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Molecular Aspects of Mucosal Repair: A Summary

Steven F. Moss[a] and Nicholas A. Wright[b]

[a]Division of Gastroenterology, Department of Medicine, St. Luke's-Roosevelt Hospital Center, Columbia University, New York, New York and [b]Histopathology Department, Royal Postgraduate Medical School, Hammersmith Hospital, London, United Kingdom

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This paper reviews the current knowledge of the molecular aspects of mucosal repair.

The mucosal lining of the gut, and epithelial cells in particular, undergo constant renewal. New cells are created from precursors in the proliferative zone, and, under the influence of "growth factors," they then undergo maturation and differentiation over the course of around a week as they move toward the lumen. Here they are lost, not by passive cell shedding into the lumen, but by an active process of cell death termed apoptosis, which may be considered the ultimate terminal differentiation event. In the mucosa of the stomach, cells also move downward, at a much slower rate, from the proliferative zone at the base of the gastric pits, down into the glands. This constant and rapid turnover of the gastrointestinal mucosa is, therefore, well suited to the prompt restoration of mucosal integrity following damage. The speakers in this session presented experimental evidence for how this may be achieved. In the discussion that ensued, the main issues were, first, the relative importance of the many factors at play in ulcer healing: is there a molecular "holy grail"? and second, the clinical relevance of the molecular events to the pathophysiology and treatment of clinically important mucosal defects.

Three main healing events occur following the experimental creation of an ulcer. The first is a rapid phase of epithelial restitution during which existing viable epithelial cells at the ulcer edge migrate inward to close the gap. Second, over the next few days, new cells are formed by proliferation to repopulate the mucosal breach. And third, new matrix is laid down, inflammatory cells are replaced by non-epithelial cells in the *lamina propria,* and this remodeling is accompanied by angiogenesis, the growth of feeding blood vessels [1]. Of course, *in vivo*, these events do not occur in this stepwise fashion, particularly when aggressive factors such as *Helicobacter pylori* are present, but nevertheless this model does provide a conceptual framework on which to place the processes of mucosal repair.

The details of the process of restitution were addressed by the first two speakers in this session. Basson presented evidence from a cell culture model of mucosal damage that the migrating epithelial cells are not merely undifferentiated and immature "foot soldiers" but instead have a specialized and polarized phenotype, uniquely adapted to movement. However, acquisition of a specialized migratory phenotype was not necessarily accompanied by loss of brush border enzyme expression (a marker of the differentiated phenotype in these Caco2 small-intestinal-like cells). In his system, a variety of factors including the extracellular matrix, peptides and the more classical growth factors were shown to induce

[a]*To whom all correspondence should be addressed:* S.F. Moss, M.D., Division of Gastroenterology, Department of Medicine, St. Luke's-Roosevelt Hospital Center, 1111 Amsterdam Avenue, New York, NY 10025. Tel: (212) 523-2409; Fax: (212) 523-3683; E-mail: smoss1@mem.po.com.

the differentiation of this migratory phenotype to varying degrees. *In vivo*, multiple factors are released so that the restituting cell population is probably heterogeneous. Interestingly, and of possible therapeutic importance, factors differed in the extent to which they induced the migratory phenotype, and this was not always linked to dedifferentiation. The ideal therapeutic agent may, therefore, be one that promotes migration but yet leaves the intact, healed epithelium relatively differentiated. Although the cells in this model were originally derived from a colon cancer line and have many of the characteristics of small intestinal epithelial cells, there is no reason not to apply these ideas to ulcer healing elsewhere in the gut.

