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TREM-1 inhibition restores impaired autophagy activity and reduces colitis in mice

Short title: TREM-1 inhibition, autophagy and colitis

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Abstract

Background and aims: Triggering receptor expressed on myeloid cells-1 [TREM-1] is known to amplify inflammation in several diseases. Autophagy and endoplasmic reticulum [ER] stress, which activates the unfolded protein response [UPR] are closely linked and defects in these pathways contribute to the pathogenesis of inflammatory bowel disease [IBD]. Both autophagy and UPR are deeply involved in host-microbiota interactions for the clearance of intracellular pathogens thus contributing to dysbiosis. We investigated whether inhibition of TREM-1 would prevent aberrant inflammation by modulating autophagy, ER stress and preventing dysbiosis.

Methods: Experimental mouse model of colitis was caused by dextran sulfate sodium treatment. TREM-1 was inhibited, either pharmacologically by LR12 peptide or genetically with *Trem-1* knock-out [KO] mice. Colon tissues and fecal pellets of control and colitic mice were used. Levels of macroautophagy, chaperone-mediated autophagy [CMA], and UPR proteins were evaluated by western blotting. The composition of the intestinal microbiota was assessed by *MiSeq* sequencing in both LR12-treated and KO animals.

Results: We confirmed that inhibition of TREM-1 attenuates the severity of colitis at clinically, endoscopically and histologically levels. We observed an increase in macroautophagy [ATG1/ULK-1, ATG13, ATG5, ATG16L1, and MAP1LC3-I/II] and in CMA [HSPA8 and HSP90AA1] while there was a decrease in the UPR [PERK, IRE-1 α and ATF-6 α] protein expression levels in TREM-1 inhibited colitic mice. TREM-1 inhibition prevented dysbiosis.

Conclusions: TREM-1 may represent a novel drug target for the treatment of IBD by modulating autophagy activity and ER stress.

Keywords

TREM-1, autophagy, inflammation, endoplasmic reticulum stress, endoscopy, inflammatory bowel disease, innovative therapy, dysbiosis, LR12 peptide, peptide-based therapy, animal models of IBD.

1. Introduction

Inflammatory bowel disease [IBD] is a chronic, remitting-relapsing inflammatory disorder that encompasses ulcerative colitis [UC] and Crohn's disease [CD].^{1,2}

Autophagy [a typical example of a genetically mediated pathway] is abnormal in IBD.³⁻⁵ There are three major autophagy pathways [microautophagy, macroautophagy and chaperone-mediated autophagy [CMA]] that differ for their mode of cargo delivery to the lysosome.⁶ Autophagy and endoplasmic reticulum [ER] stress are closely linked in the pathogenesis of IBD.⁷⁻¹⁰ ER stress is caused by the accumulation of unfolded and/or misfolded proteins in the ER arising from either primary [genetic] or secondary [environmental] factors.^{11,12} The ER stress activates the unfolded protein response [UPR] to resolve the protein folding defect and to restore cellular homeostasis. The UPR is a major inducer of autophagy to compensate the ER stress in the intestinal epithelium.^{9,13} Impaired autophagy can also promote ER stress with its downstream consequences.¹⁴ Both autophagy and UPR play important roles in intestinal homeostasis, particularly for host-microbiota interactions at the epithelial surface of the intestine in the context of IBD.^{10,15-18} During infection, microbe-associated molecular patterns [MAMPs] are detected by a family of proteins called pattern recognition receptors [PRRs] located within host cells. PRRs involved in autophagy include the Nod-like receptors [NLRs] and Toll-like receptors [TLRs].¹⁹ NLRs and TLRs in macrophages as well as other innate immune cells are closely associated with autophagy, which is highly related to the mediation of innate immune response in the context of IBD.^{20,21} The bacterial microbiota in IBD has been extensively investigated, and several groups have observed bacterial dysbiosis characterized primarily by low levels of biodiversity.²²⁻²⁴ Defects in autophagy affects several aspects of the intestinal innate and acquired inflammatory responses [including abnormalities in antigen presentation, cytokine production and bacterial clearance] that may skew the commensal composition and lead to colitis-inducing dysbiosis.^{25,26}

