



Article

# Loss of ER $\beta$ in Aging LXR $\alpha\beta$ Knockout Mice Leads to Colitis

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**Abstract:** Liver X receptors (LXR $\alpha$  and LXR $\beta$ ) are oxysterol-activated nuclear receptors that play key roles in cholesterol homeostasis, the central nervous system, and the immune system. We have previously reported that LXR $\alpha\beta$ -deficient mice are more susceptible to dextran sodium sulfate (DSS)-induced colitis than their WT littermates, and that an LXR agonist protects against colitis in mice mainly via the regulation of the immune system in the gut. We now report that both LXR $\alpha$  and LXR $\beta$  are expressed in the colonic epithelium and that in aging LXR $\alpha\beta^{-/-}$  mice there is a reduction in the intensity of goblet cells, mucin (MUC2), TFF3, and estrogen receptor  $\beta$  (ER $\beta$ ) levels. The cytoplasmic compartment of the surface epithelial cells was markedly reduced and there was a massive invasion of macrophages in the lamina propria. The expression and localization of  $\beta$ -catenin,  $\alpha$ -catenin, and E-cadherin were not changed, but the shrinkage of the cytoplasm led to an appearance of an increase in staining. In the colonic epithelium there was a reduction in the expression of plectin, a hemidesmosome protein whose loss in mice leads to spontaneous colitis, ELOVL1, a fatty acid elongase protein coding gene whose overexpression is found in colorectal cancer, and non-neuronal choline acetyltransferase (ChAT) involved in the regulation of epithelial cell adhesion. We conclude that in aging LXR $\alpha\beta^{-/-}$  mice, the phenotype in the colon is due to loss of ER $\beta$  expression.

**Keywords:** liver X receptor; estrogen receptor  $\beta$ ; colitis; inflammatory bowel diseases; plectin



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## 1. Introduction

Ulcerative colitis and Crohn's disease represent the two main types of inflammatory bowel diseases (IBDs). The etiology and underlying molecular mechanisms behind these idiopathic IBDs are currently poorly understood, but involve the activation of mucosal/epithelial cells driving an uncontrolled inflammatory response in the small and large intestines. The intestinal mucosa is in constant contact with microorganisms, and thus the intestinal epithelial barrier is a key component of the defense mechanisms required to prevent infection and inflammation. Intestinal epithelial cells play an important role in controlling the interaction between the luminal microbiota and underlying immune cells [1].

Liver X receptors (LXRs) encoded by the genes *Nhr1h3* (LXR $\alpha$ ) and *Nhr1h2* (LXR $\beta$ ) are members of the nuclear receptor family of ligand-regulated transcription factors [2,3]. LXRs are master regulators of lipid metabolism, cholesterol homeostasis, and inflammation [4,5], and as such, are involved in numerous physiological and pathological processes such as atherosclerosis, cancer, diabetes mellitus, and the loss of specific neurons in the substantia nigra, spinal cord, retina, and cochlea. LXRs are receptors with potent anti-inflammatory effects and have emerged as important targets for inflammatory diseases [6,7]. In addition to synthetic LXRs agonists, such as T0901317 and GW3965, sterol-based LXR agonists attenuate dextran sulfate colitis-induced weight loss with a reduced expression of inflammatory

markers in the large intestine of mice [8]. A recent study showed that LXR $\alpha\beta$  expression was decreased in colonic samples from patients with IBD and IL-10 deficient mice [9]. In this study, the authors concluded that LXRs exert anti-inflammatory effects in colonic epithelial cells via ABCA1.

It has been reported that genetic variants in the nuclear receptors including LXRs may indicate a risk of ulcerative colitis [10]. In a previous study, we found that either LXR $\alpha$ -, LXR $\beta$ -, or LXR $\alpha\beta$ -deficient mice were more susceptible to dextran sodium sulfate (DSS)-induced colitis. Clinical indicators of colitis including weight loss, diarrhea, and blood in feces appeared earlier and were more severe in LXR $\alpha\beta$ -deficient mice and, particularly, LXR $\beta$  protected against symptoms of colitis [11]. Furthermore, the activation of LXRs strongly suppressed the expression of inflammatory mediators. The expression of both LXR $\alpha$  and LXR $\beta$  was significantly suppressed in the inflamed colon of IBD subjects compared to the non-inflamed colon [11].

Estrogen and its receptors, in addition to their role in regulating several biological functions, contribute to the development and progression/severity of IBDs [12]. We have previously reported that estrogen receptor  $\beta$  (ER $\beta$ ) is essential for the maintenance of cellular homeostasis and for driving cellular differentiation in the colon [13]. In ER $\beta$ <sup>-/-</sup> mice, there is rapid proliferation of the epithelium and defective tight junctions characterized by a decrease in the expression of the adherens junction protein,  $\alpha$ -catenin, and the hemidesmosomal protein, plectin. ER $\beta$  deficiency impairs gut microbiota [14] and intestinal ER $\beta$  suppresses colon inflammation and tumorigenesis in both sexes [15].

