Biochimica et Biophysica Acta 1862 (2016) 166–173

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

Splenic dendritic cell involvement in FXR-mediated amelioration of DSS colitis

Vittoria Massafra [a], Noortje Ijssennagger [a], Maud Plantinga [b], Alexandra Milona [a], José M. Ramos Pittol [a], Marianne Boes [b], Saskia W.C. van Mil [a,*]

[a] Center for Molecular Medicine, UMC Utrecht, Utrecht, The Netherlands
[b] Laboratory of Translational Immunology, UMC Utrecht, Utrecht, The Netherlands

ARTICLE INFO

Article history:
Received 25 June 2015
Received in revised form 2 October 2015
Accepted 4 November 2015
Available online 7 November 2015

Keywords:
Farnesoid X receptor
Dendritic cells
Inflammatory Bowel Disease

ABSTRACT

Inflammatory Bowel Disease (IBD) is a multifactorial disorder involving dysregulation of the immune response and bacterial translocation through the intestinal mucosal barrier. Previously, we have shown that activation of the bile acid sensor Farnesoid X Receptor (FXR), which belongs to the family of nuclear receptors, improves experimental intestinal inflammation, decreasing expression of pro-inflammatory cytokines and protecting the intestinal barrier.

Here, we aimed to investigate the immunological mechanisms that ameliorate colitis when FXR is activated. We analyzed by FACS immune cell populations in mesenteric lymph nodes (MLN) and in the spleen to understand whether FXR activation alters the systemic immune response. We show that FXR activation by obeticholic acid (OCA) has systemic anti-inflammatory effects that include increased levels of plasma IL-10, inhibition of both DSS-colitis associated decrease in splenic dendritic cells (DCs) and increase in Tregs. Impact of OCA on DC relative abundance was seen in spleen but not MLN, possibly related to the increased FXR expression in splenic DCs compared to MLN DCs. Moreover, FXR activation modulates the chemotactic environment in the colonic site of inflammation, as Madcam1 expression is decreased, while Ccl25 is upregulated. Together, our data suggest that OCA treatment elicits an anti-inflammatory immune status including retention of DCs in the spleen, which is associated with decreased colonic inflammation. Pharmacological FXR activation is therefore an attractive new drug target for treatment of IBD.

1. Introduction

Inflammatory Bowel Disease (IBD) encompasses a group of disorders characterized by chronic intestinal inflammation. IBD involves dysregulation of the local mucosal immune response combined with compromised intestinal epithelial barrier function, in genetically predisposed individuals [1]. While treatment options for IBD patients are reasonably effective, they are often accompanied by significant side-effects and 40–50% of patients on currently available IBD medication fail to respond to the treatment [2,3]. Thus, these clinical observations underscore the need for novel treatment options for IBD. Considering that progression into active disease requires the recruitment of leukocytes to the intestine, current research for IBD treatment involves the targeting of selective adhesion molecules and chemokine receptors that guide leucocyte migration [4,5].

Farnesoid X Receptor (FXR) is a bile acid (BA)-activated transcription factor belonging to the family of nuclear receptors. FXR regulates BA glucose and lipid homeostasis and decreases tumor formation in liver and intestine [6]. Besides its metabolic functions, FXR activation also elicits anti-inflammatory and immune modulatory effects. We, and others have shown that FXR activation attenuates the severity of colitis in two separate murine models of IBD [7,8]. In these studies, the protection against colitis involved the repression of pro-inflammatory cytokine expression and inhibition of microbicidal genes, and thus likely modification of intestinal microbiota. FXR activation was shown to modulate *in vitro* dendritic cell (DC) differentiation and TNF production by immune cells, but the nature and extent of FXR impact on immune function in colitis *in vivo* is currently unclear.

DCs are a critical immune cell subset bridging the innate and adaptive immune responses, and are implicated in the immune dysregulation associated with colitis. Adoptive transfer of bone marrow-derived DCs exacerbated dextran sodium sulfate (DSS)-induced colitis in mice, while selective genetic ablation of DCs attenuated the severity of colitis [9]. Anti-DC therapy in colitis blocked leukocyte infiltration to the inflamed colon and controlled inflammation more effectively than targeting further downstream events, highlighting the involvement of DCs at early stages of inflammation [9]. DCs are found scattered throughout tissues, where they monitor the surroundings and

* Corresponding author: Center for Molecular Medicine, UMC Utrecht, HP 3.217, PO box 85060, 3508 AB Utrecht, The Netherlands.
E-mail address: S.W.C.vanMil@umcutrecht.nl (S.W.C. van Mil).

http://dx.doi.org/10.1016/j.bbadis.2015.11.001
0925-4439/

capture antigens. Nuclear receptors are also sensors of the local tissue environment and can relay extracellular signals into changes in gene expression. Consistently, some nuclear receptors trigger both metabolic and immunological effector functions. Nuclear receptors involved in metabolism also modulate macrophage and DC function, affecting their maturation, their ability to activate T cells and their migratory properties [10]. For example, retinoid receptors (RXR and RAR) mostly imprint a pro-inflammatory phenotype in DCs, whereas peroxisome proliferator-activated receptor (PPARγ), vitamin D receptor (VDR) and liver X receptor (LXR) have anti-inflammatory effects [10]. Yet the widespread connections between immunology and metabolism and the potential of nuclear receptor agonists in therapy for IBD are far from being fully explored.

In this study, we examined the systemic effects of FXR activation on splenic and mesenteric lymph node (MLN) immune cell populations in DSS-induced colitis. We show that FXR activation changes the relative abundance of splenic immune cell populations and affects the chemotaxis of cells in the colon during colitis. Together, our data suggest that obeticholic acid (OCA) treatment results in retention of DCs in the spleen, which is associated with decreased colonic inflammation.

2. Methods

2.1. Animals

Wild-type (WT) C57BL/6J male mice (8 weeks old) were obtained from Charles River Laboratories (Leiden, The Netherlands). Mice were fed chow diet *ad libitum* and housed in a temperature and light-controlled room. All experiments were approved by the ethics committee of the University Medical Center Utrecht.

Colitis was induced by administration of 2,5% (wt./vol) Dextran Sodium Sulfate (DSS; MW. 36,000–50,000 Da, MP Biochemicals, Santa Ana, California, U.S.) in drinking water for 8 days. Pharmacological activation of FXR was accomplished by treatment with obeticholic acid (OCA, 6-ethyl-chenodeoxycholic acid, kindly provided by Luciano Adorini, Intercept Pharmaceuticals Inc., San Diego, California, U.S.). OCA (10 mg/kg/day) or vehicle (1% methyl cellulose) were administered by oral gavage once a day from three days prior to DSS treatment, and continued until the end of the DSS-treatment. In the evening prior to the sacrifice, mice received an extra gavage of OCA. Mice were starved for 4 h prior to sacrifice. 10 mice per group were used for this experiment (groups: no DSS, DSS, and DSS + OCA). Body weight was assessed daily. Rectal bleeding was scored on a scale from 0 to 4, indicating no (0) to severe (4) rectal bleeding.

