Biomedicine & Pharmacotherapy 129 (2020) 110368

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

The anti-inflammatory and immune-modulatory effects of OEA limit DSS-induced colitis in mice

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ARTICLE INFO

Keywords:
Mesenteric lymph nodes
Intestinal barrier
PPAR-α
Inflammasome
Inflammatory bowel disease

ABSTRACT

Fatty acid ethanolamides acting on proliferator-activated receptor (PPAR)-α are among the endogenous lipid molecules that attenuate inflammatory processes and pain sensitivity. Whereas these properties are well-known for palmitoylethanolamide (PEA), the efficacy of oleoylethanolamide (OEA, first described as a satiety hormone synthesized in the jejunum) has been overlooked. In this study, we aimed to evaluate the effect of OEA administration in a mouse model of colitis. C57BL/6J mice were exposed to 2.5% dextran sodium sulphate (DSS) in drinking water for 5 days. Daily i.p. administration of 10 mg/kg OEA started 3 days before DSS and lasted for 12 days. The DSS-untreated control group received only ultrapure water. DSS mice treated with OEA had a significant improvement of disease score. OEA restored mRNA transcription of PPAR-α, of tight junctions and protective factors of colon integrity disrupted by DSS. The improvement correlated with significant decrease of colonic and systemic levels of pro-inflammatory cytokines compared to the DSS group. OEA antiinflammatory effects were mediated by the selective targeting of the TLR4 axis causing a downstream inhibition of nuclear factor kappa B (NF-κB)- MyD88-dependent and NLRP3 inflammation pathways. OEA treatment also inhibited DSS-induced increase of inflammatory cytokines levels in the mesenteric lymph nodes.

Conclusions and implications: These results underscore the validity of OEA as a potent protective and anti-inflammatory agent in ulcerative colitis that may be exploited to broaden the pharmacological strategies against inflammatory bowel disease.

1. Introduction

Lipid mediators such as palmitoylethanomide (PEA) and oleoylethanolamide (OEA) have emerged as prominent intrinsic regulators of pain and inflammation [1,2]. Host-defence cells can generate these bioactive lipids on demand and express their receptors. OEA exerts anti-inflammatory effects on LPS-stimulated, immortalized monocytes (THP-1) by activating the proliferator-activated receptor-alpha (PPAR-α) and modulates maturation of dendritic cells via activation of the transient receptor potential vanilloid 1 (TRPV1; [3]). In the intestinal tract where it is synthesised, OEA plays a key role in gut physiology and dysregulation of its metabolism is associated to intestinal diseases. Nutrients containing oleic acid enhance the biosynthetic pathways of OEA resulting in an overall increase in the lipid levels [4]. In an inflammatory condition such as colitis the intestinal proteins that transport long-chain fatty acids like oleic acid are strongly reduced. An immunohistochemical study showed that the expression of peroxisome proliferator receptor (PPAR)-α (for which OEA has high affinity) is decreased in biopsies of colonic epithelium from subjects with active colitis, relative to healthy subjects [5]. This change is accompanied by a

Abbreviations: DSS, dextran sodium sulphate; FAE, fatty acid ethanolamide; IBD, inflammatory bowel disease; NF-κB, nuclear factor-kappa-B Ocln, occludin; OEA, oleoylethanolamide; PHA, phytohaemagglutinin; PPAR-α, proliferator-activated receptor-alpha; TRPV1, transient receptor potential vanilloid 1

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https://doi.org/10.1016/j.biopha.2020.110368
Received 28 February 2020; Received in revised form 31 May 2020; Accepted 2 June 2020

marked local decrease of the fatty amide ethanolamides (FAE) synthesizing enzyme N-acyl phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) expression, and an increase of the degrading enzyme N-acylethanolamine-hydrolyzing acid amidase (NAAA) expression [5]. We recently showed that OEA reduces intestinal cytokine expression by immune cells isolated from Peyer's patches, one of the immune sensors of the intestine [6].

All these observations set up the rational for investigating the protective effect of OEA in a murine model of intestinal inflammatory disease (IBD). A large and growing number of people worldwide is diagnosed with IBD, a debilitating pathology characterized by both acute and chronic inflammation of the gastro-intestinal tract, with poorly effective treatments. In our study, we used the dextran sodium sulphate (DSS)-induced colitis. This animal model exhibits many characteristic features of IBD in humans, including severe inflammation associated with diarrhoea, weight loss, recruitment of immune cells and their subsequent activation, altered macrophage function. Therefore, DSS-induced colitis has been considered an indispensable tool to decipher the underlying mechanisms of IBD pathogenesis, as well as to evaluate potential therapeutics. Little is known though of the efficacy of OEA to counteract inflammatory colitis. The only indication of a possible role of OEA in intestinal homeostasis are the results of a clinical trial describing a significant increase of mast cells count and reduction of OEA levels in colonic biopsies of patients affected by IBD, in comparison with healthy subjects [7];(ClinicalTrial.gov NCT01370720); conversely another report describes a modest increase of OEA in the plasma of IBD patients [8].

In our study, we evaluated the effects of OEA administration in C57BL/6J mice exposed to DSS for 5 days. It has been reported that this mouse strain develops acute colitis that progresses to severe chronic inflammation even with such a short term treatment [9]. The specific purpose of our study was to investigate the systemic and intestinal protective, immunomodulatory and anti-inflammatory effect of OEA, by testing its ability to prevent gut barrier damage, to reduce inflammatory responses through TLR4/NLRP3 pathways, and inflammatory mesenteric lymph nodes microenvironment.

2. Materials and methods

2.1. Animals

All the experiments were approved by the Animal Care Committee of the Dipartimento di Neuroscienze, Psicologia, Area del Farmaco e Salute del Bambino, Sezione di Farmacologia e Tossicologia, Universitá di Firenze (I). Ethical policy of the Universitá di Firenze complies with the Guide for the Care and Use of Laboratory Animals of the Council Directive of the European Community (2010/63/EU) and the Italian Decreto Legislativo 26 (13/03/2014). C57Bl/6, 8–10 weeks old male mice (25 − 30 g) were used, bred and housed in macrolon cages in temperature-controlled rooms (20–24 °C) in a dedicated room at Centre for Laboratory Animals Università di Firenze (I). They were allowed free access to food and water and kept on a 7:00 − 19:00 light/dark cycle. Every effort was made to minimize animal suffering and to reduce the number of animals used. Animals were handled for at least 4 days before experiments begun, to let them get used to human contact and then they were randomly assigned to the experimental groups.

2.2. Induction of colitis and treatment

Experimental colitis was induced by 2.5 % wt:vol dextran sodium sulphate (DSS, 36–50 kDa, MP Biomedical) in drinking water available *ad libitum* from day 3 (D3) after the beginning of experimental observations (D0) until D7, followed by DSS-free water from D8 to D11 (end of experimental protocol). Mice were randomly divided into the following groups: 1) control mice; 2) mice receiving DSS and vehicle; 3) mice receiving DSS and OEA. OEA (Tocris Bioscience, UK) was dissolved in saline/polyethylene glycol/Tween80 (90/5/5, v/v) and administered i.p. at a dose of 10 mg/kg from D0 to D11 as a preventive therapy. The dose of OEA was within the range of i.p. doses previously reported in the literature [6,10–13]. Animals were sacrificed by cervical dislocation and sample collected immediately after.