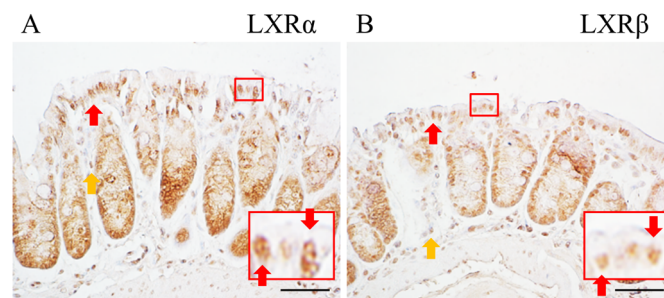
The aim of this study was to investigate the mechanisms through which loss of LXRs leads to the sensitivity of the colon to colitis. We were particularly interested in the possibility that ER $\beta$  signaling was involved because of the colonic phenotype of ER $\beta$  mice and our finding of the loss of ER $\beta$  in the lungs of LXR $\alpha\beta$ <sup>-/-</sup> mice: aging LXR $\alpha\beta$ <sup>-/-</sup> lungs developed a rare form of lung cancer after mice turned 13 months of age [16]. The hypothesis that we are testing is that loss of ER $\beta$  is a key factor in the development of the phenotype in LXR $\alpha\beta$ <sup>-/-</sup> mice because of the loss of the antiproliferative role of ER $\beta$  in epithelial cells.

In the present study, we found a colitis phenotype in LXR $\alpha\beta$ <sup>-/-</sup> mice as they age. We examined gene expression via immunohistochemistry and RNA sequencing. We now confirm that LXR $\beta$  and LXR $\alpha$  are expressed in colonic epithelial cells and that as LXR $\alpha\beta$ <sup>-/-</sup> mice age (above 12 months), there is dysfunction of the colonic epithelium and colitis, similar to that seen in plectin knockout mice. The study shows that the colonic phenotype in these aged mice is not directly due to loss of LXRs, but was indirectly caused by the loss of ER $\beta$  in the colonic epithelium, which occurs in the absence of LXRs.

## 2. Results

### 2.1. Expression of LXR $\alpha$ and LXR $\beta$ in the Colon

To detect the expression of LXRs in the colon of WT mice, we used our in-house antibodies which have been well characterized for staining LXRs in the central nervous system and other organs [17–22]. With our home-made antibodies, we found that both LXR $\alpha$  and LXR $\beta$  are expressed in the colon both in the cells at the base of the crypts, along the crypts, and in the surface epithelium (Figure 1A,B).

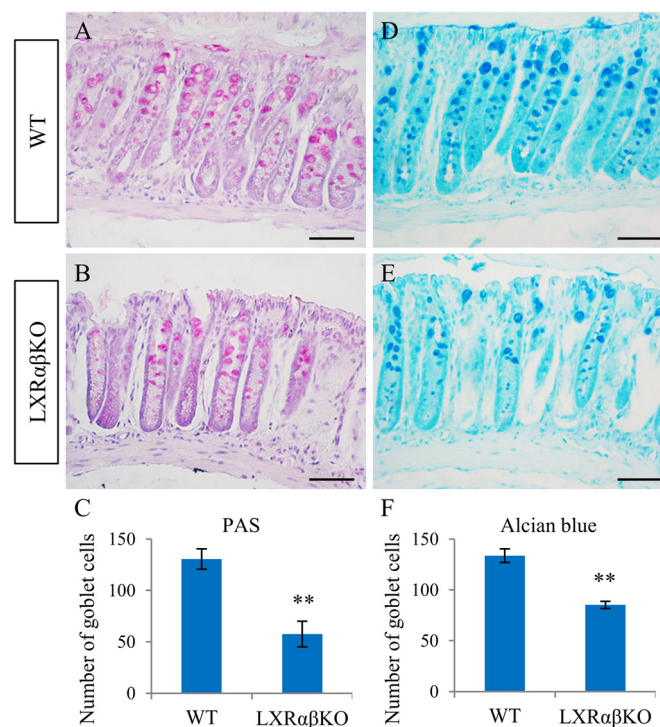


**Figure 1.** Expression of LXR $\alpha$  and LXR $\beta$  in mouse colon. In mouse colon, LXR $\alpha$  and LXR $\beta$  are expressed in the colonic epithelial cells ((A,B), respectively). Red arrows indicate positive epithelial cells and yellow arrows indicate other positive cells (macrophages). Scale bars: 50  $\mu$ m.