2.2. Intestinal permeability assay

Intestinal permeability was examined in mice after 8 days of DSS treatment, as described previously [11]. In brief, mice were gavaged with 0.6 mg/g body weight of fluorescein isothiocyanate (FITC)-conjugated dextran (Sigma, S Louis, Missouri, U.S.; molecular mass 3–5 kDa) 4 h before termination. FITC concentrations were measured in plasma (Fluorimeter Pharos FX; BioRad, Hercules, California, U.S.).

2.3. RNA extraction, cDNA synthesis and qRT-PCR analysis

Colons were harvested, washed in PBS and snap frozen. RNA was isolated using the RNeasy Micro kit (Qiagen, Venlo, The Netherlands). cDNA was generated from 1 μg of total RNA using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, California, U.S.). qRT-PCR analysis was carried out using SYBR green PCR mastermix (Roche, Basel, Switzerland) and analyzed on a MyIQ real time PCR cycler (BioRad, Veenendaal, The Netherlands). Relative expression of colonic genes is normalized to glyceraldehyde-3-phosphate dehydrogenase, whereas expression of splenic genes is normalized to hypoxanthine phosphoribosyltransferase 1. Primer sequences are listed in Supplementary Table 1.

2.4. Isolation of spleen and mesenteric lymph node (MLN) cells for flow cytometry and cell sorting

Spleen and MLNs were passed through a cell strainer to make a single-cell suspension and then stained with fluorochrome-labeled mouse antibodies. All antibody incubations were performed at 4 °C for 30 to 45 min. For detection and phenotyping of cell subsets in cell suspensions of spleen and mesenteric lymph nodes, cells were stained with monoclonal antibodies directed against CD3, MHCII, CD11c, CD19, GR-1, CD11b, CD4, CD8 and CD25 (all from Biolegend, San Diego, California, U.S.), CD69 (BD Biosciences, Franklin Lakes, New Jersey, U.S.), Foxp3 Transcription Factor Staining Buffer Set and fixable live/dead marker Aqua (eBioscience, San Diego, California, U.S.). For intranuclear staining, cells were stained using the FoxP3 staining kit (eBioscience) according to the manufacturer's protocol. Acquisition of multicolor stained samples was done on a LSRII cytometer. Cell sorting was performed on a FacsARIA II cytometer and after cell sorting, purity was checked (always >95%). Final analysis and graphical output were performed using FACS Diva™ software (BD bioscience).

2.5. Multiplex cytokine analysis

Cytokine concentrations of IFNγ, IL-2, IL-10 and IL-17A were measured in mouse plasma, using Diaclone Murine 5-plex, following the manufacturer's protocol (Diaclone, Besançon cedex, France). In brief, antibodies coupled to differentially fluorescently labeled beads were mixed in suspension with plasma samples and incubated with biotin-conjugated secondary antibody. Phycoerythrin-Streptavidin was subsequently added to the sample and detection of the cytokine-specific intensities was performed by FacsCalibur.

2.6. Immunohistochemistry and immunofluorescence

Paraffin-embedded colon tissue sections were incubated overnight with anti-CXCR3 1:250 (Bioss, Woburn, Massachusetts, U.S.) and for 1 h with secondary antibody goat anti rabbit 1:200 (Thermo Fisher Scientific, Waltham, Massachusetts, U.S.) and counterstained with Hematoxylin. The percentage of CXCR3-positve cells was calculated as a ratio over haematoxilin positive cells as quantified by ImageJ. Colon cryosections were fixed in acetone and incubated with antibody against MHCII conjugated with APC diluted 1:100 (Biolegend) and counterstained with NucGreen Dead488, following the manufacturer's protocol (Life Technologies, Carlsbad, California, U.S.). Imaging of the sections was performed using a confocal microscope (Zeiss LSM510).

2.7. Statistical analysis

All results are expressed as mean ± SEM, unless stated differently. Statistical significance was determined by unpaired non parametric T-test (Mann–Whitney test) and by Kruskal–Wallis Anova test. Statistical analyses were performed using Graphpad (version 6.02) software. Two-sided p values ($p < 0.05$) were considered significant.

3. Results

3.1. FXR activation alters the composition of splenic immune cell populations

We addressed whether amelioration of colitis induced by FXR activation involves secondary lymphoid organs. We therefore induced colitis in C57Bl6 mice by administering 2.5% DSS in the drinking water for 8 days. Three days prior to DSS treatment, we started daily OCA administration (10 mg/kg) per oral gavage until the end of the experiment, as previously described [7]. Efficient FXR activation in the colon of OCA-treated mice was evaluated by increased mRNA expression of the primary FXR target gene IBABP (Supplementary Fig. 1A). We

confirmed that FXR activation decreased rectal bleeding scores, intestinal permeability and mRNA expression of the pro-inflammatory cytokine IL-1β in the colon (Supplementary Fig. 1B–D), as previously described [7].

To understand the role of immune cells in mesenteric lymph nodes and spleen in OCA-mediated amelioration of colitis, we assayed the cellular composition of the MLNs and splenocytes by flow cytometry (Supplementary Fig. 2). Colitis induction triggered a significant decrease in splenic DCs. OCA treatment fully prevented the disease-associated decrease in splenic DCs (Fig. 1A). OCA did not alter the ratio of CD8-positive/CD11b-positive DCs, as the abundance of both DC subsets was equally rescued by OCA during colitis (Supplementary Fig. 3A). In MLNs, which are known to drain colonic tissues, DC counts were unaffected by DSS or OCA treatment (Fig. 1A), despite the significant increase in total number of live cells in the MLNs upon DSS treatment (Supplementary Fig.3B). The amount of granulocytes was higher in the MLNs and the spleen in the DSS-treated groups compared to the group receiving no DSS. OCA treatment further increased the amount of granulocytes only in the spleen (Fig. 1B). The fraction of FoxP3-positive regulatory T-cells (Tregs) increased in the MLNs as well as in the spleen upon the induction of colitis by DSS. OCA treatment resulted in a significant decrease in Tregs only in the spleen (Fig. 1C). No significant differences were observed between DSS and DSS + OCA groups in the relative fraction of B cells, CD4-positive and CD8-positive T lymphocytes either in spleen or MLNs (Supplementary Fig. 3C/D).