The triggering receptor expressed on myeloid cells [TREM] family of cell-surface receptors has recently emerged as a potential modulator of the inflammatory response.²⁷ One of the components of this family, TREM-1 is constitutively expressed on most monocytes/macrophages and neutrophils, and is upregulated by a variety of stimuli, including TLR ligands [e.g. lipopolysaccharide [LPS]], lipoteichoic acid [LTA] and pro-inflammatory cytokines [e.g. tumor necrosis factor- α [TNF α]]. TREM-1's one or more natural specific ligands have not yet been identified.²⁸⁻³⁰ Several studies have showed that TREM-1 expression is upregulated during acute inflammation or in chronic intestinal inflammatory disorders.^{28,31-36} Although TREM-1 is generally not expressed by macrophages in the healthy intestine, it is significantly upregulated in mucosal lesions in mouse models of colitis and in patients with IBD.³⁴ Previous studies have shown that TLRs activation leads to upregulation of TREM-1 expression in a myeloid differentiation factor 88 [MyD88]-dependent manner.^{37,38}

TREM-1 has also a synergistic effect on the production of pro-inflammatory mediators induced by nucleotide-binding oligomerization domain-1 and 2 [NOD1 and NOD2] ligands.^{39,40} The potential impact of TREM-1 on autophagy activity is unknown.

The potential link between TREM-1 expression, autophagy activity, ER stress/UPR and intestinal dysbiosis in IBD has never been investigated. In the present study, we first confirmed that TREM-1 inhibition [using the non-IBD related LR12 peptide] attenuated the severity of experimental colitis. We found that inhibition of TREM-1 restores impaired autophagy, reduced ER stress/UPR and re-establishes homeostasis of the gut microbiota.

2. Materials and Methods

2.1. Animals

2.1.1. *In vivo* experiments were performed as recommended by the US National Committee on Ethics Reflection Experiment [described in the Guide for Care and Use of Laboratory Animals, NIH, MD, 1985]. The experiments were performed on 25 adult male C57BL/6 mice [Janvier Labs, Le Genest-Saint-Isle, France] and 10 adult male *Trem-1* knock-out [TREM-1 KO] C57BL/6 mice [INSERM U1116, Inotrem Laboratory, Nancy, France], all aged between 7 and 9 weeks. The animals were housed at 22–23°C, with a 12h/12h light/dark cycle, and *ad libitum* access to food and water.

2.1.2. In the TREM-1 inhibition experiments with administration of LR12 peptide, three groups of C57BL/6 mice were constituted: [i] healthy mice without LR12 peptide [DSS(-)/LR12(-); n=5]; [ii] mice treated with DSS without LR12 peptide [DSS(+)/LR12(-); n=5] and [iii] mice treated with DSS and LR12 peptide [DSS(+)/LR12(+); n=5].

2.1.3. In the experiments involving *Trem-1* KO mice, four groups of animals were constituted: [i] littermate C57BL/6 wild-type mice without DSS [DSS(-)/WT; n=5]; [ii] *Trem-1* KO mice without DSS [DSS(-)/TREM-1 KO; n=5]; [iii] littermate C57BL/6 wild-type mice treated with DSS [DSS(+)/WT; n=5] and [iv] *Trem-1* KO mice treated with DSS [DSS(+)/TREM-1 KO; n=5].

2.2. Induction of colitis, treatment with TREM-1 inhibitory peptide and assessment of disease activity index

2.2.1. Colitis was induced by administration of 3% dextran sulfate sodium [DSS, molecular weight 36,000–50,000, MP Biomedical, Strasbourg, France] dissolved in water for 5 days. DSS was replaced thereafter by normal drinking water for another 5 days.

2.2.2. TREM-1 inhibitory peptide or the vehicle alone, used as control, were administered intraperitoneally 2 days before colitis induction and then once daily until the last day of DSS administration [see experimental layout in Figure 1A], at a concentration of 10 mg/kg in 200 μ L of saline. This dose was chosen after having performed dose-response experiments [supplementary Figure S1]. The LR12 peptide corresponds to a 12-amino-acid [aa] residues-long sequence from TLT-1's extracellular domain [LQEEDTGEYGCV], and was chemically

synthesized [Pepsican Presto BV, Lelystad, The Netherlands] as a C-terminally amidated peptide.