## 2.2. Decrease in the Intensity of Goblet Cells and Mucins in the Colon of LXR $\alpha\beta$ <sup>-/-</sup> Mice

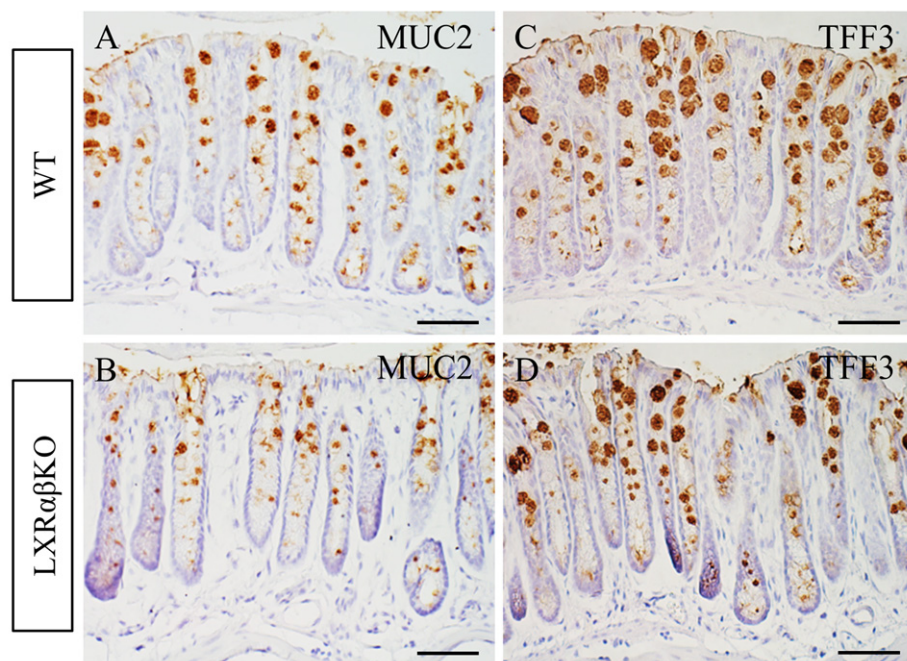
To examine the effect of LXR $\alpha\beta$  loss on colon morphology, colon sections from LXR $\alpha\beta$ <sup>-/-</sup> and their WT littermates were stained with hematoxylin/eosin. Compared with WT, the colonic epithelium of LXR $\alpha\beta$ <sup>-/-</sup> mice was flattened, with more cells (macrophages) infiltrating the lamina propria (Supplementary Figure S1). In addition, the number of proliferating cells (PCNA-positive) was higher in the colon of LXR $\alpha\beta$ <sup>-/-</sup> mice and the proliferating cells were not confined to the base of the crypts but migrated up the sides of the crypts (Supplementary Figure S2).

Goblet cells are found scattered in between enterocytes and secrete high-molecular-weight glycoprotein mucins. Alcian blue and periodic acid–Schiff staining were used to measure the number of goblet cells and mucin content in colonic mucosa. We found that compared to WT mice, the number of goblet cells was significantly reduced in the colon of LXR $\alpha\beta$ <sup>-/-</sup> mice (Figure 2A–F).



**Figure 2.** Decrease in the number of goblet cells in the colon of LXR $\alpha\beta$ <sup>-/-</sup> mice. Periodic acid–Schiff staining showed significant decrease (\*\*  $p < 0.01$ ) in the number of goblet cells in the colon of LXR $\alpha\beta$ <sup>-/-</sup> mice compared to WT (A–C). Alcian blue staining confirmed that goblet cells were significantly reduced (\*\*  $p < 0.01$ ) in LXR $\alpha\beta$ <sup>-/-</sup> mice (D–F). PAS: periodic acid–Schiff;  $n = 3$ . Scale bars (A,B,D,E): 50  $\mu$ m.

Goblet cells produce and secrete mucin 2 (MUC2) along with trefoil factor 3 (TFF3) into the lumen within the intestine cells where they participate in mucosal regeneration and repair [23]. We confirmed a significant reduction in mucin expression using antibodies for MUC2 and TFF3, respectively (Figure 3A–D). Overall, the goblet cell numbers and mucin expression were significantly reduced in the colon of  $LXR\alpha\beta^{-/-}$  mice.



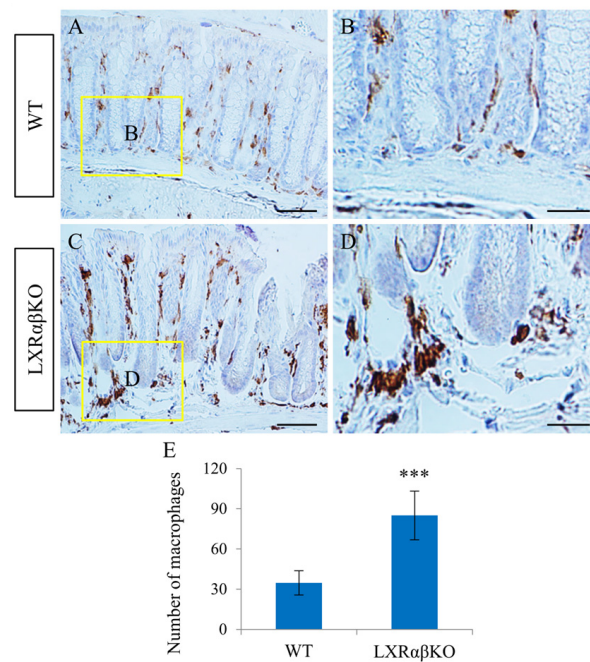
**Figure 3.** Decreased expression of MUC2 and TFF3 in the colon of  $LXR\alpha\beta^{-/-}$  mice. MUC2 was strongly expressed in the colon of WT mice (A), and its expression was decreased in the colon of  $LXR\alpha\beta^{-/-}$  mice (B). TFF3 was highly expressed in the colon of WT mice (C), and its expression was significantly decreased in the  $LXR\alpha\beta^{-/-}$  mice (D). Scale bars (A–D): 50  $\mu$ m.