The decrease in spleen DCs upon inflammation induced by DSS colitis and its intersection by OCA treatment may be due to impaired differentiation/maturation, increased cell death, or local depletion in favor of accumulation at sites of inflammation. We first addressed the possibility that OCA treatment causes changes in differentiation/maturation

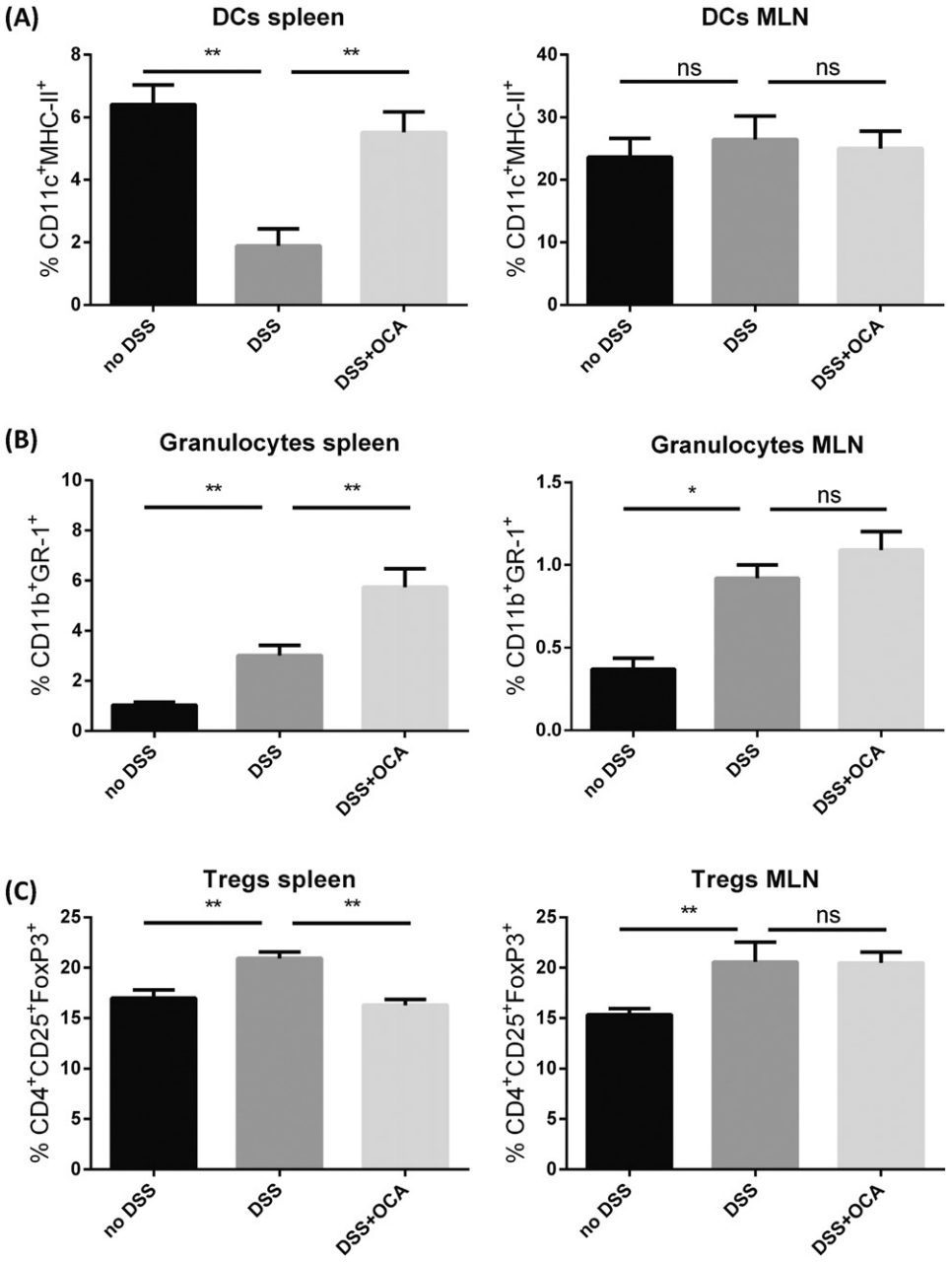


Fig. 1. FXR activation alters the composition of splenic immune cell populations. FACS analysis of DCs (A), granulocytes (B) and Tregs (C) in the spleen and in the MLNs collected from mice receiving vehicle, DSS or DSS in combination with OCA. Gating on live cells and out gating T-, B-cells and granulocytes, the relative amount of DCs ($CD11c^+MHCII^+$) is depicted. Granulocytes are expressed as percentage of $CD11b^+GR\text{-}1^+$ cells. Tregs are expressed as percentage of $CD4^+CD25^+FoxP3^+$ cells. Data are represented as mean ± SEM, n = 10 mice/group. Mann–Whitney T-test was performed to determine differences between groups. (*$p < 0.05$, **$p < 0.01$).

of the DCs, by assessment of gene expression of MHCII encoding genes H2Ab1 and H2Eb1 and APC markers CD80 and CD86 in spleen tissue (Fig. 2B). Expression of these maturation markers decreased significantly upon DSS but mRNA levels were not restored upon OCA treatment, suggesting that OCA-treatment probably does not cause persistence of DC markers due to increase of these markers per cell. Instead, low CD80/CD86 mRNA levels caused by DSS might be explained by DC migration out of the spleen, rather than by an effect on maturation, because it is expected that DSS treatment would increase DC maturation. Further analysis of FACS data revealed an increase in CD11c/MHCII-positive DC size (Forward Scatter) and granularity (Side Scatter) upon DSS treatment (Fig. 2C), which is commonly associated with increased maturation [12,13] and negatively associated with cell death [14]. Importantly, OCA treatment restored normal size and granularity of DCs, suggesting decreased maturation compared to DSS alone. We therefore conclude that alteration of DC maturation or cell death cannot explain the reduction in splenic DCs upon DSS treatment, nor the rescue by OCA. Instead, all available evidence supports that DSS treatment causes depletion of splenic CD11c/MHCII-positive DCs, a feature that OCA can counteract.

3.2. FXR activation enhances systemic anti-inflammatory cytokine production

In light of the tight functional connection between the spleen and the bloodstream, we aimed at determining the systemic effects of FXR activation, by measuring cytokine production in plasma. The plasma concentration of the pro-inflammatory cytokine IL-6 was remarkably high upon DSS treatment, irrespective of OCA administration. Interestingly, the concentration of the anti-inflammatory cytokine IL-10 was significantly increased in mice receiving OCA. No significant differences were detected in the plasma concentrations of IFNγ, IL-2 and IL-17A.