Lacy presented further insights into how rapid epithelial restitution may be modulated. In the mouse stomach, he found that a chronic and repetitive hyperosmolar insult ultimately led to an acceleration of restitution compared with the "unconditioned" mucosa and that this adaptive response was accompanied by a widening of the proliferative zone. Several other intriguing changes in the cell populations within the gastric pits and glands were observed: chronic hyperosmolar insult led to a decrease in parietal and gastrin-secreting (G) cells and a tendency to increased somatostatin-secreting (D) cell number. In addition, a new population of "vesiculated" cells, with some features of immature mucous neck cells by electron microscopy, appeared in the lower third of the gastric gland, in an area previously populated by parietal cells. What is the function of this new population? Lacy and others thought that these vesiculated cells may represent a distinct lineage and were not precursor cells, despite their proximity to the proliferative zone. Indeed, it is tempting to speculate that these cells may be ideally located to promote proliferation and healing, through the local secretion of mucosal repair proteins. It would also explain how chronic superficial injury ultimately leads to a more rapid restitutive response.

What is the role of the neuroendocrine cells in enhancing the mucosal repair process? No clear picture emerged. On a simplistic level, gastrin stimulates gastric mucosal growth while somatostatin inhibits it, so that the lack of somatostatin receptors at the edge of healing ulcers may represent an appropriate response to aid mucosal repair [2]. If gastrin and somatostatin control mucosal growth, do acid-inhibitory drugs heal ulcers through increasing circulating gastrin? This possibility, that it is increasing circulating gastrin concentrations rather than acid inhibition which stimulates ulcer healing, was considered unlikely. In rats, experimental ulcers can be healed with acid suppression induced by giving a potent antagonist of the gastrin/CCKB receptor [3], an agent that produces acid inhibition yet blocks the trophic effect of gastrin on the gastric mucosa. Interestingly, in Lacy's model there was an increased D:G cell ratio accompanying increased epithelial proliferation, which is at variance with the generally-accepted wisdom that circulating gastrin is trophic to the gastric mucosa. It also differs from the situation in human chronic gastritis due to *H. pylori*, where there is increased proliferation yet a decreased D:G ratio. Exploring the role of these gastric peptides *in vivo* is an area of potential importance, both in order to understand the processes of mucosal repair and in terms of the potential for therapy. Obviously, gastrin and somatostatin are not the only growth stimulatory and inhibitory molecules in the gastric mucosa.

Which molecules are the most important players in mucosal repair? This is likely to depend partly upon which part of the gut is injured and the nature of the injury. Playford has recently classified several of the key mucosal repair peptides according to their function [4]. In this classification, epidermal growth factor, which is capable of performing many of the functions essential for good repair, is considered an example of a luminal surveillance peptide. Although continually secreted into the lumen, it is only able to bind to its receptor, located on the basolateral surface of the epithelial cell, when mucosal integrity is breached. On the other hand, pancreatic secretory trypsin inhibitor and transforming growth factor-α are considered mucosal integrity peptides, ensuring normal barrier function. The

third group, the trefoil factors, appear to act as rapid response molecules, upregulated at times of injury. Of particular relevance to the healing process is a distinct glandular constituent — the ulcer associated cell lineage — which produces several repair peptides including trefoil peptides and epidermal growth factor as well as mucus. It has, therefore, been suggested that these cells act as a "first aid kit," pouring healing agents onto the ulcer base. The ulcer-associated lineage is present only at the site of chronic mucosal injury and probably arises from the duct region of metaplastic epithelium, such as intestinal metaplasia in the stomach. Might these be related to the vesiculated cells of Lacy's mice?

Exactly what the trefoils do in ulcer healing is unclear. Currently, this is difficult to study *in vivo* because the recombinant proteins are so expensive to produce. Pretreating rats with human spasmolytic polypeptide causes a moderate reduction in indomethacin-induced gastric damage, less impressive than similar doses of epidermal growth factor [5]. It would be interesting to examine whether they are beneficial if given after the onset of mucosal damage, since the injury itself appears to stimulate endogenous expression so markedly. The data from trefoil peptide transgenic animals are keenly awaited.