### 2.3. Increased Activation of Macrophages in the Lamina Propria of $LXR\alpha\beta^{-/-}$ Mice

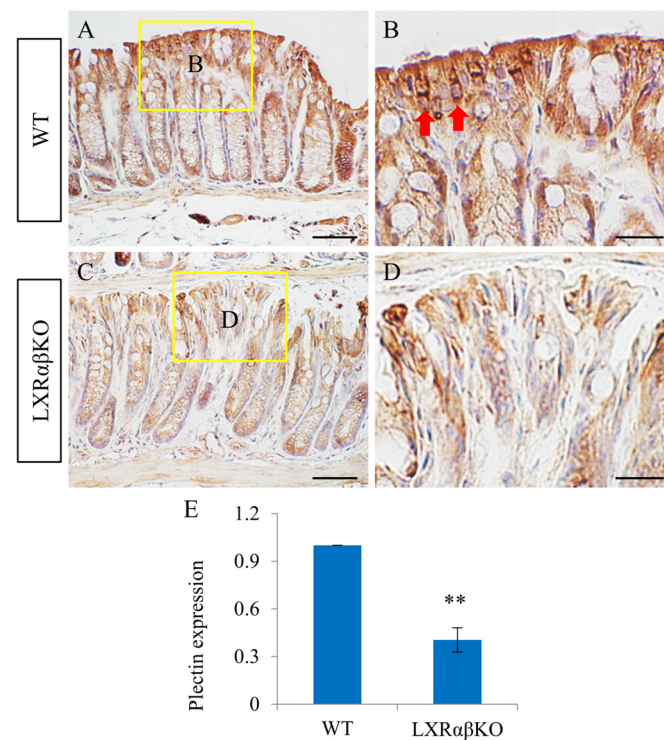
Intestinal macrophages play a key role in the gut immune system and the regulation of gastrointestinal physiology [24]. LXRs are key regulators of the immune system and macrophages [22]. There were a few macrophages (Iba1-positive) detected in the lamina propria of WT mice (Figure 4A,B). However, there were abundant macrophages in the colon of  $LXR\alpha\beta^{-/-}$  mice (Figure 4C–E). These macrophages appear to be activated, as evidenced by larger cell bodies and limited ramifications. Furthermore, there were only a few MPO-positive neutrophils in the colon of both WT and  $LXR\alpha\beta^{-/-}$  mice, and there was no difference in the number of neutrophils (Supplementary Figure S3).

### 2.4. Decreased Expression of Plectin in the Colon of $LXR\alpha\beta^{-/-}$ Mice

Plectin in intestinal epithelial cells is important for maintaining keratin filament network organization and the formation of functional hemidesmosomes, as well as intercellular junctions. We observed abundant plectin expression in the colonic epithelium of WT mice, with a marked expression of plectin in the cytoplasmic and perinuclear regions of the colon in WT mice (Figure 5A,B). However, plectin expression was significantly reduced in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice (Figure 5C,D).



