Prostanoids put a brake on necroptosis in IBD

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A form of programmed cell death, necroptosis, in intestinal epithelial cells initiates mucosal inflammation. A study now finds that prostanoid EP4 receptor signalling interferes with RIPK1/RIPK3-dependent MLKL activation, thereby inhibiting necroptosis and accelerating resolution of inflammation.

A single layer of intestinal epithelial cells is the interface between the microbiologically and chemically hostile environment of the intestinal lumen, and the sterile host tissue, which harbours at that locale the body's most extensive accumulation of immune cells. Whilst long considered an inert barrier, the intestinal epithelium has emerged as central orchestrator of mucosal immune homeostasis in health, and initiator of intestinal inflammation in disease. Necroptosis is a form of non-apoptotic programmed cell death, which, when triggered in the intestinal epithelium, can cause ileal and colonic inflammation in mice. Descriptive evidence of necroptotic cell death has been reported for the intestinal epithelium in both forms of inflammatory bowel disease (IBD), Crohn's disease (CD) and ulcerative colitis (UC).[1] In this issue of *Nature Cell Biology*, Patankar *et al* now demonstrate that prostanoid signalling, involving a receptor linked to genetic risk for CD and UC, blocks necroptosis within the intestinal epithelium and thereby promotes resolution of inflammation.[2]

The authors' starting point was the observation that mucosal transcriptomes of mice that had recovered from experimental colitis, induced by a several-day course of dextran sodium sulphate (DSS), were enriched for transcripts indicative of arachidonic and linoleic acid biosynthesis, when compared with transcriptomes obtained at peak inflammation. In publicly available datasets, they also noted a corresponding depletion of these biosynthetic pathways in the inflamed regions of patients with UC. Specific regenerative activities on the intestinal epithelium had been reported for the arachidonic acid metabolite prostaglandin E_2 (PGE_2) acting via EP4.[3] EP4, which is encoded by the *PTGER4* gene, is one of the four prostanoid receptors activated by PGE_2, and the only one highly expressed in the gastrointestinal mucosa. Patients with UC and high mucosal *PTGER4* expression exhibited notably longer flare-free periods compared with $PTGER4^{low}$ patients, and a similar trend was observed in CD.

To investigate this mechanistically, the authors activated EP4 with EP4-D, a small molecule drug, which was administered either concomitantly with DSS, or only started when colitis was already fully established at the end of the DSS course. EP4-D had a marked preventative effect on intestinal inflammation, and was also therapeutic in that it accelerated the restoration of mucosal integrity and the recovery from systemic inflammation.[2] This protective and therapeutic effect was associated with a marked decrease in the number of necroptotic cells within the epithelium. Necroptosis is executed by phosphorylation of mixed lineage kinase domain-like pseudokinase (MLKL), which, in turn, oligomerises and translocates to the plasma membrane to create pores, causing osmolysis and leakage of cellular contents into the extracellular space.[4] EP4-D indeed markedly suppressed MLKL phosphorylation in DSS colitis. A phosphoproteomic screen in intestinal organoids stimulated with tumour necrosis factor (TNF) alongside a stable isomer of PGE_2 ($dmPGE_2$) also pointed to inhibition of cell death-associated pathways, including the upstream receptor-interacting protein kinase 1 (RIPK1), which was imputed into their analysis. Extending the authors' earlier work on TNF-induced necroptosis in epithelial-specific caspase-8 deficiency,[1] they demonstrated that co-ablation of caspase-8 together with either MLKL or RIPK3, which directly phosphorylates MLKL, protected mice from DSS-induced pathology, identifying necroptosis in the epithelium as a key driver of the inflammatory process in this variation of DSS colitis. Mice with epithelial-specific caspase-8 deletion were exquisitely sensitive to even low doses of DSS and exhibited high mortality. They were almost completely protected

from colitis and mortality by EP4-D treatment, achieving a comparable degree of protection as by the genetic co-deletion of MLKL or RIPK3.

Employing intestinal organoids as a reductionistic *in vitro* model, the authors demonstrated that EP4 agonism with EP4-D or dmPGE_2 is acting directly on epithelial cells to prevent necroptotic cell death. This was the case for both caspase-8-deficient organoids as well as wild-type organoids, in which necroptosis was induced pharmacologically by a pan-caspase inhibitor alongside a SMAC (second mitochondria-derived activator of caspases) inhibitor and TNF. This protection was again associated with decreased phosphorylation of RIPK1 and MLKL.