2.2.3. Bodyweight, physical condition, stool consistency, water/food consumption and the presence of gross and occult blood in excreta and at the anus were determined daily. The DAI was also calculated daily by scoring bodyweight loss, stool consistency and blood in the stool on a 0 to 4 scale.⁴¹ The overall index corresponded to the weight loss, stool consistency and rectal bleeding scores divided by three, and thus ranged from 0 to 4.

2.3. Collection of colon tissue and fecal samples

Ten days after the initiation of colitis with DSS, the mice were sacrificed by decapitation. The colon was quickly removed, opened along its length and gently washed in PBS [2.7 mmol/L KCl, 140 mmol/L NaCl, 6.8 mmol/L Na₂HPO₄·2H₂O, 1.5 mmol/L KH₂PO₄, pH 7.4]. For histological assessment samples were fixed overnight at 4°C in 4% paraformaldehyde solution and embedded in paraffin. For protein extractions samples were frozen in liquid nitrogen [-196°C] and stored at -80°C. For the gut microbiota analysis, whole fecal pellets were collected daily in sterile tubes and immediately frozen at -80°C until analysis.

2.4. Histological assessment and scoring

Colitis was histologically assessed on 5 µm sections stained with hematoxylin-eosin-saffron [HES] stain. The histological colitis score was calculated blindly by an expert pathologist [Dr. H  l  ne Busby-Venner], as previously described.⁴²

2.5. Endoscopic assessment and scoring

Endoscopy was performed on the last day of the study, just before the mice were sacrificed [Figures 1A and 3A]. Prior to the endoscopic procedure, mice were anaesthetized by isoflurane inhalation. The distal colon [3 cm] and the rectum were examined using a rigid Storz Hopkins II miniendoscope [length: 30 cm; diameter: 2 mm; Storz, Tuttlingen, Germany] coupled to a basic Coloview system [with a xenon 175 light source and an Endovision SLB Telecam; Storz]. Air was insufflated via a 9-French gauge over-tube and a custom, low-pressure pump with manual flow regulation [Rena Air 200; Rena, Meythet, France]. All images were displayed on a computer monitor and recorded with video capture software [Studio Movie Board Plus from Pinnacle, Menlo Park, CA]. The endoscopy score was calculated from three subscores: the vascular pattern [scored from 1 to 3], bleeding [scored from 1 to 4] and erosions/ulcers [scored from 1 to 4] as previously described.⁴³

2.6. Western blot analysis

Total protein was extracted from the frozen colon samples by lysing homogenized tissue in a radioimmunoprecipitation assay [RIPA] buffer [0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS] and 1% NP-40] supplemented with protease inhibitors [Roche Diagnostics, Mannheim, Germany]. Protein was then quantified using the bicinchoninic acid assay method.

For each mouse, a total of 20 µg of protein was transferred to a 0.45 µm polyvinylidene fluoride [PVDF] or 0.45 µm nitrocellulose membrane following electrophoretic separation on

a denaturing acrylamide gel. The membrane was blocked with 5% w/v non-fat powdered milk or 5% w/v bovine serum albumin [BSA] diluted in Tris-buffered saline with 0.1% v/v Tween[®] 20 [TBST] for 1h at room temperature. The PVDF or nitrocellulose membranes were then incubated overnight at 4°C with various primary antibodies diluted in either 5% w/v non-fat powdered milk or 5% w/v BSA, TBST [supplementary Figure S2]. After washing in TBST, the appropriate HRP-conjugated secondary antibody was added and the membrane was incubated for 1h at room temperature [supplementary Figure S2]. After further washing in TBST, the proteins were detected using an ECL or ECL PLUS kit [Amersham, Velizy-Villacoublay, France]. Glyceraldehyde 3-phosphate dehydrogenase [GAPDH] was used as an internal reference control.

2.7. Enzyme-linked immunosorbent assay [ELISA] for analysis of soluble TREM-1 [sTREM-1]

At the time of animal sacrifice, whole blood from each mouse was collected into heparinized tubes. These tubes were centrifuged at 3,000 g for 10 min at 4°C to collect the supernatants, which were stored at -80°C until use. Plasma concentration of sTREM-1 was determined by a sandwich ELISA technique using the Quantikine kit assay [RnD Systems, Minneapolis, MN, USA] according to the manufacturers' instructions. Briefly, samples were incubated with a monoclonal antibody specific for TREM-1 pre-coated onto the wells of a microplate. Following a wash, to eliminate the unbound substances, an enzyme-linked polyclonal antibody specific for TREM-1 was added to the wells. After washing away the unbound conjugate, a substrate solution was added to the wells. Colour development was stopped and optical density of each well was determined within 30 min using a microplate reader [Sunrise, Tecan, Männedorf, Switzerland] set to 450 nm, with a wavelength correction set to 540 nm. All measurements were performed in duplicate and the sTREM-1 concentration was expressed in pg/ml.