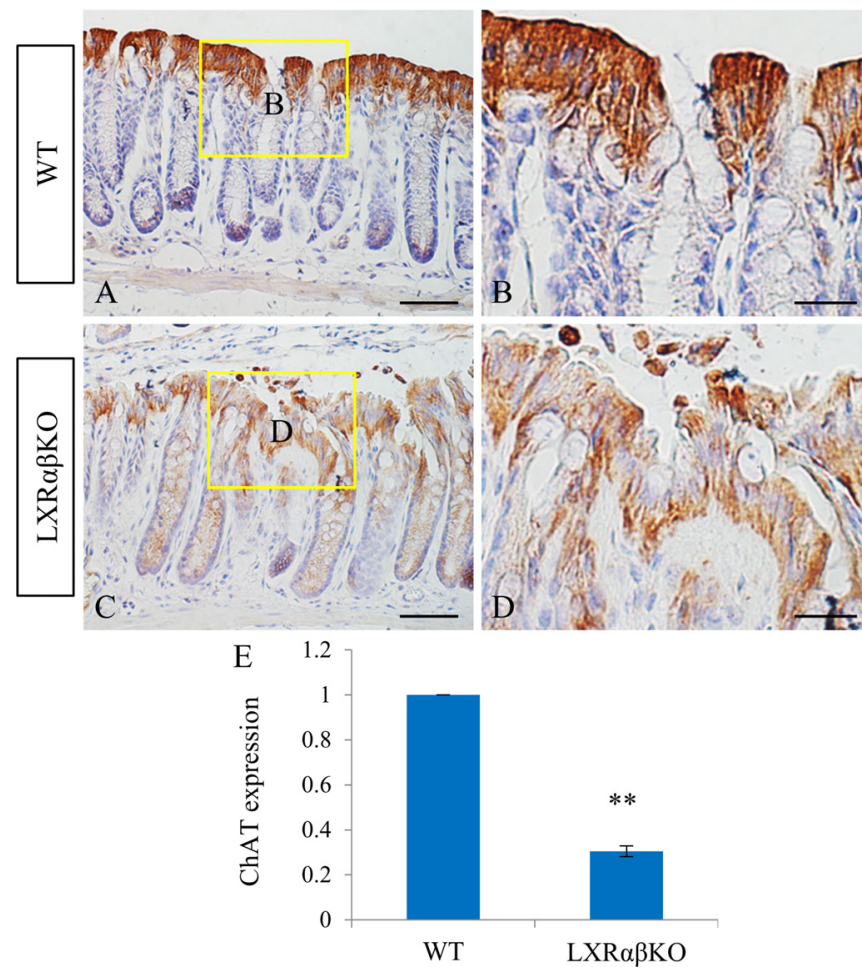
**Figure 4.** Increased macrophages in the lamina propria of  $LXR\alpha\beta^{-/-}$  mice. There were few Iba1-positive macrophages within the lamina propria of WT mice (A,B). In the colon of  $LXR\alpha\beta^{-/-}$  mice, the number of macrophages was much higher (\*\*\*) than that in WT and these macrophages appeared to be activated (C–E).  $n = 3$ . Scale bars: (A,C), 50  $\mu\text{m}$ ; (B,D), 20  $\mu\text{m}$ .



**Figure 5.** Decreased plectin expression in the colon of  $LXR\alpha\beta^{-/-}$  mice. There was a prominent expression of plectin in the cytoplasm and perinuclear regions of the colon in WT mice (A,B). Plectin expression in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice was significantly decreased (\*\*  $p < 0.01$ ) (C–E).  $n = 3$ . Red arrows indicate the expression of plectin around the nucleus. Scale bars: (A,C), 50  $\mu\text{m}$ ; (B,D), 20  $\mu\text{m}$ .

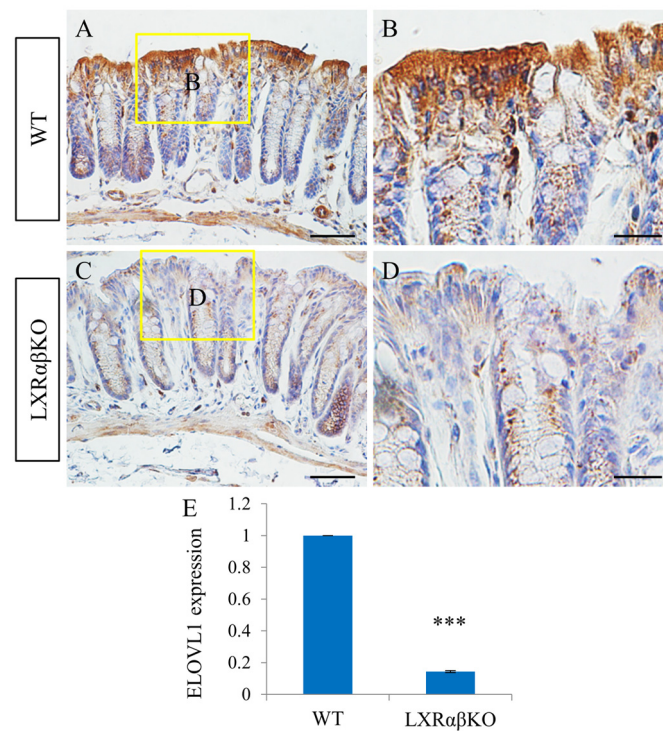
### 2.5. Decreased Expression of ChAT and ELOVL1 in the Colonic Epithelium of $LXR\alpha\beta^{-/-}$ Mice

Choline acetyltransferase (ChAT) and ELOVL fatty acid elongase 1 (ELOVL1) have been implicated in diseases of the colon. Both enzymes were very abundantly expressed in the colonic epithelium of WT mice, whereas their expression levels were significantly reduced in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice (Figures 6 and 7). In neurons and most non-neuronal cells, ChAT is the enzyme responsible for the synthesis of ACh from choline and acetyl coenzyme A [25] and ELOVL1 catalyzes the elongation of short-chain fatty acids (SCFA).