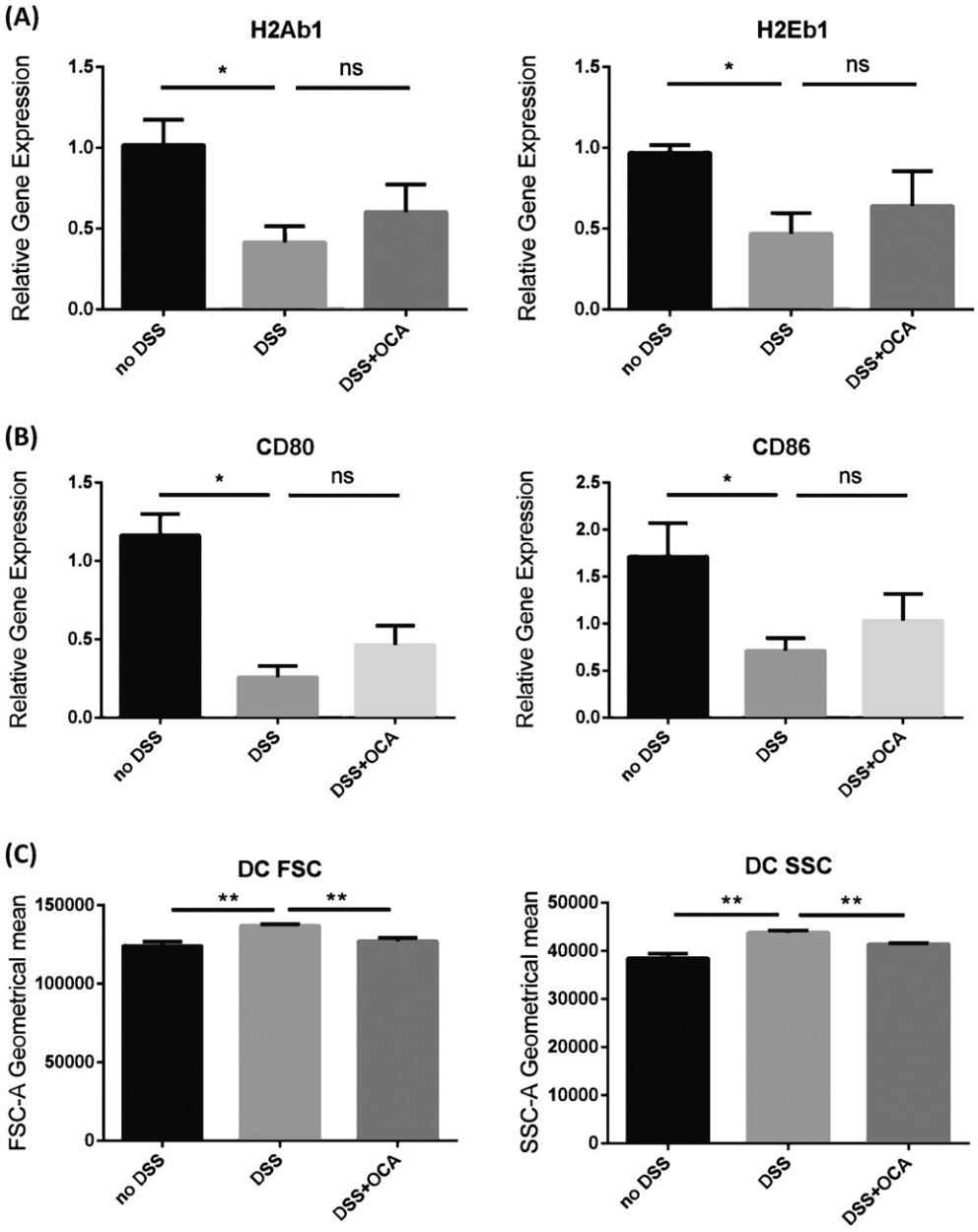


Fig. 2. OCA impacts on splenic depletion of DCs upon colitis induction. mRNA expression of MHCII encoding genes H2Ab1 and H2Eb1 (A) and of APC maturation markers CD80 and CD86 in spleen tissue of mice treated either with vehicle or DSS. Data are normalized to Hprt1 expression. (B). (C) Geometrical mean of forward and side scatter signal of DCs defined as $CD11c^{+}$ $MHCII^{+}$, derived from gating on live cells and out gating T-, B-cells and granulocytes. Data are represented as mean ± SEM. Mann–Whitney T-test was performed to determine differences between groups. (*$p < 0.05$, **$p < 0.01$).

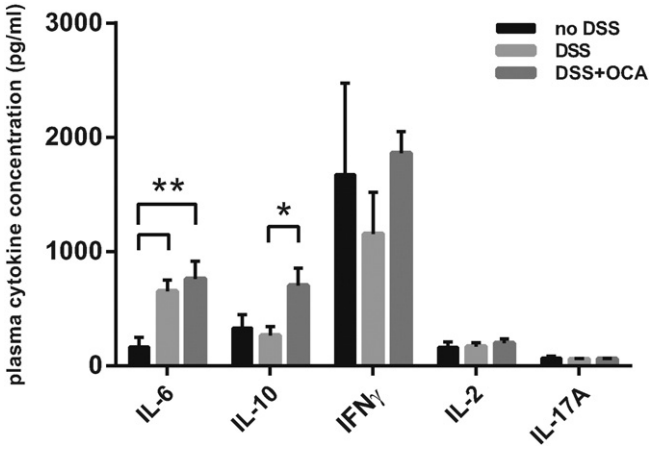


Fig. 3. FXR activation alters systemic cytokine concentration. Absolute concentration of cytokines IL-6, IL-10, IFNγ, IL-2 and IL-17A were determined in plasma of mice treated with either vehicle, DSS or DSS in combination with OCA. Data are represented as mean ± SEM, n = 6 mice/group. Mann–Whitney T-test was performed to determine differences between groups. (*$p < 0.05$, **$p < 0.01$).

(Fig. 3). In conclusion, administration of OCA resulted in higher systemic levels of the anti-inflammatory cytokine IL-10.

3.3. FXR is highly expressed in splenic DCs

FXR activation increased systemic levels of the anti-inflammatory cytokine IL-10, and abrogated the decrease in splenic DCs and increase in Tregs in DSS-treated mice. We therefore hypothesized that FXR is expressed in a subset of splenic cells. In order to investigate which immune cells express FXR, RNA was isolated from FACS-sorted subpopulations of immune cells in MLNs and spleen (Supplementary Fig. 4). FXR expression is lower in immune cells than in liver and intestinal cells (data not shown), but it is significantly higher in splenic DCs compared to lymph node DCs and splenic and MLN B, CD4-positive and CD8-positive cells (Fig. 4). Based on these data and data in Fig. 1, we propose that FXR expressing splenic DCs respond to OCA treatment in DSS-treated colitis, potentially resulting in their retention in the spleen.

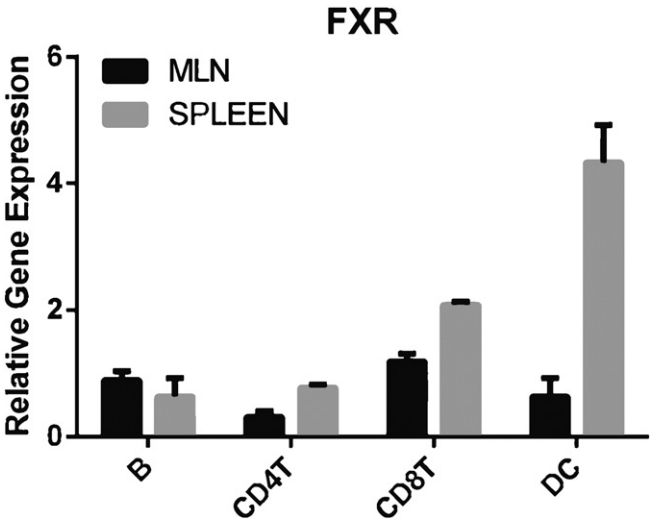


Fig. 4. FXR is expressed in splenic DCs. mRNA expression of the nuclear receptor FXR in FACS-sorted subsets of immune cells derived from either spleen or MLNs. Wild type healthy BL/6 mice were used for this purpose. RT-qPCR was performed in technical duplicate. Data are normalized to Gapdh expression. Data are represented as mean ± SD.