2.3. Evaluation of experimental colitis

Mice were weighted and food consumption measured daily from D0 to D11. Presence of blood and stool consistency were determined daily as previously described [14]. Scores were determined as follows: a) weight change (0: weight loss < 1 % compared with starting weight; 1: weight loss between 1 and 5 %; 2: weight loss between 6 and 15 %; 4: weight loss > 15 %); b) blood in stool: 0, no visible blood; 2, blood visible in stools; 4, frank bleeding from the anus and/or fresh or dried blood around the anus; c) stool consistency: 0, normal; 2, pasty and semi-formed stools that do not adhere to the anus; 4, liquid stools (may adhere to the anus). The disease activity index (DAI) was determined by adding the scores obtained in the 3 above-mentioned parameters and by dividing the sum by 3. At day 12 mice were sacrificed, tissues were collected and colon length measured.

2.4. Serum parameters

Blood was collected by cardiac puncture. Sera were obtained by centrifugation at 1500 × g at 4 °C for 15 min and stored at −70 °C until use. Serum interleukin (IL)-1, IL-6, IL-10, (Thermo Scientific, Rockford, IL, USA), tumour necrosis factor-α (TNF-α) (Biovendor R&D, Brno, Czech Republic) were determined using commercially available ELISA kits. Lipopolysaccharide (LPS) was measured using the Limulus amebocyte lysate (LAL QCL-1000; Lonza Group Ltd., Basel, Switzerland) technique.

2.5. Real-Time semi-quantitative PCR

Total RNA, isolated from colon, was extracted following protocols as previously reported (Lama et al. 2019). Each cDNA sample (500 ng) was mixed with 2X QuantiTech SYBRGreen PCR Master Mix and primers, *Tnfa, Nlrp3*, *Il1b*, *Il6*, *Myd88*, *Ocln*, *Tijp1*, *Muc2, Pparα* (Qiagen, Hilden, Germany). The relative amount of each studied mRNA was normalized to *Actb* as a housekeeping gene, and the data were analysed according to the $2^{-\Delta\Delta Ct}$ method. Real-Time PCR was performed by CFX96 instrument (Bio-Rad, Segrate, Italy).

2.6. Western blotting

After homogenization, the colon was lysed and total protein lysates underwent SDS-PAGE as described [14]. Filters were probed with polyclonal antibody against anti-NFκB (Cell Signaling Technology, Danvers, MA, USA), anti-TLR4 and anti-IκBα (Santa Cruz Biotechnology, Dallas, TE, USA). To evaluate NF-κB activation, NF-κB p65 and IκBα were measured in nuclear and cytosolic extracts, respectively, as previously described [15]. Western blot analysis for β-Actin (Sigma-Aldrich, Milan, Italy) was performed to ensure equal sample loading. Bands were detected by ChemiDoc imaging instrument (Bio-Rad, Segrate, Italy).

2.7. Mesenteric lymph nodes isolation and cytokines determination

Mice were sacrificed by cervical dislocation without anaesthesia. Mesenteric lymph nodes were isolated from mice intestine and transferred in a 15 ml tube with PBS + 1% Pen/Strep (Lonza, Germany). Mesenteric lymph nodes were next mechanically dissociated on a 70 μm cell strainer (Falcon, USA) placed over 50 ml tube. During dissociation, filters were washed with RPMI + 1 % Pen/Strep. Cells flowed through the filter and were suspended in 15 ml of RPMI, 10 % FBS, 1% Pen/

Strep, 0,01 % β-mercaptoethanol, 1 % L-glutamine, 1 % Na-Pyr and 1 % Hepes (complete medium). Cells were next centrifuged for 10 min at 1300 rpm and at room temperature. The supernatants were discarded and pellets were re-suspended in complete medium. Mesenteric lymph nodes cells were counted with Thomas chamber using Trypan blue dye to exclude dead cells. Mesenteric lymph nodes experiments were done in triplicate to ensure reliability of single values. Cells were then plated at the concentration of 1×10^5 cells/100 µl (1×10^6 cells/mL) in 96-well plate in the presence of PHA (5 ug/mL), (Sigma-Aldrich, USA) or vehicle for 48 h, in a 37 °C and 5% CO_2 incubator. After incubation cell supernatants were collected to determine the following soluble factors: IFNγ, IL-4, IL-6, I-L10, IL-17, TNF-α, CXCL2, by Luminex Technologies (Procartaplex, Life Technologies, USA).

2.8. Data and statistical analysis

Sample size was determined by power analysis based on effect sizes previously reported for similar compounds and protocols [14] by using Gpower software. Measurements and data analysis were performed by operators not aware of the experimental groups the samples belonged to. In some circumstances, normalization was employed to control for unwanted sources of variability. Experiments were done in triplicate to ensure reliability of single values. Statistical analysis was performed by one-ANOVA followed by Bonferroni's post-hoc test for multiple comparisons. Differences among groups were considered significant at values of $p < 0.05$. Analyses were performed using GraphPad Prism 7 (GraphPad Software, San Diego, CA, USA). The data and statistical analysis comply with the recommendations on experimental design and analysis in pharmacology [16].

3. Results

3.1. OEA treatment reduced DSS-induced colon tissue damage and disease activity index

The treatment with OEA significantly attenuated the development of colitis evaluated as DAI during the 11 days of the protocol when compared to mice receiving DSS and vehicle (Fig. 1A). Body weight and food consumption were also monitored throughout the experimental period (Fig. 1B, C). Despite of food consumption being not significantly different among experimental groups, DSS-treated mice lost weight with respect to controls and OEA did not prevent this effect. This is not unexpected, given the hypophagic properties of OEA [13]. Diarrhoea was present in DSS + Veh treated mice and OEA ameliorated significantly this parameter, as values were not significantly different from controls up to day 9 (Fig. 1D). DSS-induced bleeding was also significantly reduced by OEA treatment (Fig. 1E). The beneficial effects of OEA were also evident in the excised colon samples, as it partially prevented DSS-induced colon shortening (Fig. 1F).

3.2. OEA counteracted systemic and local inflammation induced by DSS

The DSS challenge triggered a strong systemic inflammation induced by the pathogen-associated molecular pattern (PAMP), LPS (Fig. 2A). Plasma LPS levels were evaluated as an indirect measurement of gut permeability to endotoxin. As shown in Fig. 2A, OEA pre-treatment partially prevented DSS effect. The detrimental effect of DSS was also displayed by increased serum levels of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6 (Fig. 2B–D) and the reduced levels of anti-inflammatory IL-10 (Fig. 2E) compared to controls. OEA treatment partially (Fig. 2C–E) or completely (Fig. 2D) prevented the onset of the systemic inflammation. The beneficial effects induced by OEA administration on systemic inflammation mirrored a strong improvement of colon tissue damage. As shown in Fig. 3, DSS treatment increased significantly mRNA expression of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6 in the colon mucosa. OEA abolished DSS-induced intestinal inflammatory profile, as there were no significant differences between DSS-OEA treated mice and controls.

