Regional and Conditional Variability of FXR: New lessons on Ileal Inflammation and Gut Barrier Functions

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Editorial Focus Article

Bile acids are amphipathic molecules synthesised in the liver and transported to the small intestine, where they emulsify ingested lipids to facilitate enzymatic digestion and absorption across the intestinal mucosa. However, they also act as bioactive signalling molecules, binding to membrane-expressed and nuclear receptors. Ligand-induced activation of intracellular nuclear receptors, such as the Farnesoid X receptors (FXR), results in transactivation of networks of target genes and subsequent activation of a variety of physiological processes, including changes in gut barrier function (1), the modulation of immune responses and interoceptive signalling from the gut to the brain

(2). Conflicting reports implicate FXR effects as context specific, particularly for colitis, by gut region, by insult and by metabolic and metabolite status (ably reviewed by (3)).

Ubiquitously expressed, FXRs are found at high levels in the liver, intestines, kidney and adrenal glands with well-defined roles in bile acid homeostasis and metabolic regulation. The research article by **O'Guinn, Handler** *et al*., "FXR Deletion Attenuates Intestinal Barrier Dysfunction in Murine Acute Intestinal Inflammation.", published in this edition of AJP GI, has focussed on the role of bile acid signalling via FXR in the context of inflammation-induced barrier dysfunction in the terminal ileum. To date, research in the gut has mainly focussed on the interaction between FXR and inflammatory insults in the colon. Indeed, FXR agonists protect the integrity of the colonic barrier in the face of pro-inflammatory insults, such as Dextran Sodium Sulphate and trinitrobenzenesulfonic acid-induced colitis, likely through the inhibition of pro-inflammatory cytokine release (4). A consequence of inflammation-induced injury to the intestinal epithelial layer is barrier disruption, primarily by disturbing expression of junction proteins. Breakdown of the epithelial barrier may, in turn, exacerbate epithelial injury and inflammation.

Facilitating the study aims, the authors have used FXR knock-out mice, which are characterised by impaired bile acid homeostasis, dysregulation of circulating cholesterol, phospholipids and triglycerides (5). Ileal integrity is reported to be compromised in these animals (6) and they exhibit increased susceptibility to colonic inflammation (7). O'Guinn, Handler *et al*., have focussed on the terminal ileum, an intestinal region physiologically important in bile acid homeostasis, as apical sodium-dependent bile acid transporters, that facilitate the efficient re-uptake of bile acids which are then returned to the liver via the portal vein, are exclusively expressed in this region. Moreover, it is a region that is often resected in patients with Crohn's disease, resulting in increased overspill of bile acids into the colon. In contrast to previous work in colonic tissue which indicated that FXR knockout mice exhibit morphological alterations in the intestinal wall that mimicked pathological changes characteristic of chronic inflammation, in addition to a pro-inflammatory cytokine phenotype (7), baseline barrier permeability in the small intestine was not found to differ to wildtype mice. Moreover, gene expression of a variety of inflammatory cytokines and chemokines were comparable in unstimulated small intestinal tissue from wildtype and FXR

knockout mice. Others have demonstrated that organ-specific deletion of hepatic FXR resulted in enhancement of the protective mucosal barrier and reduced symptoms of colitis (8). This study adds to the complexity of the interactions between FXR and immune molecules, demonstrating that in addition to organ-specific distinctions in the role of FXR in regulating inflammatory responses, the role of FXR in baseline intestinal function also varies in the proximal and distal intestinal regions.

Under pathophysiological conditions, FXR is implicated in the initial inflammatory insult that frequently leads to breakdown of the integrity of the gut barrier, dysregulation of the inflammatory cascade and subsequent pathological processes that contribute to Crohn's disease, ulcerative colitis and other gastrointestinal disorders. In this study, the authors have evoked an inflammatory response using intraperitoneal injection of lipopolysaccharide (LPS) as an innate immune challenge. The authors have focussed on the acute immune response to the inflammatory insult. However, one strength of this study is that the experimental design has included two different timepoints (2 hours and 16 hours) within this acute process, with the goal of detecting different activities within the immune response that occur at the onset and at later stages. Chemokine release is detected within an hour of LPS exposure, which recruits immune cells to the site of inflammation, and then, within the first 1-4 hours, release cytokines. These markers of the immune response subside after 4–6 hours. Thereafter, pathological changes in the tissue begin to become apparent.