**Figure 6.** Decreased ChAT expression in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice. There was abundant expression of ChAT in the colonic epithelium of WT mice (A,B). However, ChAT expression was markedly decreased (\*\*  $p < 0.01$ ) in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice (C–E).  $n = 3$ . Scale bars: (A,C), 50  $\mu$ m; (B,D), 20  $\mu$ m.

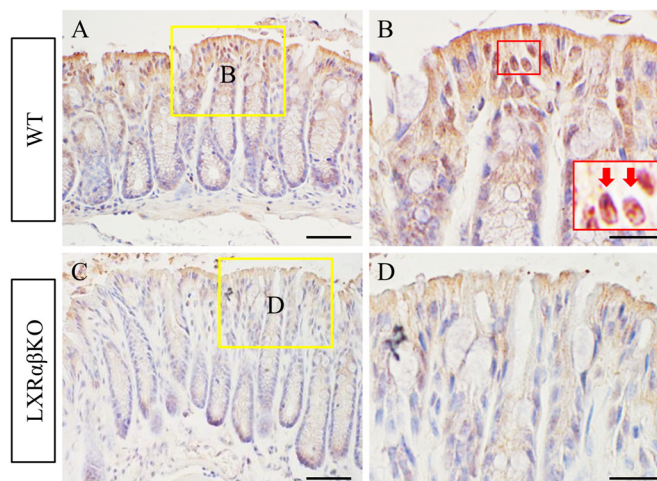
Adherens junctions (expressing  $\beta$ -catenin,  $\alpha$ -catenin, and E-cadherin), together with tight junctions, form an apical junctional complex that regulates intestinal epithelial cell-to-cell adherence and barrier homeostasis [26].  $\beta$ -catenin is well expressed in the colon of both WT and  $LXR\alpha\beta^{-/-}$  mice (Supplementary Figure S4A,B), and the expression of  $\beta$ -catenin in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice appeared to be increased somewhat due to the shrinkage of the epithelium cytoplasm. The expression and localization of  $\alpha$ -catenin and E-cadherin in the colon of WT and  $LXR\alpha\beta^{-/-}$  mice did not significantly change (Supplementary Figure S4C–F).



**Figure 7.** Decreased ELVOL1 expression in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice. ELVOL1 was highly expressed in the colonic epithelium of WT mice (A,B). In the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice, ELVOL1 expression was significantly reduced (\*\* $p < 0.001$ ) (C–E).  $n = 3$ . Scale bars: (A,C), 50  $\mu\text{m}$ ; (B,D), 20  $\mu\text{m}$ .

### 2.6. ER $\beta$ Expression in the Colon of WT and $LXR\alpha\beta^{-/-}$ Mice

ER $\beta$  was expressed in the colon surface epithelium and cells at the base of the crypts of WT mice (Figure 8A,B). However, ER $\beta$  expression was lost in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice (Figure 8C,D).



**Figure 8.** Loss of ER $\beta$  in the epithelium of  $LXR\alpha\beta^{-/-}$  mice. There was a very strong nuclear expression of ER $\beta$  in the colonic epithelium of WT mice (A,B). The expression of ER $\beta$  was significantly reduced in the colonic epithelium of  $LXR\alpha\beta^{-/-}$  mice (C,D). Red arrows indicate ER $\beta$ -positive cells. Scale bars: (A,C), 50  $\mu\text{m}$ ; (B,D), 20  $\mu\text{m}$ .

### 3. Discussion

In the present study, we report that LXR $\alpha$  and LXR $\beta$  are expressed in the colonic epithelial cells and that loss of LXR $\alpha\beta$  leads to a phenotype of colitis as mice age. This colitis is characterized by loss of goblet cells, increased macrophage activation, and decreased ER $\beta$  expression in the colon.

Plectin, a cytoskeletal linker protein, binds to and interconnects intermediate filaments and attaches them to junctional complexes at the cell membrane, different organelles, and inner membrane systems. Plectin also interacts with actomyosin structures and microtubules [27]. It functions as a mediator of keratinocyte mechanical stability in the skin, primarily through linking intermediate filaments to hemidesmosomes [28]. Plectin expression was reduced in the colons of ulcerative colitis patients and negatively correlated with the severity of colitis. Plectin plays an important role in maintaining the integrity of the intestinal epithelium [29]. Mechanistically, plectin deficiency in intestinal epithelial cells leads to aberrant keratin filament network organization and the formation of dysfunctional hemidesmosomes and intercellular junctions. In the present study, we found that the knockout of LXR $\alpha\beta$  resulted in significantly reduced plectin expression in the colon. Thus, the colonic phenotype of LXR $\alpha\beta^{-/-}$  mice is at least partially mediated by the downregulation of plectin, and effects on the entire integrin  $\alpha 6$ /plectin/keratin 18 axis are to be expected.