3.3. OEA limited DSS-induced inflammatory immune cell recruitment

We also determined the effect of OEA on the TLR4/NF-κB complex in the colon mucosa, as this classical pathway controls the induction of pro-inflammatory cytokines and chemokines. DSS-challenged mice showed a significantly increased expression of TLR4 protein, the main LPS receptor in the gut (Fig. 4A). Furthermore, DSS induced the nuclear translocation of the p65 subunit of NF-κB (Fig. 4B) and decreased the cytosolic expression of IκBα, the inhibitory protein of NF-κB (Fig. 4C). These effects were completely prevented by OEA treatment, as there were no significant differences between DSS-OEA treated mice and

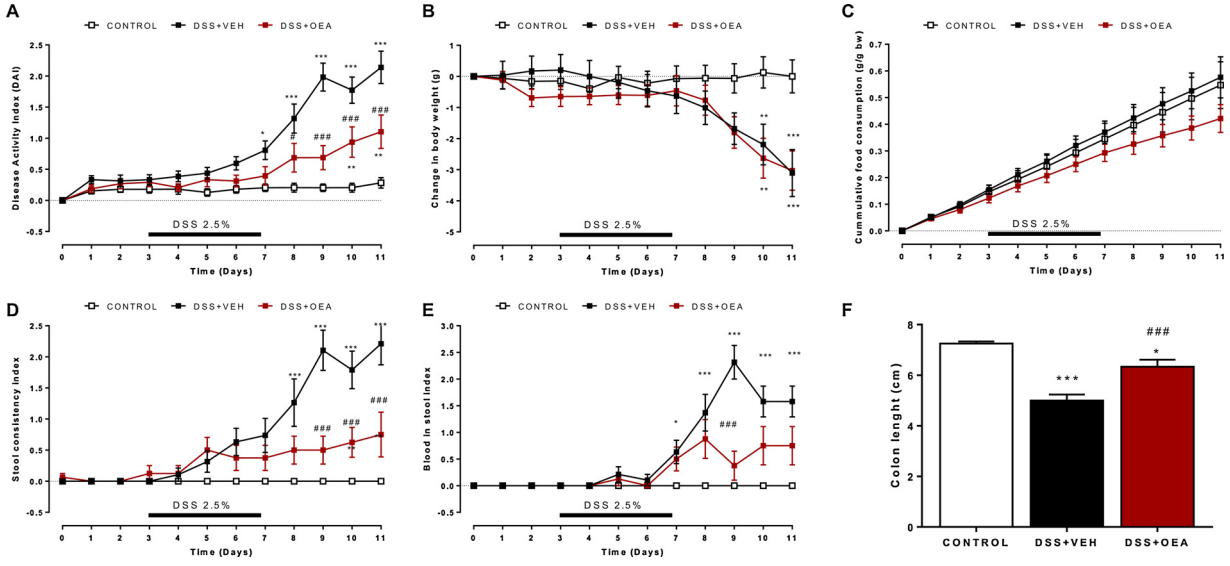

Fig. 1. Effects of OEA on DSS-induced tissue damage and body weight change. (A) Disease activity index (DAI) during the 11 days of experimental protocol. (B) Time course of changes in body weight and (C) cumulative food consumption. (D) Time course of stool consistency and (E) Blood in stool indexes. (F) Colon length expressed in cm; samples were excised at day 11. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, vs control; ### $p < 0.001$ vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test. N = 13-19 in panels A, B, C, D, E; N = 10-15 in panel F).

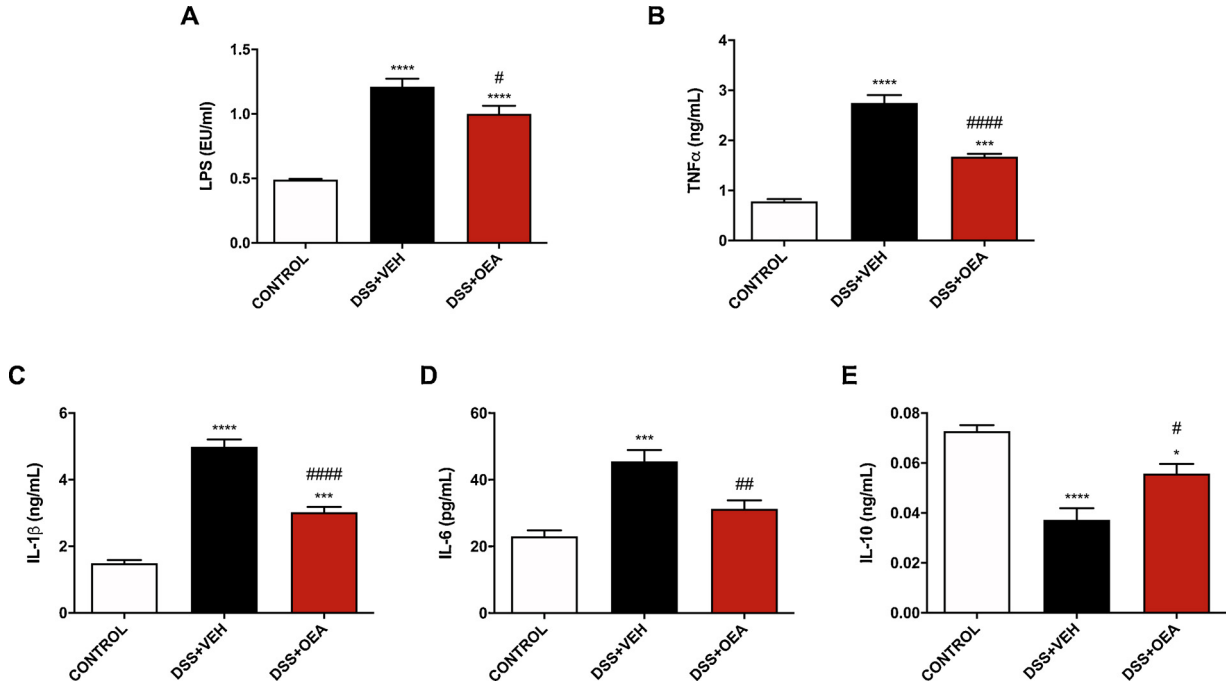

Fig. 2. Effects of OEA on DSS-induced systemic inflammation. DSS significantly increased serum levels of LPS (A), TNF-α(B), IL-1β(C), IL-6(D), and decreased the levels of anti-inflammatory IL-10 (E). OEA treatment limited all these effects. Results are shown as mean ± SEM (n = 5). ***$p < 0.001$, ****$p < 0.0001$, vs controls; #$p < 0.05$, ##$p < 0.01$, #### $p < 0.0001$ vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test.

controls. As shown in Fig. 4D, the DSS-induced pro-inflammatory profile involved the Myeloid differentiation primary response (Myd)88b-dependent signalling pathway, that mediates the early-phase activation of NF-κB. Furthermore, DSS altered gut immune response inducing mRNA expression of NACHT, LRR and PYD domains-containing protein 3 (NLRP3; Fig. 4E). The beneficial effects of OEA were reflected on all gut immune response to PAMPs tested, as well as on the transcription of the inflammasome, as there were no significant differences between DSS-OEA treated mice and controls.