2.8. Reverse transcription-quantitative polymerase chain reaction

Total RNA was purified from the frozen colon samples with the RNeasy Lipid Tissue kit following the recommendation of Qiagen [Courtaboeuf, France], which includes treatment with DNase. To check for possible DNA contamination of the RNA samples, reactions were also performed in the absence of Omniscript RT enzyme [Qiagen]. Reverse transcription was performed using PrimeScript[™] RT Master Mix [TAKARA Bio, USA] according to the manufacturer's recommendations with 200 ng of RNA in a 10 µL reaction volume. PCR was then carried out from 2 µL of cDNA with SYBR[®] Premix Ex Taq[™] [Tli RNaseH Plus] [TAKARA Bio, USA] according to the manufacturer's recommendations in a 20 µL reaction volume, with reverse and forward primers at a concentration of 0.2 µM. Specific amplifications were performed using the following primers: TREM-1, forward 5'-CTGTGCGTGTCTTTGTC-3' and reverse 5'-CTTCCCGTCTGGTAGTCT-3'. Quantification was performed with RNA polymerase II [Pol II] as an internal standard with the following primers: forward 5'-AGCAAGCGGTTCCAGAGAAG-3' and reverse 5'-TCCCGAACACTGACATATCTCA-3'. Temperature cycling for TREM-1 was 30 s at 95°C followed by 40 cycles consisting of 95°C for 5 s and 59°C for 30 s. Temperature cycling for

RNA polymerase II was 30 s at 95°C followed by 40 cycles consisting of 95°C for 5 s and 60°C for 30 s. Results were expressed as arbitrary units by calculating the ratio of crossing points of amplification curves of TREM-1 and internal standard by using the $\delta\delta C_t$ method.

2.9. Microbiota analysis

2.9.1 For the pharmacologically [with LR12 peptide treatment] inhibition of TREM-1, total DNA was extracted from three pooled fecal pellets from each group of mice [day 0 to day 10; n=33 samples], in accordance with a previously validated protocol.⁴⁴ For microbiota analysis by MiSeq sequencing, the V3-V4 region [519F-785R] of the 16S rRNA gene was amplified with the primer pair S-DBact-0341-b-S-17/S-D-Bact-0785-a-A-21.⁴⁵ The following quality filters were applied: minimum length=300 base pairs [bp], maximum length=600bp and minimum quality threshold=20. This filtering yielded an average [range] of 25600 reads/samples [14,553-35,490] for further analysis. High-quality reads were pooled, checked for chimeras [using uchime⁴⁶], and grouped into operational taxonomic units [OTUs] [based on a 97% similarity threshold] using USEARCH 8.0.⁴⁷ Singletons and OTUs representing less than 0.02% of the total number of reads were removed, and the phylogenetic affiliation of each OTU was assessed with Ribosomal Database Project's taxonomy⁴⁸ from the phylum level to the species level. The mean [range] number of detected OTUs per sample was 324 [170-404].

2.9.2. In the experiments involving *Trem-1* KO mice, similar methods were applied but total DNA was extracted from individual fecal pellets of each mouse from the four groups of animals at baseline [before DSS treatment] and at day 10 [after DSS treatment] [n=37 samples]. Following MiSeq sequencing of the V3-V4 region of the 16S rRNA gene, yielding 2,143,457 raw reads, quality filtering was applied [minimum length=200bp, maximum length=600bp and minimum quality threshold=20] and an average [range] of 11,560 reads/samples [7,560-18,495] was kept for further analysis. The mean [range] number of detected OTUs per sample was 599 [131-798].

DNA sequence reads from this study have been submitted to the NCBI under the Bioproject ID PRJNAXXXX and are available from the Sequence Read Archive [Biosamples accession numbers SAMXXXXXX-SAMXXXXXX].