3.4. FXR activation modulates colonic chemotaxis events in favor of amelioration of colitis

We next addressed whether splenic DC depletion upon DSS treatment associates with an increase in migratory cells in the colon and whether OCA can counteract such immune cell infiltration. Intestinal MHCII/CD103-positive DCs express the chemokine receptor CXCR3 and migrate to the colon in response to inflammatory stimuli during a *Cryptosporidium parvum* infection [15]. In our study, the percentage of CXCR3-positive cells was significantly increased upon DSS treatment, correlating with an increased influx of CXCR3-positive cells during colonic inflammation. OCA treatment tended to reverse the increase in CXCR3 expression in the colon (Fig. 5A/B), possibly contributing to the OCA-dependent decrease of recruitment of immune cells to the colon, including DCs. Also MHCII staining and CD11c mRNA expression were increased upon DSS treatment, and showed a trend to decrease upon OCA (Supplementary Fig. 5). To exclude that the changes in these DC markers are not due to changes in macrophage infiltration in the colon, as these markers are also expressed on macrophages, we determined gene expression of macrophage specific markers F4/80 and CD68. In support, neither of these two markers showed any enrichment upon DSS treatment or rescuing effect upon OCA (Fig. 5C/D), collectively underscoring that OCA treatment may cause a reduction in DC counts in the colon.

To investigate further the local chemotaxis events affected by FXR activation, we determined gene expression levels of chemokine receptors and chemokine ligands in the colon (Fig. 5E–I). Madcam1 is a chemokine expressed in cytokine activated endothelial cells within the MLNs and in the lamina propria of the intestine. Colonic expression of Madcam1 increased significantly upon DSS treatment, and this increase was partially prevented by OCA treatment (Fig. 5E). The receptor for Madcam1, α4β7, an integrin expressed in T cells recruited to inflamed sites in the gut. Colonic expression of Itga4 and Itgb7 (encoding for the integrin α4β7) was not affected by OCA (Fig. 5F/G). Disruption of α4β7-Madcam axis is known to ameliorate symptoms of colitis [16]. Thus, inhibition of Madcam1 expression may contribute to OCA treatment-induced amelioration of colitis.

Ccl25 is another chemokine with gut homing effects produced by small and large intestinal epithelial cells [17]. Ccl25 binds the chemokine receptor Ccr9. Tregs rely on Ccl25-Ccr9 axis for homing to intestine during ileitis [18]. Expression of Ccl25 increased upon DSS and increased even further upon OCA treatment in DSS-treated mice, whereas changes in Ccr9 expression were modest (Fig. 5H/I). Unlike deficiency of the Madcam1-α4β7 axis which ameliorates colitis [16], disruption of Ccl25-Ccr9 axis worsens the symptoms of colitis [17]. Thus, OCA-induced improvement of colitis may depend on the increase in Ccl25 expression and the consequent recruitment of Tregs inhibiting the inflammatory process.

Together, these data suggest that FXR acts as a modulator of colonic chemotaxis of immune cells resulting in a decrease in colitis inflammatory symptoms.

4. Discussion

IBD is thought to primarily result from a damaged mucosal barrier as a consequence of genetic factors or exogenous agents. The recurrent episodes of intestinal inflammation are promoted by the penetration of bacterial products through the damaged mucosal layer [1]. DSS-induced mouse colitis is a well-established model for studying human colitis, which encompasses a DSS-dependent damaging effect on the colonic epithelium, responsible for allowing bacterial translocation and therefore activating macrophages and neutrophils [19,20]. However, recent studies question the role of bacterial translocation as primary event in the development of inflammation [21,22]. Instead, DC activation is now considered a direct trigger after epithelial damage, sufficient to trigger production of chemokines and pro-inflammatory cytokines [9].