Finally turning to the human colon carcinoma cell line HT29, the authors corroborated that EP4-D inhibited pharmacologically-induced necroptosis and RIPK1 phosphorylation. EP4 agonism prevented the RIPK1/RIPK3-dependent oligomerisation and plasma-membrane translocation of MLKL, and required TGFβ-activated kinase-1 (TAK1) as demonstrated by its pharmacological inhibition. TAK1 is a critical molecular switch downstream of TNF, which regulates cell death via caspase-8. Consistent with an important role of TAK1 in EP4-mediated protection, TAK1 inhibition partially prevented the protective effect of EP4-D in DSS colitis. Corroborating the translational potential of EP4 agonism in IBD, EP4-D prevented pharmacologically-induced necroptosis in both colonic biopsies and cultured organoids.

EP4 agonism preventing epithelial necroptosis and consequently intestinal inflammation is significant for several reasons. Necroptosis has been linked to major genetically-affected risk pathways in inflammatory bowel disease (IBD), such as defective ATG16L1-dependent autophagy and pathological endoplasmic reticulum stress.[5-7] Several genomic risk loci point to pathways involved in programmed cell death mechanisms as predisposing for 'polygenic' IBD,[8] and some being causative for monogenic very-early onset IBD (VEOIBD). Caspase-8 deficiency itself has been linked to VEOIBD.[9] Epithelial necroptosis has also been observed upon combined deficiency of A20 (*Tnfaip3*) and ABIN-1 (*Tnip1*), which are risk genes not only linked to IBD but multiple other immunopathologies.[10] Conceptually interesting is also that reduced expression of a histone methyltransferase (SETDB1), which has been observed in IBD, triggered necroptosis and ileocolitis in mice due to reactivation of endogenous retroviruses in intestinal stem cells.[11] Western diet, correlatively implicated in the marked global increase in incidence and prevalence of IBD, has also been reported to trigger, via the TSC1/mTOR pathway, epithelial necroptosis in mouse models.[12]

PTGER4 is the most plausible, and closest, risk gene in proximity to a gene desert that is strongly associated with increased risk for both Crohn's disease and ulcerative colitis.[8] Whilst *Ptger4* is also expressed in *bona fide* immune cells, it is primarily expressed within the intestinal epithelium. Germline deletion of *Ptger4*, or EP4 antagonism, had previously been reported to increase susceptibility to DSS colitis, associated with an epithelial regeneration defect and mucosal immune cell infiltration.[13] Epithelial cell-specific deletion was sufficient to observe these phenotypes.[14] The work presented in the current paper hence suggests that prevention of necroptosis might be a critical mechanism of mucosal protection afforded by PGE_2-activated EP4 signalling in the mucosa. The precise mechanism of how activation of EP4, which is a G protein-coupled receptor that acts via Gαs, β-arrestin and Src, affects TAK1 activation remains to be determined. The presented results indicate that mechanisms other than via TAK1 may also be involved in restraining necroptosis. The beneficial effects on regeneration may further include pathways unrelated to this form of cell death, rendering a particularly effective approach to restore mucosal integrity.

Needless to say, for actual human CD and UC, the relative importance of necroptosis amongst mechanisms demonstrated to instigate mucosal inflammation, and/or prevent its resolution in experimental preclinical models, is impossible to predict with certainty. After all, the only studies of actual human disease, where cause-effect relationships are *a priori* resolved, are 'perturbation experiments in man', hence clinical trials, and germline genetic association studies. The latter, mechanistically interrogated in animal models, imply an important role of necroptosis in CD and UC. The authors' identification of EP4 agonism-mediated protection from necroptosis provide a rationale for testing this in a human 'perturbation experiment'. Indeed, therapeutically interfering with the EP4 pathway has been attempted with an investigational drug, ONO-4819, in moderately active UC.[15] However, this study included only 4 and 3 patients receiving a fixed dose of ONO-4819 and placebo, respectively, and hence was by no means powered to ascertain the intervention's effects. Locally-acting EP4 agonists that act directly on the intestinal epithelium would overcome the systemic side effects observed with currently available systemic agents. [1267]