Interestingly, although in the LXR $\alpha\beta^{-/-}$  mice there is increased proliferation, there is no significant change in crypt length. This is different from mice bearing an intestinal-epithelial-cell-specific loss of  $\alpha 6$  integrin [30], or mice with Keratin 8 deletion [31]. Integrin  $\alpha 6$  knockout mice spontaneously develop colitis, which degenerates over time into infiltrating adenocarcinoma [30]. Mutant crypts in the distal colon of  $\alpha 6$  integrin knockout mice were twice as long, and proliferation rates in the colon and rectum were significantly increased. Keratin 8 gene disruption led to colorectal hyperplasia with an increased number of gut epithelial cells, and more than five-fold elongated villi [31]. Mice lacking plectin in intestinal epithelial cells spontaneously developed colitis characterized by the extensive detachment of intestinal epithelial cells from the basement membrane and increased intestinal permeability, and exhibited increased crypt proliferation, severe crypt damage, and increased goblet cells [29]. The LXR $\alpha\beta^{-/-}$  mouse colon, therefore, most closely resemble plectin knockout mice.

Acetylcholine (ACh) is not only a neurotransmitter but also exists in non-neuronal cholinergic systems. Components of the non-neuronal cholinergic system, including ChAT, are located in intestinal epithelial cells, crypt-villus organoids, immune cells, intestinal stem cells, and vascular endothelial cells [32]. Upon exposure of the SCFA, propionate, which is produced by gut bacteria, ACh is released [33]. ACh is a key regulator of epithelial tightness in the proximal colon [34], and in patients with ulcerative colitis, ChAT expression is reduced in endocrine and epithelial cells of the mucosa [35]. Thus, the non-neuronal cholinergic system plays a significant role in intestinal epithelial homeostasis by protecting the colonic epithelium from changes in the luminal environment [36,37]. In the present study, the expression levels of ChAT in the colonic epithelium of LXR $\alpha\beta^{-/-}$  mice were significantly decreased, indicating a disturbance of the non-neuronal cholinergic system. The mechanisms through which LXRs regulate the cholinergic system are not fully understood.

ELOVL1 is involved in the elongation of saturated fatty acids and monounsaturated fatty acids. ELOVL1 is essential for permeability barrier formation, as ELOVL1-deficient mice die shortly after birth due to epidermal barrier defects [38]. LXRs have been implicated in the control of fatty acid synthesis and subsequent lipogenic processes in several tissues.

The intestinal mucosa is in continuous contact with microorganisms, and the intestinal epithelial barrier is a critical component in the defense against infection and inflammation. MUC2, which is produced by the goblet cells, is the major component of the intestinal mucus and protects the intestinal tract from self-digestion and numerous microorganisms [39]. TFF3 is expressed with MUC2 in intestinal goblet cells where it participates in mucosal regeneration and repair [23,40]. In the present study, we observed a significant reduction in



the number of goblet cells and decreased expression levels of MUC2 and TFF3 in the colon of  $LXR\alpha\beta^{-/-}$  mice, suggesting a compromised intestinal barrier in  $LXR\alpha\beta^{-/-}$  mice.

One possible explanation for the changes in the colonic mucosa as mice age is the rapid turnover of the surface epithelium with an overall consequence on a less-well-differentiated surface epithelium. Late-onset phenotype characteristics of  $LXR^{-/-}$  mice have been reported in dopaminergic neurons in the substantia nigra [41], large motor neurons in the ventral horn of the spinal cord [42], retinal ganglion cells in the eye [21], spiral ganglion neurons in the cochlea [22], and squamous cell lung cancer, a rare subtype of non-small cell lung cancer, in  $LXR\alpha\beta^{-/-}$  mice [16,43]. In the case of non-small cell lung cancer, there was a loss of ER $\beta$  expression which contributed to increased proliferation.

ER $\beta$  regulation of inflammation and its protective role in colitis and colitis-associated colorectal cancer (CRC) have been extensively studied in terms of other different mechanisms [44]. 17 $\beta$ -Estradiol induces the expression of MUC2 and tight junction molecules and inhibits pro-inflammatory cytokines to exert anti-inflammatory effects on the intestinal barrier through the ER $\beta$  signaling pathway [45]. Arctigenin, a phytoestrogen, can maintain the integrity of the mucus barrier by inhibiting the apoptosis of goblet cells via ER $\beta$  signaling [46]. Orally administered estrogen receptor modulator (SERM2) could alleviate DSS-induced colitis in male mice and promote anti-inflammatory phenotypes of immune cells [47]. However, the possible regulation of ER $\beta$  on plectin, ELOVL1, and ChAT in colitis still needs more animal model experiments and clinical studies to investigate.