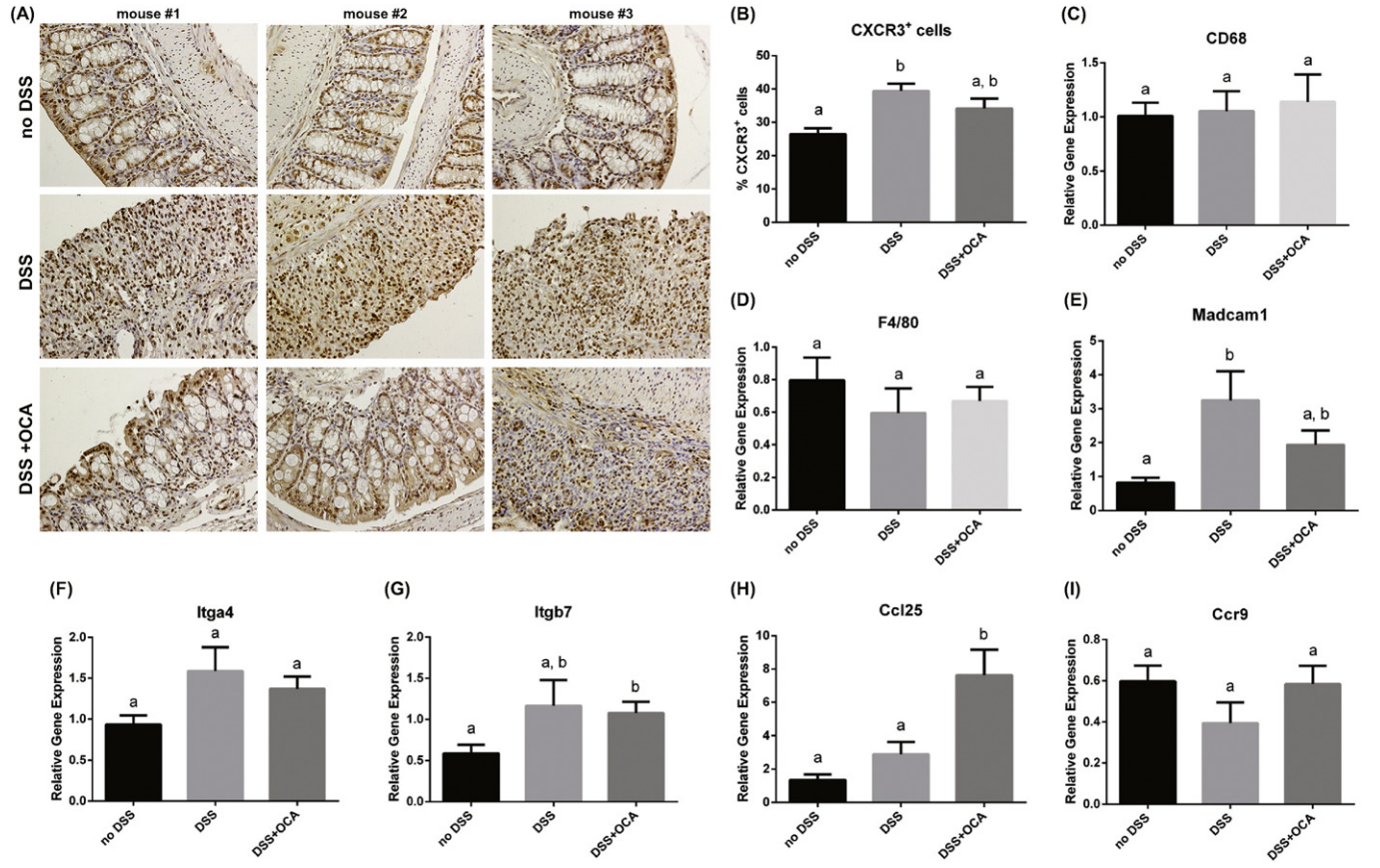


Fig. 5. FXR activation modulates colonic chemotaxis events in favor of amelioration of colitis. (A) Representative immunohistochemistry pictures of CXCR3$^+$ cells in paraffin-embedded colon sections collected from mice treated with either vehicle or DSS or DSS in combination with OCA. Sections of 3 different mice are depicted. (B) Relative amount of CXCR3$^+$ over hematoxylin-stained cells was determined using ImageJ; 10 independent microscope fields per section were analyzed (in 2 non consecutive sections/mouse; 10 mice/group). (C) mRNA expression of Madcam1 and of Itga4 and Itgb7 (encoding for the gut homing integrin α4β7) and (D) of Ccl25 and its receptor Ccr9. (E) mRNA expression of macrophage markers F4/80 and CD68 in colon tissue of mice treated either with vehicle or DSS or DSS + OCA. Data are normalized to Gapdh expression. (A–E) Data are represented as mean ± SEM. Kruskal-Wallis Anova test was performed for multiple comparison analysis.

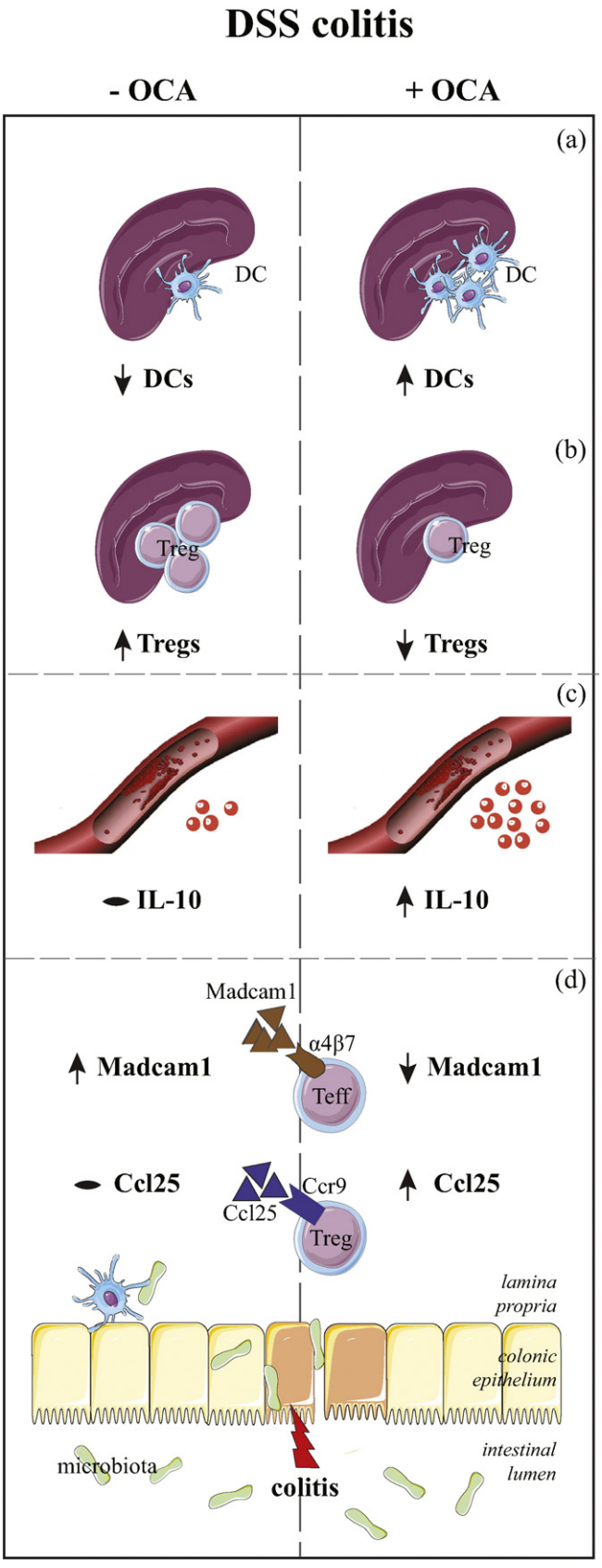
DCs are key sentinels able to sense pathogen and danger signals in the intestinal epithelium and deliver them to the lymphoid tissues, orchestrating an appropriate immune response to maintain mucosal homeostasis [9,23]. DCs gain a functional phenotype able to enhance or attenuate the severity of DSS colitis, depending on the locally perceived stimuli [24]. Here we show that activation of the metabolic nuclear receptor FXR i) rescued depletion of splenic DCs and prevented local increases in Tregs, ii) increased the plasma concentration of IL-10, an anti-inflammatory cytokine, iii) collectively and consistently shows a trend for DC markers to be decreased in the colon, iv) decreased gene expression of intestinal homing factor for effector T cells Madcam1, and v) increased gene expression of intestinal homing factor for Tregs Ccl25 in DSS colitis (Fig. 6).

MLNs host immunological changes relevant for mounting an inflammatory response in DSS colitis [25]. The relative amounts of granulocytes and Tregs were increased in the MLNs during DSS colitis (Fig. 1B/C). This is in line with a previous study [25], reporting that the percentage of CD11b/CD11c-positive phagocytes, and CD4/CD25-positive Tregs increased in MLNs upon DSS treatment. Although FXR activation improved the symptoms of colitis (Supplementary Fig. 1) in line with previous studies [7,8], we did not observe significant immunological changes in the MLNs compared to the DSS group, suggesting that FXR mediated protection from colitis may rely on the involvement of different secondary lymphoid tissues, such as the spleen.

DSS treatment has been reported to cause splenomegaly [26,27], and increase splenic Tregs and CD4/CD49-positive NK [25]. We report an increase in Tregs and a decrease in DCs in the spleen during DSS colitis. Tregs probably increase to restrain a dysregulated inflammatory response, and the OCA-mediated decrease in Tregs may therefore reflect the reduced need to limit intestinal inflammation. Analysis of DC morphology and surface-expressed activation markers suggested that DCs are likely more mature upon DSS treatment and OCA reverses this increase in maturation, suggesting that the decrease in DCs in the spleen cannot be explained by DC maturation differences. Of note, it has also been shown that the spleen stores reservoirs of monocytes readily deployable to migrate to injured tissue during acute inflammation [28,29]. Together, the data suggest that OCA-mediated FXR activation may cause retention of monocyte DC progenitors in the spleen, which lowers their influx into the colonic inflammatory site, and thereby reduces colitis symptoms.