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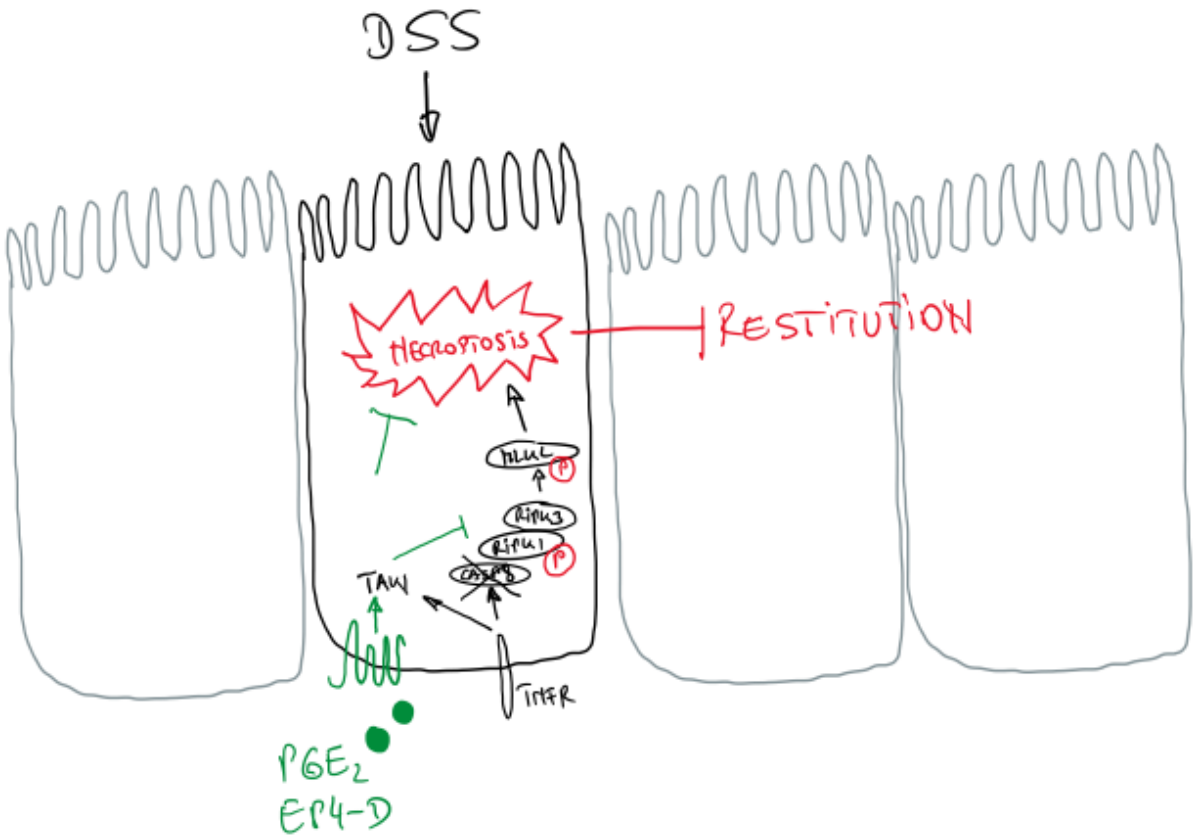
Figure 1. EP4 agonism blocks epithelial necroptosis and thereby promotes mucosal restitution.

Ligand (prostaglandin E_2) or pharmacologically (EP4-D)-triggered signalling via EP4, a G protein coupled receptor encoded by *PTGER4*, inhibits intestinal epithelial necroptosis elicited in the course of dextran sodium sulphate (DSS)-induced colitis. This is achieved by inhibiting the activation and plasma membrane translocation of the necroptosis executioner myosin light chain kinase (MLKL), which acts downstream of TNF-induced receptor-interacting protein kinase 1 (RIPK1)/RIPK3 signalling when caspase-8 is compromised. This protective mechanism, which is at least partly dependent on EP4-induced signalling via TGFβ-activated kinase-1 (TAK1), prevents local and systemic inflammation, and promotes restitution to mucosal homeostasis.



The authors declare no competing interests.