One other factor contributing to the immaturity of the colonic surface epithelium is the loss of ER $\beta$  expression as  $LXR\alpha\beta^{-/-}$  mice age. We have previously shown a role for ER $\beta$  in the organization and architectural maintenance of the colon, and loss of ER $\beta$  leads to colitis in mice. The ER $\beta^{-/-}$  mouse colon was characterized by increased proliferation, an increase in the migration of proliferating cells toward the luminal surface, a decrease in apoptosis, and poorer differentiation of the surface epithelium, as measured via the expression of cytokeratin 20,  $\alpha$ -catenin, and plectin [13]. Tight junction defects were confirmed via electron microscopy. Furthermore, our results indicate that rapidly proliferating colonic epithelial cells in ER $\beta^{-/-}$  mice are lost by increased shedding and not by increased apoptosis. The late development of colitis in  $LXR\alpha\beta^{-/-}$  mice coincided with the loss of ER $\beta$ .

## 4. Materials and Methods

### 4.1. Animals

In this study, 12- and 18-month-old ( $n = 3$ ,  $n = 2$ ) WT mice and 12- and 15-month-old ( $n = 3$ ,  $n = 2$ )  $LXR\alpha\beta^{-/-}$  mice were used for histological assays.  $LXR\alpha\beta^{-/-}$  mice with the C57BL/6 genetic background have been described [20,43,48]. Mice were housed in a room of standard temperature ( $22 \pm 1$  °C) with a regular 12 h light, 12 h dark cycle and given free access to water and standard rodent chow. All animal experiments were carried out according to the National Research Council's Guide for the Care and Use of Laboratory Animals and in accordance with the University of Houston guidelines on the use of laboratory animals. Effort was made to minimize the number of animals used and their suffering. All mice were anesthetized deeply with CO<sub>2</sub> and transcardially perfused with 0.1 M PBS followed by 4% paraformaldehyde. Colon tissues were dissected and post-fixed overnight at 4 °C using the same fixative. After fixation, colons were processed for paraffin sections.

### 4.2. Histopathological Analysis

Swiss-roll colon sections (5  $\mu$ m thick) were processed for hematoxylin/eosin (Sigma-Aldrich, St. Louis, MO, USA), periodic acid-Schiff (Chromaview, Kalamazoo, MI, USA), and Alcian blue (Electron Microscopy Sciences, Hatfield, PA, USA) staining. Staining was performed according to the manufacturer's instructions. The stained sections were imaged using an Olympus BX43 microscope at different magnifications. The number of goblet cells in the colon (40 $\times$  images) was statistically analyzed.

#### 4.3. Immunohistochemistry

The immunohistochemistry protocol was the same as that reported previously [21]. Paraffin sections were processed accordingly and then incubated overnight with antibodies specific for LXR $\alpha$  (1:1000; home-made), LXR $\beta$  (1:1000; home-made), LXR $\alpha\beta$  (1:100; Life Span Biosciences), MUC2 (1:2000; ab272692), TFF3 (1:5000; ab300427), Iba1 (1:1000; ab178846), Myeloperoxidase (1:500; ab45977), PCNA (1:8000; ab29),  $\beta$ -catenin (1:500; ab32572),  $\alpha$ -catenin (1:100; sc7894), E-cadherin (1:200; sc7870), Plectin (1:100; BD Transduction), ChAT (1:1500; ab178850), ELOVL1 (1:200; LS-B13651), and ER $\beta$  (1:100; in-house antibody mapping the C-terminus part). Primary antibodies were replaced with BSA as negative controls. Sections were incubated with biotinylated secondary antibody (1:200) for 1 h at room temperature, and then the Vectastain Avidin-Biotin Complex kit (Vector Laboratories, Newark, CA, USA) was used according to the manufacturer's instructions, followed by 3,3'-diaminobenzidine staining (Thermo Scientific, Waltham, MA, USA) and counter staining with Mayer's hematoxylin (Sigma-Aldrich, St. Louis, MO, USA). Images were acquired and analyzed using an Olympus BX43 microscope.

#### 4.4. Quantification of Immunostaining

Images were acquired with an Olympus BX43 microscope and saved as TIF files. Immunostaining results were examined and analyzed using ImageJ software [22]. Images were first grayscaled and then thresholded to 8-bit black and white. The integrated density of plectin, ChAT, and ELOVL1 was quantified and normalized, respectively.

#### 4.5. Data Analysis

Data are expressed as mean  $\pm$  SD. The statistical significance of differences between groups was evaluated using Student's *t*-test.  $p < 0.05$  was considered to be statistically significant.

### 5. Conclusions

In conclusion, LXRs play an important role in regulating the function of the mouse colonic epithelium, and the inactivation of LXR $\alpha\beta$  causes colitis similar to that seen in plectin knockout, but the direct regulator of the genes most changed in the LXR $\alpha\beta$ <sup>-/-</sup> mouse colon was ER $\beta$ .

**Supplementary Materials:** The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/ijms241512461/s1>.

**Author Contributions:** X.S., M.W. and J.-Å.G. designed the research; X.S., W.W., Y.D., M.W., I.N., P.A. and M.V. performed the research; X.S., M.W. and J.-Å.G. analyzed the data; and X.S., M.W. and J.-Å.G. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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**Data Availability Statement:** Data and materials used to support the findings of this study are available from the corresponding author upon request.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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