2.10. Statistical analysis

A two-tailed Student *t* test was used to compare two groups and a one-way analysis of variance [ANOVA] was used to compare three or more groups. Bonferroni or Tamhane *post hoc* tests were applied, depending on the homogeneity of the variance. The threshold for statistical significance was set to $p < 0.05$. The statistical language R was used for data visualization and to perform abundance-based principal component analysis [PCA] and inter-class PCA associated with Monte-Carlo rank testing on the bacterial genera.

3. Results

3.1. Inhibition of TREM-1 by LR12 peptide prevents colonic inflammation in experimental colitis.

Previous studies using a 17-aa residues long TREM-1 antagonistic peptide [LP17] have shown that inhibition of TREM-1 decreases inflammation in DSS-induced experimental colitis.^{34,35} Here, as TREM-1 antagonist we used a shorter, potentially less antigenic peptide [LR12] with five aa residues deleted at the C-terminal. DSS-induced colitic mice were treated either LR12 peptide or vehicle [referred to respectively as DSS(+)/LR12(+) and DSS(+)/LR12(-) treatments] and 2,4,6-Trinitrobenzenesulfonic acid [TNBS]-induced colitic mice were treated either LR12 peptide or scrambled [Sc] LR12 peptide [referred to respectively as DSS(+) // LR12(+) and DSS(+) // ScLR12(+) treatments] [Figure 1 and supplementary Figure S3, respectively]. Peptide treatments starting from 2 days before and until the end of the DSS administration [Figure 1A] or until day 3 after TNBS administration at day 0 [supplementary Figure S3A]. LR12 peptide significantly attenuated the expression of TREM-1 *in vivo* [supplementary Figure S4]. We first confirmed that TREM-1 inhibition can indeed reduce intestinal inflammation after the onset of colitis.

Results showed that during the course of colitis the percentage of bodyweight loss [a typical sign of acute disease] was significantly reduced in DSS(+)/LR12(+) mice than in DSS(+)/LR12(-) animals [up to ~12% and ~30% of the initial bodyweight, respectively; $p=0.001$] [Figure 1B]. The disease activity index [DAI], that remained null for the DSS(-)/LR12(-) healthy group, was remarkably lower in DSS(+)/LR12(+) mice in comparison with the DSS(+)/LR12(-) group [$p<0.001$] [Figure 1C]. Similar protective effect of LR12 peptide on the clinical course of colitis was observed in TNBS-induced acute colitis [supplementary Figure S3B and C]. At the end of the protocol at day 10, we performed colonoscopy in all the experimental groups. While the DSS(-)/LR12(-) healthy group revealed a normal vascular pattern with clearly defined capillary trees and the absence of bleeding, erosions or ulcers, the DSS(+)/LR12(-) group presented a completely obliterated vascular network, with visible blood in the lumen, and fibrin-covered ulcers which resulted in a significantly increased endoscopic score [$p<0.001$] [Figure 1D]. In contrast, the colon of DSS(+)/LR12(+) mice closely resembled that of healthy animals, with a regular vascular pattern, a translucent mucosa and no signs of ulcerations or liquid blood [Figure 1D], thus leading to a reduced endoscopic score when compared to the DSS(+)/LR12(-) group [$p<0.001$]. Similar beneficial effect exerted by LR12 peptide was observed at the histological level. In fact, histopathological analysis of colon biopsies collected at day 10 revealed marked damage to the crypt architecture, with inflammatory cell infiltration, severe ulceration, and a loss of mucosal secretion in DSS(+)/LR12(-) mice [Figure 1E]. The mean percentages of tissue occupied by different inflammatory cell subtypes was calculated for each group. The colon of

DSS(+)/LR12(-) mice revealed a higher inflammatory infiltrate with 13% of polymorphonuclear neutrophils [PMNs], 15% of macrophages, 70% of lymphocytes, 2% of plasmocytes and 0% of mastocytes, when compared with DSS(-)/LR12(-) healthy group which presented 5% of PMNs, 8% of macrophages, 78% of lymphocytes, 8% of plasmocytes and 1% of mastocytes. Treatment with LR12 peptide was associated with significantly less mucosal damage, as assessed by the histological scores [$p<0.001$] [Figure 1E], and a decrease in inflammatory infiltrate that represented by 8% of PMNs, 7% of macrophages, 79% of lymphocytes, 5% of plasmocytes and 1% of mastocytes. Notably, colonic expression levels of pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 that are known to be increased in this acute model of colitis,⁴⁹ were significantly reduced in the DSS(+)/LR12(+) group [$p<0.001$ for TNF- α and IL-6 and $p=0.022$ for IL-1 β], when compared with DSS(+)/LR12(-) animals, as shown by western blot analysis [Figure 1F]. Overall, these results indicate that *in vivo* TREM-1 inhibition by LR12 peptide attenuates inflammation and tissue damage in acute colitis.