3.4. OEA modulated immune cytokines and chemokines in mesenteric lymph nodes

In order to study the immune adaptive response, we evaluated cytokine production by T lymphocytes isolated from mesenteric lymph nodes, after stimulation with the mitogen phytohaemagglutinin (PHA). As shown in Fig. 5, DSS significantly increased the production of the pro-inflammatory cytokines IFN-γ, IL-6, IL-17, TNF-α, IL-4 and of the regulatory cytokine IL-10 compared to control mice. Neutrophil recall chemokine CXCL2 was also significantly increased by DSS treatment. The administration of OEA prevented the DSS effect on IFN-γ, IL-6 IL-17, CXCL2 as results were not different from controls. OEA decreased TNF-α, IL-4, IL-10 significantly with respect to both DSS + VEH and Controls.

3.5. Effects of OEA on intestinal barrier integrity

The local and systemic pro-inflammatory profile induced by DSS entails a disruption of the intestinal barrier. Tight junction status is a good marker for barrier integrity loss. Therefore, we investigated the expression of two main tight junction proteins in the distal colon, occludin (Ocln) and tight junction protein-1 (Tijp1) which are involved in preserving the gut integrity [17]. The administration of DSS induced a significant reduction of these tight junction proteins, and their levels were restored by OEA treatment (Fig. 6A, B). Moreover, OEA prevented DSS-induced reduction of mucin 2 (Muc2) which is involved in mucus production and triggered the transcription of Trefoil factor 3 (Tff3) an essential protein in gut protection (Fig. 6C, D). In addition, we measured PPAR-alpha expression in the various experimental conditions to investigate if this receptor confers protection against intestinal injury [18]. As shown in Fig. 6E, DSS treatment significantly decreased PPAR-α mRNA expression, and OEA completely prevented this effect.

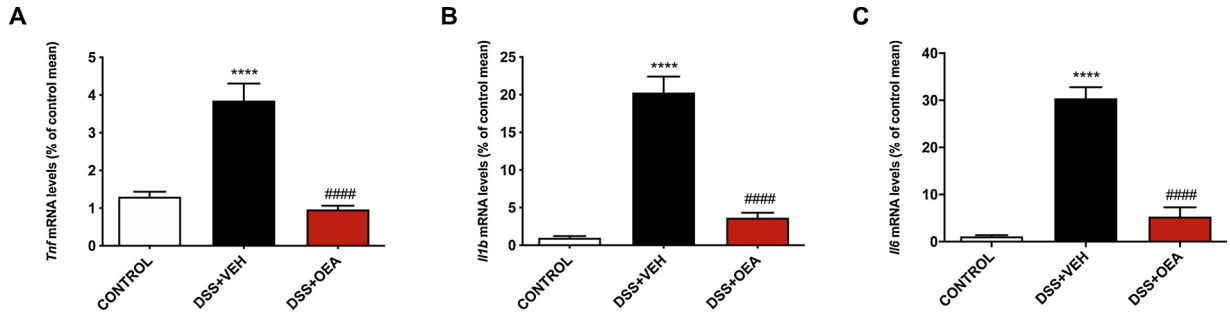

Fig. 3. OEA reduced DSS-induced colon inflammation. DSS significantly increased colon mucosa transcription levels of TNF-α (A), IL-1β (B) and IL-6 (C). OEA treatment prevented these effects. Results are shown as mean ± SEM (n = 5). ****$p < 0.0001$, vs controls; ####$p < 0.0001$ vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test.

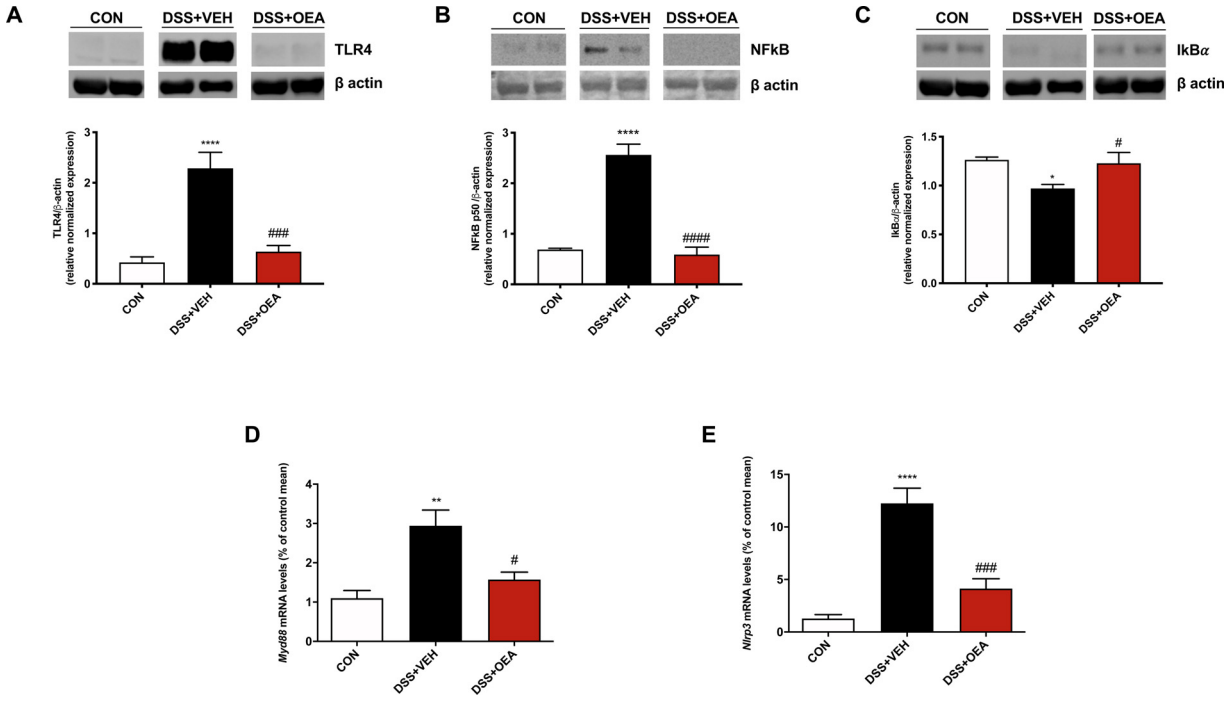

Fig. 4. Effects of OEA on gut immune response altered by DSS. DSS significantly increased TLR4 (A), increased the nuclear level of NF-κB p50 subnunit (B), whereas it decreased cytosolic expression of IκBα (C). Cropped Western blots are shown for each parameter. Furthermore, DSS augmented the transcription of Myd88 (D) and the inflammasome NLRP3 (E). OEA limited the protein levels and mRNA expression of all parameters. Results are shown as mean ± SEM (for Western blot, n = 4 and for Real Time PCR, n = 6). $*p < 0.05$, $**p < 0.01$, $****p < 0.0001$, vs control; $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#\# p < 0.0001$ vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test.