In this study, barrier permeability remained intact in wildtype and FXR knockout mice two hours after administration of LPS. However, 16 hours after the LPS challenge, increased epithelial leakiness was evident in wildtype mice. This was accompanied by disruption to the mucosal architecture. In contrast, after 16 hours, breakdown of the intestinal barrier and villus disruption were less severe in FXR knockout mice, findings that suggested that ablation of FXR resulted in a protective effect in small intestinal tissue. This contrasts with the general agreement that activation of FXR evokes a protective effect, mainly through modulation of pro- and anti-inflammatory cytokine secretion (4). The authors, however, bolstered their conclusions by challenging the FXR mice to two additional pro-inflammatory insults and found that FXR knockout mice were also protected from structural damage induced by dithizone/Klebsiella induced acute intestinal injury and also by Caecal Ligation Puncture (CLP). Although the

mechanisms underpinning the protective effects of FXR deletion are likely to differ for each of the challenges, this provides strong evidence that the small intestine of FXR knockout mice are protected from pro-inflammatory challenges. Further experiments to confirm the divergent effects of LPS, dithizone/Klebsiella and/or CLP in the small and large intestine of the same FXR knockout mice would further strengthen the conclusions that FXR differentially modulates the immune response in different regions of the intestine. Furthermore, it would provide opportunities to understand the mechanisms underpinning the contrasting modulatory effects of FXR on inflammation in the small and large bowel.

Transcriptomics analysis of mucosal tissue from WT and FXR knockout mice detected a plethora of changes in gene expression in untreated FXR knockout mice. In FXR null mice treated with LPS innate immune-related Gene Ontology (GO) Biological Process terms were underrepresented when compared to wildtype mice. This was interpreted as a potential dampening of the immune response in the ileum of FXR knockout mice which had been exposed to LPS. More focussed experiments explored the impact of FXR deletion on LPS-induced cytokine and chemokine expression. In contrast to the significant changes in membrane permeability and epithelial morphology that were evident only at 16 hours in FXR knockout mice, *Il6* alone was increased as compared to the wildtype mice at this time point. However, at 2 hours, LPS-induced mucosal expression of pro-inflammatory cytokines, *Il1α, Il1β* and *Tnf* was blunted in FXR knockout mice. Anti-inflammatory *Il10,* which is important in intestinal would healing, was also diminished at 2 hours, which could further potentiate the pro-inflammatory response. Although chemokine functions include leukocyte proliferation, survival, differentiation and degranulation, the dominant biological role is in chemoattraction of leukocytes. Rapid release of chemokines to a challenge such as LPS (7) orchestrates directional movement of leukocytes to the site of injury or inflammation. Mucosal expression of *Cxcl1* and *Ccl2* was delayed in FXR knockout mice, and the authors concluded that delayed chemokine release could underlie decreased expression and secretion of pro-inflammatory cytokines. This could subsequently result in protection of intestinal integrity noted in FXR knockout mice 16 hours after exposure to LPS. To focus on the mechanisms of epithelial barrier integrity, this study examined the expression of tight junctions, which consist of transmembrane and intracellular scaffold proteins and are critical to the regulation of intestinal permeability. Exposure to LPS induced

internalisation or re-localisation of the tight junction proteins occludin and ZO-1 within 2 hours in WT but not FXR knockout mice. Thus, preservation of tight junction proteins in the small intestine aligns with suppressed cytokine release and protection from inflammation-induced epithelial permeability in FXR knockout mice.

In the context of bidirectional crosstalk between inflammatory cytokines and FXR, which has previously been shown to be organ-specific and dependent upon whether inflammation has been studied in acute or chronic phases, this study has further highlighted the complexity of the role of FXR in inflammation-mediated changes within the intestine. Indeed, evidence suggests that FXR targeted, FGF15 and its synthetic analogue (M52) may alleviate gut regional colitis, although this effect is lost in a global FXR knock out (9). They also reported crosstalk between FXR and glucocorticoid receptor to influence immune and cyclooxygenase responses. Receptor crosstalk may be critical in this setting. A further consideration to strengthen the conclusions could involve exploration of the direct and indirect impacts of the FXR knockout-associated increases in the bile acid pool, as bile acids that are microbially modified are known to influence immune cell differentiation and responsivity (10). Furthermore, while the authors of this study did recognise the importance of alterations in the microbiota of FXR knockout mice, further investigation into the consequences of FXR deletion to both microbes and their bile acid modifications could provide further valuable insight into bile acid interactions with the immune responses. Whether FXR is protective or deleterious appears to be dependent upon whether inflammation is in the proximal or distal colon, revealing further questions as to how FXR modulates intestinal integrity in the face of inflammatory challenges.

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