

## Proefschriftsamenvatting I. Renes, Rotterdam

### **The intestinal epithelium during damage and regeneration: *Cell type-specific responses in experimental colitis and after cytostatic drug treatment.***

The thesis of Ingrid consists of 2 parts; the first part deals with the colonic functions of rats during acute inflammation induced by dextran sulfate sodium (DSS) treatment, and the second part describes the small intestinal functions of rats after treatment with the cytostatic drug methotrexate (MTX). There are several remarkable similarities between the DSS-model and the MTX-model concerning the characteristics of the induced intestinal damage and the control of this damage. DSS is a toxic agent that inhibits proliferation, induces apoptosis, and elicits a strong inflammatory reaction. As a consequence, DSS treatment leads to loss of crypt and surface epithelium and ulcerations. The cytostatic agent MTX primarily inhibits proliferation and induces apoptosis, leading to loss of crypts and surface epithelium. MTX does not primarily induce an inflammatory reaction. The colonic epithelium and the small intestinal epithelium respond to these alterations in several ways. There are at least 3 main points of similarity in the response of the small intestinal and colonic epithelium, respectively. **Firstly, both colonic and small intestinal damage lead to flattening of epithelial cells lining the crypts and surface cuffs/villi implying the occurrence of epithelial restitution.** It is clear that epithelial restitution is of utmost importance to both maintenance of the epithelial barrier as well as restoration of the epithelial barrier. As such epithelial restitution can be divided in two fundamental different processes: 1) flattening of cells to **maintain** the epithelial barrier and 2) flattening of cells to **restore** the epithelial barrier. Maintenance of epithelial barrier occurs during inhibition of epithelial proliferation and induction of apoptosis, before ulcers are present. This process is seen during DSS treatment and after MTX treatment. Restoration of the epithelial barrier takes place when ulcers are present, thus at the end of and after DSS treatment.

**Interestingly, the flattening of epithelial cells coincided with the down-regulation of most of the enterocyte specific differentiation markers** (*i.e.* SI, SGLT1, Glut2 and -5, and i- and l-FABP in the small intestine after MTX treatment, and CA I and -IV, NHE2 and -3 and l-FABP in the colon during/after DSS treatment). We hypothesize that down-regulation of these genes/functions that are less essential for survival of the cells during/after a severe insult is programmed, *i.e.* a hardwired genetic reaction of the epithelium, to conserve energy to survival of the cells and ultimately for survival of the organism. In this way the conserved energy can be used to induce a phenotypic shift of the enterocytes to prepare them for a critical role in epithelial restitution and mucosal defense. Supportive to this hypothesis is the fact the down-regulation of the differentiation markers by MTX and DSS is gene specific, *i.e.* the genes are down-regulated in different extent and/or at different time points. Furthermore, the maintenance of AP activity by the enterocytes in both epithelial damage models also supports the hypothesis described above.

**The second response of the epithelium, which occurs after colonic and after small intestinal damage, is a rapid hyper-proliferation to restore epithelial barrier function.** The hyper-proliferation after an epithelial insult is most likely a general

mechanism of the small intestine as well as colon to restore epithelial barrier function, because it is seen in *in vitro* epithelial wound models, in epithelial damage models, in both of our epithelial damage models, and in patients with UC. In the DSS colonic damage model epithelial hyper-proliferation is seen while DSS is still administered and only in severely damaged areas (*i.e.* in crypts adjacent to ulcerations). This is fundamentally different from the small intestinal damage induced by MTX, where hyper-proliferation is equally distributed along the entire epithelium.

**The third response of the colonic and small intestinal epithelium to damage is maintenance of goblet cell-specific Muc2 and TFF3 expression after damage.** Moreover, goblet cells accumulated in the surface/villus epithelium and/or in the crypts during/after DSS treatment and after MTX treatment. This indicates that goblet cells are selectively spared from apoptosis and extrusion and that epithelial cells preferentially differentiate along the goblet cell lineage after severe epithelial damage. As a consequence of the goblet cell accumulation in the surface/villus epithelium and/or the crypts during/after DSS treatment and after MTX treatment, the expression levels of goblet cell-specific Muc2 and TFF3 are maintained or even up-regulated. These data imply that goblet cells and in particular Muc2 and TFF3 are of critical importance to control and/or restore epithelial damage induced by DSS and MTX.

In summary, we conclude that both the small intestine and colon have at least 3 specific mechanisms to control epithelial damage after an insult: 1) epithelial restitution concomitant with down-regulation of most of the enterocyte-specific differentiation markers and maintenance/up-regulation of epithelial defense, 2) epithelial hyper-proliferation, 3) maintenance of goblet cell-specific Muc2 and TFF3 expression after damage in combination with goblet cell sparing and preferential differentiation into goblet cells. These epithelial responses after an intestinal insult are most likely genetically hardwired responses for survival of the intestine and ultimately for the survival of the organism. In other words the evolution has led to ‘concerted’ reactions of the intestine after an epithelial insult, that together lead to the best result, *i.e.* survival of the organism.