3.2. Inhibition of TREM-1 by LR12 peptide restores impaired autophagy activity during experimental colitis.

The relationship between TREM-1 and autophagy has never been characterized. To examine whether and how inhibition of TREM-1 by LR12 peptide regulates autophagy pathways during the acute phase of experimental colitis, we analyzed by western blot analysis the colonic expression of various macroautophagy and CMA proteins at day 10 [Figure 2A and B]. The expression of mTOR, known to be a negative regulator of autophagy,⁵⁰ was remarkably high in the DSS(+)/LR12(-) group, relative to healthy DSS(-)/LR12(-) mice. LR12 peptide treatment was found to significantly reduce the expression of mTOR as assessed by densitometric quantification [$p<0.001$] [Figure 2A]. This finding suggests that TREM-1 may regulate autophagy activity during DSS-induced colitis *via* mTOR signaling. With regard to macroautophagy, the expression of proteins like ATG1/ULK-1 and ATG13, involved in the initiation of autophagosome formation,⁵¹ and ATG5, ATG16L1 and MAP1LC3-I/II, involved in the membrane elongation and expansion of the forming autophagosome,⁵² was significantly reduced in the DSS(+)/LR12(-) mice when compared with the healthy DSS(-)/LR12(-) group [Figure 2A]. Interestingly, treatment of colitic mice with LR12 peptide was able to rescue in a significantly manner the expression of these proteins at basal levels [Figure 2A; $p\leq 0.017$ for the DSS(+)/LR12(+) group versus DSS(+)/LR12(-) animals] suggesting that in terms of macroautophagy, TREM-1 is involved in early and later phases of autophagosome formation under inflammatory conditions. Conversion from MAP1LC3-I to MAP1LC3-II status, judged by the MAP1LC3-II/MAP1LC3-I ratio, has been extensively used as a marker of macroautophagy activation.^{53,54} We observed that, the MAP1LC3-II/MAP1LC3-I ratio was significantly higher in DSS(+)/LR12(+) mice than in the DSS(+)/LR12(-) group [$p=0.017$], confirming that TREM-1 controls macroautophagy activation during experimental colitis [Figure 2A].

With regard to CMA, we observed reduced expression levels of HSPA8 protein, involved in the substrate targeting and the regulation of the dynamics of the CMA translocation

complex,⁵⁵ in the colon of DSS(+)/LR12(-) mice versus DSS(-)/LR12(-) animals. However, inhibition of TREM-1 by LR12 peptide was able to increase the amount of HSPA8 protein at basal levels, similar to those observed in healthy animals [Figure 2B]. The expression levels of HSP90AA1, another protein involved in CMA and which is in coordination with HSPA8,⁵⁵ were comparable in the DSS(-)/LR12(-) and DSS(+)/LR12(-) groups. In contrast, the amount of this protein was remarkably higher in DSS(+)/LR12(+) mice [$p < 0.001$], suggesting that TREM-1 plays a role in the modulation of CMA activation during DSS-induced experimental colitis [Figure 2B]. Altogether, these results suggest that [i] both macroautophagy and CMA are impaired in experimental colitis and [ii] the inhibition of TREM-1 by treatment with LR12 peptide can restore the activity of these two forms of autophagy.

3.3. Inhibition of TREM-1 by LR12 peptide reduces endoplasmic reticulum [ER] stress and induces unfolded protein response [UPR] during experimental colitis.