4. Discussion

Current treatments of intestinal inflammatory diseases have short-term efficacy and induce severe side effects; thus, the search for new therapeutic targets is relentless and represents a challenge for physicians and pharmacologists. Crohn's disease and ulcerative colitis are the major forms of IBD characterized by bowel inflammation and abdominal pain. Over the last decade the development of animal models of intestinal inflammation, such as DSS-induced colitis, has facilitated significant advances in our understanding of human pathologies. In our study, we found that OEA has a potent protective effect against all macroscopic and pathological changes of DSS colitis. The main findings of our study are the following: DSS-induced ulcerative colitis produces local gut inflammation and alteration of colonic tight junctions complex that presumably allows the contact of bacteria with mesenteric lymph nodes, the passage of LPS to the bloodstream and systemic

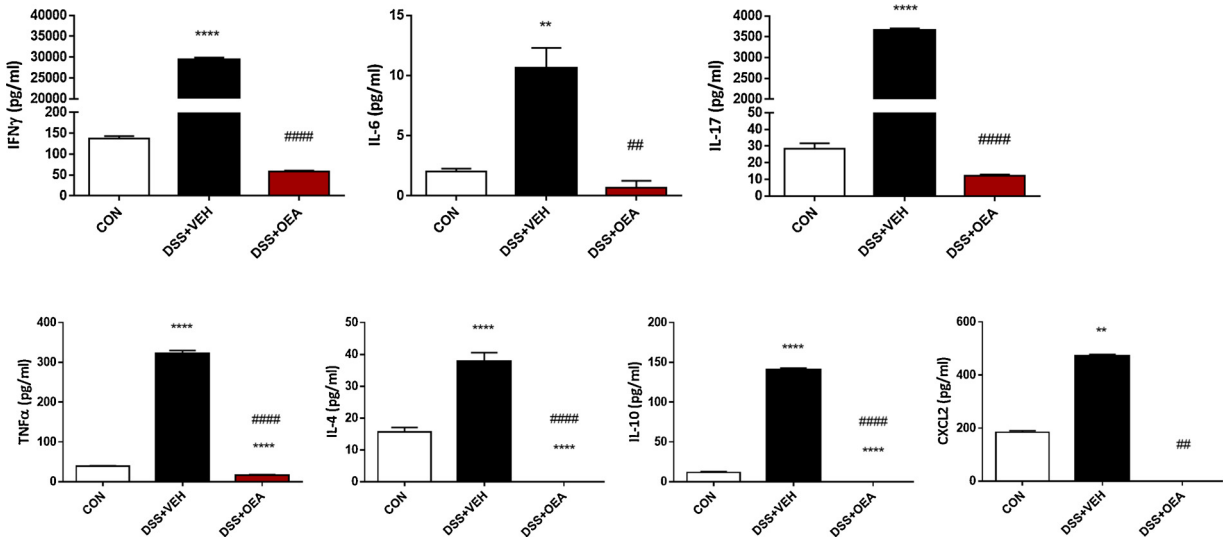

Fig. 5. OEA prevented DSS-induced changes of cytokines and chemokines secretion in intestinal mesenchymal lymph nodes. Protein production was determined by Luminex technology on cells supernatants. Cells were activated by PHA (5 μg/mL). DSS treatment increased the release of pro-inflammatory cytokines IFN-γ, IL-6, IL-17, TNF-α, IL-4 and the chemokine CXCL2, and the regulatory cytokine IL-10. OEA completely abolished the mesenteric lymph nodes inflammatory profile. Each histogram represents mean value ± SEM of protein concentration (pg/mL) of 3 samples for each group from 6 independent experiments. $**p < 0.01$; $***p < 0.001$; $****p < 0.0001$, vs control; $\#\#p < 0.01$; $\#\#\#\# p < 0.0001$ vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test.

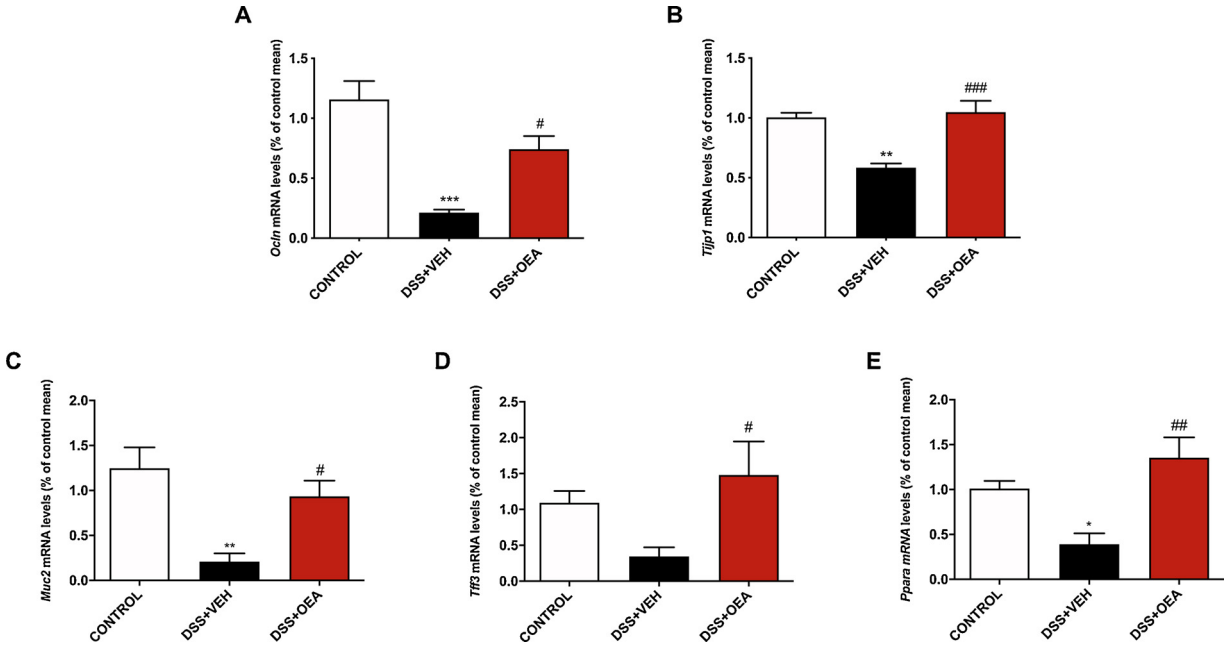

Fig. 6. OEA improved gut integrity of DSS mice. DSS decreased mRNA expression of two main tight junctions, occluding (A) and zonulin-1 (TIJP1; B), and increased mRNA levels of mucin (Muc (C) and Tff3 (D). DSS also decreased the expression of PPAR-α (E). OEA prevented all DSS induced changes. Results are shown as mean ± SEM (n = 5). $^{**}p < 0.01$, $^{***}p < 0.001$, vs CON; $\#p < 0.05$, $\#\#p < 0.01$, vs DSS. Comparison among groups were made by one-way analysis of variance followed by Bonferroni's post hoc test.

inflammation; OEA pre-treatment significantly reduced system inflammation; prevented colonic inflammation, the production of pro-inflammatory cytokine in mesenteric lymph nodes, and preserved the alterations of gut integrity induced by DSS.

