.

Acknowledgement is given to the Medical Research Council for funding RJP as a MRC Clinical Scientist.

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