Another signaling pathway that has also emerged in IBD pathophysiology is the UPR, which is induced by ER stress.^{11,56} The UPR and autophagy are directly intimately intertwined and are deeply involved in IBD pathogenesis.⁵⁷⁻⁵⁹ The relationship between the UPR, autophagy and TREM-1 is unknown. To examine whether and how inhibition of TREM-1 by LR12 peptide regulates the ER stress and induced UPR during the acute phase of experimental colitis, we analyzed by western blot analysis the colonic expression of the three ER stress sensor proteins [e.g. inositol-requiring transmembrane kinase endonuclease-1 α [IRE-1 α], protein kinase RNA-like endoplasmic reticulum kinase [PERK] and activating transcription factor-6 α [ATF-6 α]], that initiate the UPR, at day 10 [Figure 3A and B]. The expression of these three proteins sensors on the ER membrane [PERK, IRE-1 α , and ATF-6 α] and also their active forms [p-PERK and p-IRE-1 α] was remarkably high in the DSS(+)/LR12(-) group, relative to healthy DSS(-)/LR12(-) mice [Figure 3A] as assessed by densitometric quantification which show the activity of PERK and IRE-1 α judged by p-PERK/PERK and p-IRE-1 α /IRE-1 α ratios respectively, and an increase of ATF-6 α expression [Figure 3B]. Interestingly, treatment of colitic mice with LR12 peptide was able to statistically reduce the expression of these proteins [p-PERK, PERK, p-IRE-1 α , IRE-1 α , and ATF-6 α] and their activity [p-PERK/PERK and p-IRE-1 α /IRE-1 α ratios] at basal levels [Figure 3A and B; $p < 0.001$ for the DSS(+)/LR12(+) group versus DSS(+)/LR12(-) animals]. Altogether, these results suggest that [i] the ER stress-induced UPR occurs in DSS-induced experimental colitis and activates PERK, IRE-1 α , and ATF-6 α and [ii] the inhibition of TREM-1 by treatment with LR12 peptide can reduce this ER stress and also induces UPR.

3.4. Genetically deletion of TREM-1 in mice prevents colonic inflammation in acute colitis.

To confirm the protective effect of TREM-1 inhibition, we studied DSS-induced colitis in littermates TREM-1 wild-type [WT] and knock-out [TREM-1 KO] mice [Figure 4A]. Results

showed that DSS(+)/TREM-1 KO mice are protected from colitis, when compared to DSS(+)/WT animals; this was observed both in terms of percentage of body weight loss [Figure 4B; up to ~30% for WT and ~12% for TREM-1 KO of the initial body weight, $p<0.001$], and DAI scores [Figure 4C; $p<0.001$]. Endoscopic analysis of the colon of WT and TREM-1 KO mice without DSS revealed a healthy mucosa with normal vascular pattern, and no visible blood in both groups, with no significant differences [Figure 4D, left panel]. On the contrary, upon DSS exposure, while WT mice showed an intricate vascular pattern, several ulcerations and mucosal damage, the endoscopic pattern of TREM-1 KO mice resulted more similar to that of healthy mice [Figure 4D, left panel]. The endoscopic scores reflected these observations; in fact, DSS-treated WT mice had a significantly increased score than colitic TREM-1 KO [$p=0.002$] [Figure 4D, right panel]. Histopathological analysis revealed damage to the crypt architecture, higher inflammatory cell infiltration [represented by 30% of PMNs, 27% of macrophages, 42% of lymphocytes, 1% of plasmacytes, and 0% of mastocytes], and severe ulceration in DSS(+)/WT mice. In contrast, DSS(+)/TREM-1 KO animals showed a significantly attenuated histological score, associated with reduced mucosal damage and lower immune cell infiltration [represented by 3% of PMNs, 8% of macrophages, 88% of lymphocytes, 1% of plasmacytes, and 0% of mastocytes] similar to that observed in both WT and TREM-1 KO healthy mice [Figure 4E]. Moreover, colitic TREM-1 KO mice displayed reduced expression levels of pro-inflammatory cytokines, such as TNF- α , IL-1 β and IL-6, when compared to colitic WT mice, as demonstrated by western blot densitometric analyses [Figure 4F; $p=0.001$ for TNF- α and $p<0.001$ for IL-1 β and IL-6]. Overall, these findings confirmed what observed with the LR12 peptide treatment, thus further highlighting a key role of TREM-1 in acute phase of colitis and the therapeutic potential of TREM-1 inhibition in IBD.