The mucosal barrier disruption may indeed be one of the first events to trigger subsequent cascade of processes that evolve in DSS-induced colonic, lymphatic and systemic inflammatory response.

Our results strongly suggest that OEA may prevent the disruption of the mucosal barrier, as it normalized mRNA expression of the tight junction proteins occludin and zonuline-1, and promoted the expression of protective factors such as mucin and Tff3. These data are in agreement with the observation that the inflamed tissues of patients with IBD [19] and of DSS animals [14] showed impairment of tight junction proteins and local inflammation, also determined by the increase of IFN-γ. IFN-γ plays a pivotal role on barrier permeability impairment in IBD, triggering the internalization of TJ proteins [20]. The strong reduction of IFN-γ in intestinal mesenchymal lymph nodes supports the positive effect of OEA on the recovery of intestinal integrity.

In pathological conditions, tight junctions are disrupted exposing TLR4 localized mainly in the basolateral surfaces of the enterocytes of the small intestine to pathogens, triggering the inflammatory response. In turn, inflammation may exacerbate barrier dysfunction, as TLR4 activation and the pro-inflammatory cytokine TNF-α modulate colonic tight junction permeability [21]. In this regard, TLR4 expression is upregulated in patients with IBD [22], in particular Crohn's disease and ulcerative colitis [23,24]. We found that OEA completely prevented DSS-induced activation of the TLR4 cascade in the colon hence reduced colon inflammation, by restoring the levels of MyD88, the cytosolic adaptor protein that transfers signals between TLR4 and NFK-bB, NF-kB and IKK.

In our experimental condition, OEA prevented the activation of the inflammasome NLRP3, maintaining the levels of inflammatory cytokines within the controls' range. In this regard, it was reported that in NLRP3 knockout mice oral administration of DSS produced a less severe colitis than in wild-type mice and lower levels of pro-inflammatory cytokines in the colon tissue [25].

In our model, ulcerative colitis is associated with increased serum levels of TNF-α, IL-1β, IL-6 that are considered as peripheral markers of inflammation. The anti-inflammatory profile afforded by OEA pre-treatment was also extended to systemic actions in the bloodstream, as we observed reduced levels of serum LPS and pro-inflammatory cytokines, mirroring colonic anti-inflammatory effects. The study of inflammation and functional profile of mesenteric lymph node T cells may provide information on ulcerative colitis pathogenesis. Surveillance against pathogens is performed by both innate and the adaptive immune systems. Mucosal Th cells regulate gut homeostasis and along with dendritic cells and macrophages have a prominent role both in the acute and chronic phases of colitis [26]. In DSS mice, OEA blunted the mesenteric lymph nodes immune adaptive response reducing cytokine and chemokine levels. Although we did not check mesenteric lymph node T cells phenotype, the absence of IL-10 production in DSS + OEA treated animals suggests that $CD4^+CD25^+FoxP3^+$ T cells (Treg) are not increased and that the anti-inflammatory effect might be mainly mediated by antigen presenting cells as dendritic cells. These results are in agreement with our previous observation that OEA polarises gut-specific immune responses towards an anti-inflammatory profile in healthy mice [6].

Previous data reported that OEA treatment induced a similar anti-inflammatory and protective effect of the gut barrier in alcohol binging rats [27], although in these animals OEA administration did not reduce plasma LPS nor prevented bacterial translocation to the mesenteric lymph nodes.

In our model, OEA's mechanisms of action are presumably multifarious. OEA binds with high affinity to PPAR-α, which is abundantly expressed in enterocytes. In our study, OEA completely restored mRNA expression of PPAR-α in the colon mucosa, which is presumably responsible for the overall protective effect of OEA on DSS-induced damages. These results are in accord with previous observations demonstrating that activation of PPAR-α protects the intestinal mucosa from injuries of various nature [18,28].

PPARs including PPAR-α, are involved in the response to treatment in patients with ulcerative colitis and PPAR-α low gene expression in human mucosa confers a higher risk of ulcerative colitis [29]. It is well known that PPAR-α plays a role in controlling mucosal tissue

homeostasis and PPAR-α agonists improve DNBS- and DSS-induced colitis [30–32]. However, we cannot exclude other mechanisms of action. For instance, some of the homeostatic effects of OEA are mediated by the activation of TRPV1. In Caco-2 cell monolayer used to assess permeability, OEA increased the transepithelial resistance when applied to the apical membrane and capsazepine, a TRPV1 antagonist, blocked this effect [33]. Moreover, OEA downregulates TLR4/NF-κB pathway leading to dendritic cells maturation through the activation of TRPV1 [34]. Activation of these receptors may contribute to the anti-inflammatory properties of OEA in DSS induced colitis. OEA is also a medium-potency agonist for GPR119 [35], but presumably this receptor is not directly involved in intestinal barrier maintenance nor inflammatory responses (reviewed in [36]). However, the activation of GPR119 mediates the release of GLP-1, a peptide that increases in DSS colitis [37] and is endowed with anti-inflammatory properties [38].

Recently, analysis of faecal biodiversity showed that OEA treatment modifies the intestinal microbial composition of healthy mice [18]. As changes in the microbiota are associated with intestinal [39], immunological and metabolic diseases [40], OEA may as well afford a protective action in the intestine by mitigating the dysbiosis that follows not only DSS treatment in animals, but also IBD in humans [41].

Beyond its preventive anti-inflammatory effects showed by our data, it is well-known that food intake promotes OEA formation in the small intestine, in particular nutrients containing oleic acid enhance the biosynthetic pathways of OEA resulting in an overall increase in the lipid levels [42]. A recent study reported that extra-virgin olive oil, that contains high levels of oleic acid, the precursor of OEA, and phenols, displays a protective effect in liver dysfunction and gut inflammation in DSS-induced colitis in mice [43]. Therefore, stimulating endogenous OEA synthesis with dietary intervention or supplementing OEA itself may contribute to the successful treatment of inflammatory-based intestinal diseases.

Author contributions

AL, GP, RA, CP, BR, MPM, performed experiments and analysed data. AL, GP, CB, GMR, RM and MBP contributed to study design and data analysis. MBP and RM wrote the manuscript.

Declaration of Competing Interest

None.