The number of trefoil peptides isolated is growing, and evidence was presented that each region of the gut may have its own particular trefoil, as for the mucin genes. Indeed, mucin genes and trefoil expression are linked geographically and, in lower animals, are encoded by the same gene, which may explain their co-expression in mammals. Under normal circumstances, mucin and trefoils are probably coordinately regulated, but perhaps in disease states they may be uncoupled, or produced at a site remote from where they are normally produced, giving rise to the "molecular metaplasia" proposed by Podolsky.

What are the stimuli from the damaged mucosa that promote cell division and repopulation of the gland from the stem cell or its slightly more differentiated progeny? How is this process regulated? Unlike the details of lineage determination and cell differentiation in the bone marrow, these events are much less well understood in the gastric gland. Similarly, we know next to nothing about how apoptosis is regulated in the gut. It will be important to understand what switches on and off epithelial cell proliferation and apoptosis. For example, could gastric atrophy be due to excessive *H. pylori*-induced apoptosis? Is cancer the result of too little apoptosis or too much proliferation?

Although this session mainly discussed the role of epithelial cells in mucosal repair, the role of the non-epithelial cells should not be overlooked. Cells in the *lamina propria* may be important not only in "filling the gap," but they may also be responsible for producing some of the repair molecules. For example, hepatocyte growth factor is produced by gastric fibroblasts in cell culture and appears to be an extremely potent mitogen and motogen for epithelial cells [6]. Cell migration is typically accompanied by a reduction in cell-cell interactions, as highlighted by Pignatelli, and mucosal repair must also involve alterations in the interaction of cells with the extracellular matrix, typically mediated by integrin molecules. In addition, mucosal repair is likely to be dependent upon the regulation of matrix protein synthesis and its degradation by collagenases and metalloproteinases. How this is regulated normally and how this regulation is altered when there is mucosal damage is not well understood. Finally, an important and relatively recently recognized component of the repair process is angiogenesis. Interestingly, basic fibroblast growth factor, which is potently angiogenic as well as being mitogenic to a variety of cell types, also speeds epithelial restitution [7], and this peptide is currently being studied in therapeutic trials [1]. It is noteworthy that sucralfate protects basic fibroblast growth factor from destruction by gastric acid — yet another potential mechanism by which this drug may heal ulcers.

Although most work presented in this session was on the molecular mechanisms of mucosal repair, it is important not to neglect some important clues from studying how mucosal repair occurs *in vivo*. Most chronic gastroduodenal ulceration in man is associated

with chronic infection with *H. pylori* or non-steroidal anti inflammatory drug ingestion and heals spontaneously when these precipitating factors are removed. Furthermore, ulcer healing can usually be achieved even in the presence of these factors by inhibiting acid secretion. What does this tell us about the mucosal repair process? Perhaps the concomitant inhibition of peptic activity which accompanies acid inhibition is important, suggested Hirschowitz. The proteolytic action of pepsin on matrix proteins will delay healing if matrix deposition is involved in mucosal repair. The fact that mucosal repair occurs, albeit slowly, after *H. pylori* eradication alone and without acid inhibition, suggests that the organism itself must inhibit mucosal repair. Dissecting the effect of *H. pylori* from the effect of the accompanying inflammatory process is not easy, which may be why this area has been so far little studied. Epithelial cell function can be modulated by cytokines in many different ways, but *in vivo,* chronic *H. pylori* infection appears to increase epithelial proliferation. The effect of *H. pylori* on restitution, matrix deposition and angiogenesis will be an interesting area to explore.

In conclusion, we should remember that the therapeutic agents that we currently use to heal ulcers were selected empirically, on the basis of symptom relief and healing efficacy *in vivo*. If ultimately we wish to apply the knowledge derived from experimental models and cell culture studies to enhance mucosal repair clinically, we will need to narrow the gap between the more basic sciences and clinical studies. This session discussed some of the areas where this may be occurring and highlighted others that may be promising to explore.

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