IMMUNE TOLERANCE

Arming the front line with TLR9

...the separation of apical TLR signals from basolateral TLR signals ... could contribute to homeostasis in the intestinal tract.

Differential signalling from Toll-like receptor 9 (TLR9) on the apical and basolateral surfaces of intestinal epithelial cells (IECs) might provide one explanation for the normal immune tolerance to commensal bacteria in the gut, according to a study in *Nature Cell Biology*. IECs are highly polarized structures as a result of the markedly different environments that they interact with at the apical surface (the microflora of the intestinal lumen) and the basolateral surface (the lamina propria). Lee *et al.* investigated how such polarity affects the immune response to TLR ligands.

Polarized IECs secrete interleukin-8 (IL-8) into the basolateral environment after basolateral, but not apical, TLR9 stimulation with the ligand ISS-ODN (immunostimulatory sequence oligodeoxynucleotide). Consistent with this, ISS-ODN activated nuclear factor- κ B (NF- κ B) from the basolateral

surface only. The authors showed that TLR9 is expressed on both basolateral and apical surfaces of IECs (compared with its endosomal location in immune cells) and that TLR9 ligands can be taken up both apically and basolaterally. Therefore, the differential response to ISS-ODN must result from different signalling pathways in response to apical and basolateral TLR9 stimulation.

Whereas IKK (I κ B kinase) activation and I κ B α (inhibitor of NF- κ B) phosphorylation and ubiquitylation were similar in response to apical and basolateral TLR9 stimulation, I κ B α was not degraded by the proteasome after apical TLR9 stimulation. This stabilization of I κ B α prevented NF- κ B activation in apically stimulated cells. The NF- κ B1 precursor p105 was also not degraded after apical TLR9 stimulation compared with basolateral stimulation. Because the ubiquitylation of both p105

and I κ B α is mediated by the ubiquitin ligase β -TrCP, the authors suggest that there might be a general failure to degrade β -TrCP-ubiquitylated proteins after apical TLR9 stimulation.

The authors propose that the inhibited NF- κ B response to apical TLR9 stimulation could

induce a state of unresponsiveness to pro-inflammatory TLR signalling from both apical and basolateral IEC surfaces by increasing the NF- κ B-activation threshold. IECs that were apically pre-stimulated with ISS-ODN gradually lost the IL-8 response to basolateral TLR9 stimulation, as well as to apical TLR2 and basolateral TLR3 or TLR5 stimulation. Apical pre-stimulation with ISS-ODN resulted in the accumulation of ubiquitylated I κ B α and the failure to activate NF- κ B after secondary stimulation.

To assess the physiological relevance of this apical TLR9-mediated 'tolerance' in IECs, Lee *et al.* looked at the susceptibility of *TLR9*^{-/-} mice to colonic inflammation induced by DSS (dextran-sulphate sodium salt). NF- κ B was activated at earlier time points after administration of DSS in *TLR9*^{-/-} mice compared with wild-type mice; moreover, the *TLR9*^{-/-} mice had larger areas of epithelial ulceration accompanied by inflammatory-cell infiltrates.

Therefore, the separation of apical TLR signals from basolateral TLR signals, owing to the polarized structure of IECs, could contribute to homeostasis in the intestinal tract.

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