Acknowledgments

This research was supported by ERA-HDHL Project AMBROSIAC and Fondazione Ente Cassa di Risparmio Firenze to MBP.

References

[1] S. Pontis, A. Ribeiro, O. Sasso, D. Piomelli, Macrophage-derived lipid agonists of PPAR-α as intrinsic controllers of inflammation, Crit. Rev. Biochem. Mol. Biol. 51 (1) (2016) 7–14.
[2] R. Russo, C. Cristiano, C. Avagliano, C. De Caro, G. La Rana, G.M. Raso, R.B. Canani, R. Meli, A. Calignano, Gut-brain axis: role of lipids in the regulation of inflammation, pain and CNS diseases, Curr. Med. Chem. 25 (32) (2018) 3930–3952.
[3] L. Yang, H. Guo, Y. Li, X. Meng, L. Yan, Dan Zhang, S. Wu, H. Zhou, L. Peng, Q. Xie, X. Jin, Oleoylethanolamide exerts anti-inflammatory effects on LPS-induced THP-1 cells by enhancing PPARα signaling and inhibiting the NF-κB and ERK1/2/AP-1/STAT3 pathways, Sci. Rep. 6 (2016) 34611.
[4] J. Fu, G. Astarita, S. Gaetani, J. Kim, B.F. Cravatt, K. Mackie, D. Piomelli, Food intake regulates oleoylethanolamide formation and degradation in the proximal small intestine, J. Biol. Chem. 282 (2) (2007) 1518–1528.
[5] J. Suárez, Y. Romero-Zerbo, L. Márquez, P. Rivera, M. Iglesias, F.J. Bermúdez-Silva, M. Andreu, F. Rodríguez de Fonseca, Ulcerative colitis impairs the acylethanolamide-based anti-inflammatory system reversal by 5-aminosalicylic acid and glucocorticoids, PLoS One 7 (5) (2012) e37729.
[6] M. Di Paola, E. Bonechi, G. Provensi, A. Costa, G. Clarke, C. Ballerini, C. De Filippo, M.B. Passani, Oleoylethanolamide treatment affects gut microbiota composition and the expression of intestinal cytokines in Peyer's patches of mice, Sci. Rep. 8 (1) (2018) 14881.
[7] G. Barbara, C. Cremon, V. Annese, G. Basilisco, F. Bazzoli, M. Bellini, A. Benedetti, L. Benini, F. Bossa, P. Buldrini, M. Cicala, R. Cuomo, B. Germanà, P. Molteni, M. Neri, M. Rodi, A. Saggioro, M.L. Scribano, M. Vecchi, G. Zoli, R. Corinaldesi, V. Stanghellini, Randomised controlled trial of mesalazine in IBS, Gut 65 (1) (2016) 82–90.
[8] M. Grill, C. Högenauer, A. Blesl, J. Haybaeck, N. Golob-Schwarzl, N. Ferreirós, D. Thomas, R. Gurke, M. Trötzmüller, H.C. Köfeler, B. Gallé, R. Schicho, Members of the endocannabinoid system are distinctly regulated in inflammatory bowel disease and colorectal cancer, Sci. Rep. 9 (1) (2019) 2358.
[9] S. Melgar, A. Karlsson, E. Michaëlsson, Acute colitis induced by dextran sulfate sodium progresses to chronicity in C57BL/6 but not in BALB/c mice: correlation between symptoms and inflammation, Am. J. Physiol. Gastrointest Liver Physiol. 288 (6) (2005) G1328–38.
[10] G. Provensi, R. Fabbri, L. Munari, A. Costa, E. Baldi, C. Bucherelli, P. Blandina, M.B. Passani, Histaminergic neurotransmission as a gateway for the cognitive effect of oleoylethanolamide in contextual fear conditioning, Int. J. Neuropsychopharmacol. 20 (5) (2017) 392–399.
[11] A. Costa, C. Cristiano, T. Cassano, C.A. Gallelli, S. Gaetani, C. Ghelardini, P. Blandina, A. Calignano, M.B. Passani, G. Provensi, Histamine-deficient mice do not respond to the antidepressant-like effects of oleoylethanolamide, Neuropharmacology 135 (2018) 234–241.
[12] H. Umehara, R. Fabbri, G. Provensi, M.B. Passani, The hypophagic factor oleoylethanolamide differentially increases c-fos expression in appetite regulating centres in the brain of wild type and histamine deficient mice, Pharmacol. Res. 113 (Pt A) (2016) 100–107.
[13] G. Provensi, R. Coccurello, H. Umehara, L. Munari, G. Giacovazzo, N. Galeotti, D. Nosi, S. Gaetani, A. Romano, A. Moles, P. Blandina, M.B. Passani, Satiety factor oleoylethanolamide recruits the brain histaminergic system to inhibit food intake, Proc. Natl. Acad. Sci. U. S. A. 111 (31) (2014) 11527–11532.
[14] R. Simeoli, G. Mattace Raso, C. Pirozzi, A. Lama, A. Santoro, R. Russo, T. Montero-Melendez, R. Berni Canani, A. Calignano, M. Perretti, R. Meli, An orally administered butyrate-releasing derivative reduces neutrophil recruitment and inflammation in dextran sulphate sodium-induced murine colitis, Br. J. Pharmacol. 174 (11) (2017) 1484–1496.
[15] A. Lama, C. Annunziata, L. Coretti, C. Pirozzi, F. Di Guida, A. Nitrato Izzo, C. Cristiano, M.P. Mollica, L. Chiariotti, A. Pelagalli, F. Lembo, R. Meli, G. Mattace Raso, N-(1-carbamoyl-2-phenylethyl) butyramide reduces antibiotic-induced intestinal injury, innate immune activation and modulates microbiota composition, Sci. Rep. 9 (1) (2019) 4832.
[16] M.J. Curtis, S. Alexander, G. Cirino, J.R. Docherty, C.H. George, M.A. Giembycz, D. Hoyer, P.A. Insel, A.A. Izzo, Y. Ji, D.J. MacEwan, C.G. Sobey, S.C. Stanford, M.M. Teixeira, S. Wonnacott, A. Ahluwalia, Experimental design and analysis and their reporting II: updated and simplified guidance for authors and peer reviewers, Br. J. Pharmacol. 175 (7) (2018) 987–993.
[17] M.A. Odenwald, J.R. Turner, Intestinal permeability defects: is it time to treat? Clin. Gastroenterol. Hepatol. 11 (9) (2013) 1075–1083.
[18] R. Di Paola, D. Impellizzeri, A. Torre, E. Mazzon, A. Cappellani, C. Faggio, E. Esposito, F. Trischitta, S. Cuzzocrea, Effects of palmitoylethanolamide on intestinal injury and inflammation caused by ischemia-reperfusion in mice, J. Leukoc. Biol. 91 (6) (2012) 911–920.
[19] T. Suzuki, Regulation of intestinal epithelial permeability by tight junctions, Cell. Mol. Life Sci. 70 (4) (2013) 631–659.
[20] M. Scharl, G. Paul, K.E. Barrett, D.F. McCole, AMP-activated protein kinase mediates the interferon-gamma-induced decrease in intestinal epithelial barrier function, J. Biol. Chem. 284 (41) (2009) 27952–27963.
[21] R. Al-Sadi, S. Guo, D. Ye, M. Rawat, T.Y. Ma, TNF-α Modulation of intestinal tight junction permeability is mediated by NIK/IKK-α axis activation of the canonical NF-κB pathway, Am. J. Pathol. 186 (5) (2016) 1151–1165.
[22] A.S. Vamadevan, M. Fukata, E.T. Arnold, L.S. Thomas, D. Hsu, M.T. Abreu, Regulation of Toll-like receptor 4-associated MD-2 in intestinal epithelial cells: a comprehensive analysis, Innate Immune 16 (2) (2010) 93–103.
[23] T. Horng, G.M. Barton, R.A. Flavell, R. Medzhitov, The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors, Nature 420 (6913) (2002) 329–333.
[24] A. Levin, O. Shibolet, Toll-like receptors in inflammatory bowel disease-stepping into uncharted territory, World J. Gastroenterol. 14 (33) (2008) 5149–5153.
[25] C. Bauer, P. Duewell, C. Mayer, H.A. Lehr, K.A. Fitzgerald, M. Dauer, J. Tschopp, S. Endres, E. Latz, M. Schnurr, Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome, Gut 59 (9) (2010) 1192–1199.
[26] L.J. Hall, E. Faivre, A. Quinlan, F. Shanahan, K. Nally, S. Melgar, Induction and activation of adaptive immune populations during acute and chronic phases of a murine model of experimental colitis, Dig. Dis. Sci. 56 (1) (2011) 79–89.
[27] M. Antón, A. Rodríguez-González, A. Ballesta, N. González, A. Del Pozo, F.R. de Fonseca, M.L. Gómez-Lus, J.C. Leza, B. García-Bueno, J.R. Caso, L. Orio, Alcohol binge disrupts the rat intestinal barrier: the partial protective role of oleoylethanolamide, Br. J. Pharmacol. 175 (24) (2018) 4464–4479.
[28] E. Mazzon, C. Crisafulli, M. Galuppo, S. Cuzzocrea, Role of peroxisome proliferator-activated receptor-alpha in ileum tight junction alteration in mouse model of restraint stress, Am. J. Physiol. Gastrointest Liver Physiol. 297 (3) (2009) G488–505.
[29] J.K. Yamamoto-Furusho, M. Jacintez-Cazares, J. Furuzawa-Carballeda, G. Fonseca-Camarillo, Peroxisome proliferator-activated receptors family is involved in the response to treatment and mild clinical course in patients with ulcerative colitis, Dis. Markers 2014 (2014) 932530.
[30] S. Cuzzocrea, R. Di Paola, E. Mazzon, T. Genovese, C. Muià, T. Centorrino, A.P. Caputi, Role of endogenous and exogenous ligands for the peroxisome