FXR has been shown to be expressed in immune cells derived from peripheral blood mononuclear cells (PBMCs) and immortalized immune cells [30,31]. Here we show that FXR is highly expressed in mouse splenic DCs and we propose that it may have a role in modulation of DC function upon colitis induction. Considering that FXR activation can favor polarization of anti-inflammatory rather than of pro-inflammatory monocytes in liver inflammation [32], further studies are needed to establish whether FXR is preferentially expressed in DCs carrying a pro-inflammatory or anti-inflammatory phenotype during colitis. FXR has also been described to inhibit NF-κB transcriptional activity [7]. It could then be plausible that by FXR activation in DCs or monocyte DC progenitors, the signaling cascade that leads to migration to the colon is suppressed due to an FXR-mediated reduction in NF-κB signaling in DCs. However, our experimental setup precluded us from

analysis whether OCA directly impacts on splenic immune cells or acts on the intestine, thereby indirectly altering the necessity of the splenic DCs to react to the inflammation.



Depletion of colonic DCs has proven to be beneficial for colitis, whereas adoptive transfer of DCs worsens the symptoms [9]. This indicates that DCs are critical in this model of colitis and implies that inhibition of their migration to the colon would be protective against DSS colitis features. Intriguingly, the chemokine receptor CXCR3, which is responsible for gut homing of DCs [15] was increased in mice with DSS colitis, and decreased by OCA treatment. CXCR3 is reported to be upregulated in IBD patients. In addition, ablation of CXCR3 attenuates the progression of DSS-induced colitis [33]. Taken together, these observations indicate that protection of OCA against colitis could involve colonic depletion of DCs and impaired expression of chemokine receptors. However, further cytofluorimetry-based assays of cells freshly isolated from colon are needed to validate that OCA actually impairs specific CXCR3-positive DC infiltration.

As the chemokine environment present in the colon is of utmost importance in shaping the immunological response, we determined the colonic expression of genes having gut or lymphoid tissue homing effects. α4β7-Madcam1 interaction guides effector T cells to the inflamed colon and the induction of migratory phenotype in effector T cells is dependent on DCs [34]. In our study, expression of Madcam1 increased in colitic mice and this increase was partially prevented by OCA treatment. These results provide insight into the potential effects of FXR activation on migratory properties of immune cells during colitis. Of note, targeting Madcam1 has been proven to be beneficial for colitis, as blocking Madcam1 *in vivo* reduces leukocyte extravasation and reverses both chronic and acute colitis [16,35]. These observations support the beneficial role of FXR in counteracting gut homing signaling of immune cells fostering the inflammatory process.

The Ccr9-Ccl25 interaction is also ascribed as causal for gut homing effects, but targets different immune cells. Indeed, Tregs have been shown to be more dependent on Ccl25/Ccr9 than effector T cells for homing to the intestine [18]. Moreover, unlike α4β7-Madcam1 interaction, ablation of Ccr9-Ccl25 interaction exacerbates colitis, causes an imbalance in DC subpopulations (increase of plasmatcytoid/conventional DCs ratio) and leads to accumulation of inflammatory monocytes in the lamina propria of the colon and in the gut-associated lymphoid tissue [17]. Ccl25 levels in large intestine have been described to increase during the recovery period of DSS-treated mice, confirming a regulatory role of Ccr9–Ccl25 interactions during large intestinal inflammation [17]. In our study, expression of Ccr9 cells underwent a modest decrease upon DSS and went back to normal levels upon OCA treatment, whereas Ccl25 expression was strongly increased upon OCA treatment. These results point to an FXR-dependent generation of strong chemotactic signals for Tregs, which can contain the inflammatory response and accelerate the recovery from colitis. It has yet to be determined whether intestinal cells expressing FXR produce Ccl25 or if FXR impacts on DC function, as bridging event for the recruitment of Tregs. Interestingly, DSS colitis increased IL-6 secretion systemically, whereas FXR activation counteracts inflammatory stimuli *via* systemic IL-10 increase. This is a remarkable finding, considering that IL-10 knockout mice develop colitis [36] and IL-10 administration reverses experimental colitis [37].

The extent of disease state in mice receiving DSS treatment is quite variable, depending on mouse strain [38], animal housing conditions and intestinal microbiota [39]. This probably explains why the FXR-mediated protection against DSS colitis was less pronounced than

Fig. 6. Schematic representation of immunological effects of FXR activation during DSS colitis. Similar to IBD, DSS compromises the integrity of intestinal mucosal barrier, allowing bacterial translocation from the lumen to the lamina propria, where specialized antigen presenting cells react to the presence of danger signals. The inflammatory process involves the spleen where the amount of DCs reduces and Tregs increase. FXR activation reverses the effects of DSS colitis, probably *via* abrogation of the depletion of DCs (a) and the increase in Tregs in the spleen (b). At the systemic level, FXR activation causes an increase in anti-inflammatory cytokine IL-10 (c). In the colon, FXR activation attenuates the DSS-dependent increase of the gut homing chemokine Madcam1, which binds α4β7 on effector T cells and enhances the production of Ccl25 (d), attracting Tregs to contain the inflammation.

previously described [7]. Yet, the direction of changes induced by OCA treatment results in consistent beneficial effects across all measurements performed, pointing towards lowering the propagation of colonic inflammation to secondary lymphoid tissues. Our results strengthen our interest to further investigate the molecular mechanisms underlying these improvements and further encourage investigation of FXR agonists as treatment option in IBD patients.

In conclusion, our results support that FXR activation, previously shown to be beneficial for DSS colitis, impacts on the splenic immune response during colitis and can shift the balance in the colon and blood in favor of anti-inflammatory responses. Our work provides novel support to the emerging role of FXR in regulation of immune responses, broadening the well-established knowledge of its functions as metabolic sensor.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbadis.2015.11.001.

Transparency document

The Transparency document associated with this article can be found, in online version.

Author conflict of interest disclosures

There is no conflict of interest to disclose.

Acknowledgments

Grant support: SWCvM is supported by the Netherlands Organization for Scientific Research (NWO) Project VIDI (917.11.365), FP7 Marie Curie Actions IAPP (FXR-IBD, 611979), the Utrecht University Support Grant, Wilhelmina Children's Hospital Research Fund.

We thank Sameh Youssef Hassan and Ellen Willemsen for expert technical assistance and Luciano Adorini for critical reading of the manuscript.

