

## 7 Summary

### Restitution of single-cell lesions in colon epithelium

Restitution of single-cell defects, a frequent event in epithelia with high turnover, is poorly understood. Morphological and functional changes were recorded in the colonic cell line HT-29/B6 and in native colon from mice, using intravital time-lapse video microscopy, confocal fluorescence microscopy, and conductance scanning techniques.

After artificial single-cell loss from an HT-29/B6 colonic cell monolayer, the basal ends of adjacent cells extended. Concurrently, the local conductive leak (initial: 0.64  $\mu\text{S}$ ; median,  $n = 17$ ) associated with the defect sealed with an exponential time course (from 0.45  $\mu\text{S}$ , 2 min post lesion, to 0.16  $\mu\text{S}$ , 8 min post lesion). Between 3 and 10 min post-lesion, a band of actin arose around the gap, which colocalized with a ring of ZO-1 and occludin. Hence, tight junction proteins bound to the actin band facing the gap, and competent tight junctions assembled in the adjoining cell membranes forming an intact epithelial barrier.

Closure and sealing were inhibited when actin polymerization was blocked by cytochalasin D, delayed following decrease of myosin-ATPase activity by 2,3-butandione monoxime, and blocked after myosin light chain kinase-inhibition by ML-7. The Rho-associated protein kinase inhibitor Y-27632 did not affect restitution. After loosening of intercellular contacts in low  $\text{Ca}^{2+}$  Ringer's, the time course of restitution was not significantly altered. Albeit epithelial conductivity was 12-fold higher in low  $\text{Ca}^{2+}$  Ringer's than in controls, under both conditions the repaired epithelium assumed the same conductivity as distant intact epithelium.

In conclusion, epithelial restitution of single-cell defects comprises rapid closure by an actinomyosin purse-string mechanism and simultaneous formation of a functional barrier from tight junction proteins also associated with the purse string.

It is known that growth factors (e.g. EGF) often stimulate migration and proliferation during restitution of larger defects. The investigated growth factor EGF had no effect on single-cell restitution. Thus, EGF does not modulate the actinomyosin purse-string mechanism.

For the first time, measurements of single-cell restitution were investigated in native mouse colon. Restitution in native colonic tissue was much faster than in the investigated cell model HT-29/B6. Between the beginning of the measurement (1.5 min post lesion) and 2.5 min after setting the lesion, the conductance,  $g^{leak}$ , decreased by 92 % (from 7.84 to 0.73  $\mu$ S; n = 13).

The effect of the proinflammatory cytokine TNF- $\alpha$ , which is found to be increased in patients with chronic inflammatory bowel diseases, on single-cell restitution was investigated. With TNF- $\alpha$  alone restitution was delayed in the cell model HT-29/B6. In native colonic tissue, only TNF- $\alpha$  together with IFN- $\gamma$  delayed single-cell restitution. Only 74 % (from 9.83 to 1.56  $\mu$ S; n = 10) of the defect were repaired within the time course from 1.5 min to 2.5 min after setting the lesion. After TNF- $\alpha$  incubation alone, the time course of restitution was not changed.

This results demonstrate that cytokines released during inflammation, TNF- $\alpha$  and IFN- $\gamma$ , delay the recovery of barrier function after the loss of an epithelial cell. This adds to other effects of the cytokines that are known to impair epithelial barrier function, e.g. apoptosis, ion secretion and the alteration of structure and function of the tight junctions.

Because the cytokines are released during inflammatory bowel diseases, impairment of epithelial restitution must be assumed to aggravate diarrheal mechanism in inflammatory bowel diseases. Thus, therapy of IBD by TNF- $\alpha$  antibodies additionally works by accelerating the repair mechanisms in the inflamed colon.