proliferators activated receptors alpha (PPAR-alpha) in the development of inflammatory bowel disease in mice, Lab. Invest. 84 (12) (2004) 1643–1654.
[31] Y.T. Azuma, K. Nishiyama, Y. Matsuo, M. Kuwamura, A. Morioka, H. Nakajima, T. Takeuchi, PPARα contributes to colonic protection in mice with DSS-induced colitis, Int. Immunopharmacol. 10 (10) (2010) 1261–1267.
[32] F. Borrelli, B. Romano, S. Petrosino, E. Pagano, R. Capasso, D. Coppola, G. Battista, P. Orlando, V. Di Marzo, A.A. Izzo, Palmitoylethanolamide, a naturally occurring lipid, is an orally effective intestinal anti-inflammatory agent, Br. J. Pharmacol. 172 (1) (2015) 142–158.
[33] M.A. Karwad, T. Macpherson, B. Wang, E. Theophilidou, S. Sarmad, D.A. Barrett, M. Larvin, K.L. Wright, J.N. Lund, S.E. O'Sullivan, Oleoylethanolamine and palmitoylethanolamine modulate intestinal permeability in vitro via TRPV1 and PPARα, FASEB J. 31 (2) (2017) 469–481.
[34] E. Yao, G. Zhang, J. Huang, X. Yang, L. Peng, X. Huang, X. Luo, J. Ren, R. Huang, L. Yang, Y. Zhou, R. Zhuo, Y. Zhao, X. Jin, Immunomodulatory effect of oleoylethanolamide in dendritic cells via TRPV1/AMPK activation, J. Cell. Physiol. 234 (10) (2019) 18392–18407.
[35] H.A. Overton, A.J. Babbs, S.M. Doel, M.C. Fyfe, L.S. Gardner, G. Griffin, H.C. Jackson, M.J. Procter, C.M. Rasamison, M. Tang-Christensen, P.S. Widdowson, G.M. Williams, C. Reynet, Deorphanization of a G protein-coupled receptor for oleoylethanolamide and its use in the discovery of small-molecule hypophagic agents, Cell Metab. 3 (3) (2006) 167–175.
[36] J.W. Yang, H.S. Kim, Y.W. Choi, Y.M. Kim, K.W. Kang, Therapeutic application of GPR119 ligands in metabolic disorders, Diabetes Obes. Metab. 20 (2) (2018) 257–269.
[37] H. Lan, H.V. Lin, C.F. Wang, M.J. Wright, S. Xu, L. Kang, K. Juhl, J.A. Hedrick, T.J. Kowalski, Agonists at GPR119 mediate secretion of GLP-1 from mouse enteroendocrine cells through glucose-independent pathways, Br. J. Pharmacol. 165 (8) (2012) 2799–2807.
[38] D.B.R. Insuela, V.F. Carvalho, Glucagon and glucagon-like peptide-1 as novel anti-inflammatory and immunomodulatory compounds, Eur. J. Pharmacol. 812 (2017) 64–72.
[39] N. Kamada, S.U. Seo, G.Y. Chen, G. Núñez, Role of the gut microbiota in immunity and inflammatory disease, Nat. Rev. Immunol. 13 (5) (2013) 321–335.
[40] M. Bruchard, R. Boidot, F. Ghiringhelli, F. Végran, Transcriptome analysis of TH2 CD4(+) T cells differentiated from wild-type and NLRP3KO mice, Genom Data 5 (2015) 314–315.
[41] M.F. Neurath, Host-microbiota interactions in inflammatory bowel disease, Nat. Rev. Gastroenterol. Hepatol. (2019).
[42] D. Piomelli, A fatty gut feeling, Trends Endocrinol. Metab. 24 (7) (2013) 332–341.
[43] M. Cariello, A. Contursi, R.M. Gadaleta, E. Piccinin, S. De Santis, M. Piglionica, A.F. Spaziante, C. Sabbà, G. Villani, A. Moschetta, Extra-virgin olive oil from apulian cultivars and intestinal inflammation, Nutrients 12 (4) (2020).