References

[1] D.K. Podolsky, Inflammatory bowel disease, N. Engl. J. Med. 347 (2002) 417–429.
[2] R.J. Farrell, D. Kelleher, Glucocorticoid resistance in inflammatory bowel disease, J. Endourol. 178 (2003) 339–346.
[3] S. Ben-Horin, U. Kopylov, Y. Chowers, Optimizing anti-TNF treatments in inflammatory bowel disease, Autoimmun. Rev. 13 (2014) 24–30.
[4] D.K. Podolsky, Selective adhesion-molecule therapy and inflammatory bowel disease—a tale of Janus? N. Engl. J. Med. 353 (2005) 1965–1968.
[5] E. Wendt, S. Keshav, CCR9 antagonism: potential in the treatment of inflammatory bowel disease, Clin. Exp. Gastroenterol. 8 (2015) 119–130.
[6] R.M. Gadaleta, M. Cariello, C. Sabba, et al., Tissue-specific actions of FXR in metabolism and cancer, Biochim. Biophys. Acta 2015 (1851) 30–39.
[7] R.M. Gadaleta, K.J. van Erpecum, B. Oldenburg, et al., Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease, Gut 60 (2011) 463–472.
[8] P. Vavassori, A. Mencarelli, B. Renga, et al., The bile acid receptor FXR is a modulator of intestinal innate immunity, J. Immunol. 183 (2009) 6251–6261.
[9] B.E. Berndt, M. Zhang, G.H. Chen, et al., The role of dendritic cells in the development of acute dextran sulfate sodium colitis, J. Immunol. 179 (2007) 6255–6262.
[10] M. Kiss, Z. Czimmerer, L. Nagy, The role of lipid-activated nuclear receptors in shaping macrophage and dendritic cell function: from physiology to pathology, J. Allergy Clin. Immunol. 132 (2013) 264–286.
[11] E. Cario, G. Gerken, D.K. Podolsky, Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function, Gastroenterology 132 (2007) 1359–1374.
[12] C.Q. Xia, K.J. Kao, Effect of CXC chemokine platelet factor 4 on differentiation and function of monocyte-derived dendritic cells, Int. Immunol. 15 (2003) 1007–1015.
[13] G. Hartmann, G.J. Weiner, A.M. Krieg, CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 9305–9310.
[14] Z. Darzynkiewicz, S. Bruno, G. Del Bino, et al., Features of apoptotic cells measured by flow cytometry, Cytometry 13 (1992) 795–808.
[15] L. Lantier, S. Lacroix-Lamande, L. Potiron, et al., Intestinal CD103+ dendritic cells are key players in the innate immune control of *Cryptosporidium parvum* infection in neonatal mice, PLoS Pathog. 9 (2013), e1003801.
[16] S. Farkas, M. Hornung, C. Sattler, et al., Blocking MAdCAM-1 in vivo reduces leukocyte extravasation and reverses chronic inflammation in experimental colitis, Int. J. Color. Dis. 21 (2006) 71–78.
[17] M.A. Wurbel, M.G. McIntire, P. Dwyer, et al., CCL25/CCR9 interactions regulate large intestinal inflammation in a murine model of acute colitis, PLoS One 6 (2011), e16442.
[18] J.D. Wermers, E.N. McNamee, M.A. Wurbel, et al., The chemokine receptor CCR9 is required for the T-cell-mediated regulation of chronic ileitis in mice, Gastroenterology 140 (2011) 1526–1535 (e3).
[19] B. Chassaing, J.D. Aitken, M. Malleshappa, et al., Dextran sulfate sodium (DSS)-induced colitis in mice, Curr. Protoc. Immunol. 104 (2014) (Unit 15 25).
[20] I. Okayasu, S. Hatakeyama, M. Yamada, et al., A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice, Gastroenterology 98 (1990) 694–702.
[21] S. Kitajima, M. Morimoto, E. Sagara, et al., Dextran sodium sulfate-induced colitis in germ-free IQI/Jic mice, Exp. Anim. 50 (2001) 387–395.
[22] M. Fukata, K.S. Michelsen, R. Eri, et al., Toll-like receptor-4 is required for intestinal response to epithelial injury and limiting bacterial translocation in a murine model of acute colitis, Am. J. Physiol. Gastrointest. Liver Physiol. 288 (2005) G1055–G1065.
[23] M. Rescigno, M. Urbano, B. Valzasina, et al., Dendritic cells express tight junction proteins and penetrate gut epithelial monolayers to sample bacteria, Nat. Immunol. 2 (2001) 361–367.
[24] K. Abe, K.P. Nguyen, S.D. Fine, et al., Conventional dendritic cells regulate the outcome of colonic inflammation independently of T cells, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 17022–17027.
[25] A. Hakansson, N. Tormo-Badia, A. Baridi, et al., Immunological alteration and changes of gut microbiota after dextran sulfate sodium (DSS) administration in mice, Clin. Exp. Med. 15 (2015) 107–120.
[26] R. Zhang, S. Ito, N. Nishio, et al., Dextran sulphate sodium increases splenic Gr1(+)CD11b(+) cells which accelerate recovery from colitis following intravenous transplantation, Clin. Exp. Immunol. 164 (2011) 417–427.
[27] Y. Wang, G. Han, Y. Chen, et al., Protective role of tumor necrosis factor (TNF) receptors in chronic intestinal inflammation: TNFR1 ablation boosts systemic inflammatory response, Lab. Investig. 93 (2013) 1024–1035.
[28] M.A. Ingersoll, A.M. Platt, S. Potteaux, et al., Monocyte trafficking in acute and chronic inflammation, Trends Immunol. 32 (2011) 470–477.
[29] F.K. Swirski, M. Nahrendorf, M. Etzrodt, et al., Identification of splenic reservoir monocytes and their deployment to inflammatory sites, Science 325 (2009) 612–616.
[30] A.B. Schote, J.D. Turner, J. Schiltz, et al., Nuclear receptors in human immune cells: expression and correlations, Mol. Immunol. 44 (2007) 1436–1445.
[31] B. Renga, M. Migliorati, A. Mencarelli, et al., Reciprocal regulation of the bile acid-activated receptor FXR and the interferon-gamma-STAT-1 pathway in macrophages, Biochim. Biophys. Acta 2009 (1792) 564–573.
[32] R.H. McMahan, X.X. Wang, L.L. Cheng, et al., Bile acid receptor activation modulates hepatic monocyte activity and improves nonalcoholic fatty liver disease, J. Biolumin. Chemilumin. 288 (2013) 11761–11770.
[33] B. Chami, A.W. Yeung, C. van Vreden, et al., The role of CXCR3 in DSS-induced colitis, PLoS One 9 (2014), e101622.
[34] B. Johansson-Lindbom, M. Svensson, M.A. Wurbel, et al., Selective generation of gut tropic T cells in gut-associated lymphoid tissue (GALT): requirement for GALT dendritic cells and adjuvant, J. Exp. Med. 198 (2003) 963–969.
[35] S. Kato, R. Hokari, K. Matsuzaki, et al., Amelioration of murine experimental colitis by inhibition of mucosal addressin cell adhesion molecule-1, J. Pharmacol. Exp. Ther. 295 (2000) 183–189.
[36] P. Kiesler, I.J. Fuss, W. Strober, Experimental models of inflammatory bowel diseases, Cell Mol. Gastroenterol. Hepatol. 1 (2015) 154–170.
[37] M. Sasaki, J.M. Mathis, M.H. Jennings, et al., Reversal of experimental colitis disease activity in mice following administration of an adenoviral IL-10 vector, J. Inflamm. 2 (13) (2005) (Lond).
[38] J.L. Knod, K. Crawford, M. Dusing, et al., Mouse strain influences angiogenic response to dextran sodium sulfate-induced colitis, J. Surg. Res. 190 (2014) 47–54.
[39] O. Rossi, M.T. Khan, M. Schwarzer, et al., Faecalibacterium prausnitzii strain HTF-F and its extracellular polymeric matrix attenuate clinical parameters in DSS-induced colitis, PLoS One 10 (2015), e0123013.