3.5. TREM-1 deletion restores impaired autophagy activity during experimental colitis.

Western blot and densitometric analyses of the above-mentioned macroautophagy- and CMA-related proteins were performed in WT and TREM-1 KO mice, both under steady-state [no DSS] and inflammatory conditions [DSS administration]. mTOR expression was remarkably higher in DSS(+)/WT mice, in comparison with both healthy WT and TREM-1 KO mice. On the contrary, TREM-1 deletion displayed significantly reduced mTOR expression [$p<0.001$], when compared to colitic WT mice [Figure 5A], thus confirming what observed with LR12 peptide treatment. In terms of macroautophagy, while we found the expression levels of ATG1/ULK-1, ATG13, and MAP1LC3-I/II lower in DSS(+)/WT mice than in the two WT and TREM-1 KO healthy groups, the amount of these proteins was restored at the basal levels in DSS(+)/TREM-1 KO animals, thus resulting significantly higher in this group than in DSS(+)/WT mice [$p\leq 0.001$] [Figure 5A]. Moreover, as observed for the LR12 peptide treatment, the MAP1LC3-II/MAP1LC3-I ratio was significantly higher in the DSS(+)/TREM-1 KO animals than in the DSS(+)/WT mice [$p=0.001$], thus suggesting that TREM-1 controls macroautophagy activation in the acute phase of experimental colitis [Figure 5A].

With regard to CMA, expression levels of HSPA8 and HSP90AA1 proteins were lower in the DSS(-)/TREM-1 KO mice than in the DSS(-)/WT group. However, while expression of these two proteins were lower in the DSS(+)/WT mice than in either of the two control healthy groups, amount of both HSPA8 and HSP90AA1 was significantly increased in DSS(+)/TREM-1 KO animals than in the DSS(+)/WT group [Figure 5B; $p=0.014$ for HSPA8 and $p=0.04$ for HSP90AA1], suggesting that not only ligands binding to TREM-1 are necessary to control CMA activation in a setting of experimental colitis, but that TREM-1 *per se* is a key modulator of these processes.

3.6. Deletion of TREM-1 in mice reduces endoplasmic reticulum [ER] stress and induces unfolded protein response [UPR] in acute colitis.

Western blot and densitometric analyses of the above-mentioned ER stress and UPR sensors-related proteins were performed in WT and TREM-1 KO mice, both under steady-state [no DSS] and inflammatory conditions [DSS administration]. The expression of the three proteins sensors on the ER membrane [PERK, IRE-1 α , and ATF-6 α] and also their active forms [p-PERK and p-IRE-1 α] was remarkably higher in DSS(+)/WT mice, in comparison with both healthy WT and TREM-1 KO mice [Figure 6A] as assessed by densitometric quantification which show the activity of PERK and IRE-1 α judged by p-PERK/PERK and p-IRE-1 α /IRE-1 α ratios respectively, and an increase of ATF-6 α expression [Figure 6B]. On the contrary, TREM-1 deletion displayed a significantly reduced expression of these proteins [p-PERK, PERK, p-IRE-1 α , IRE-1 α , and ATF-6 α] and activity [p-PERK/PERK and p-IRE-1 α /IRE-1 α ratios] at basal levels [$p<0.001$], when compared to colitic WT mice [Figure 6A and B], thus confirming what was observed with LR12 peptide treatment [see above]. Moreover, as observed for the LR12 peptide treatment, the p-PERK/PERK and p-IRE-1 α /IRE-1 α ratios were significantly lower in the DSS(+)/TREM-1 KO animals than in the DSS(+)/WT mice [$p<0.001$], thus confirming that TREM-1 modulate the ER stress and induced UPR activation in the acute phase of experimental colitis [Figure 6A and B].

3.7. Inhibition of TREM-1, either pharmacologically by LR12 peptide or genetically in TREM-1 KO mice, prevents disease-related changes in intestinal microbiota during acute colitis.

Dysbiosis of the gut microbiota aggravates intestinal inflammation in IBD.⁶⁰ To investigate whether inhibition of TREM-1 by LR12 peptide could modulate this dysbiosis, we analyzed the operational taxonomic units [OTUs] richness and the taxonomic composition of the bacterial community in fecal pellets from three groups of mice: DSS(-)/LR12(-),

DSS(+)/LR12(-), and DSS(+)/LR12(+). Although DSS treatment was associated with low microbial richness at day 10 [Figure 7A], the richness in colitic mice receiving LR12 peptide [observed OTUs: 282] was closer to that found in the control group [observed OTUs: 335] [Figure 7A]. The DSS treatment had the greatest effect on the Bacteroidetes [Figure 7B], and was associated with low proportions of bacteria from the Porphyromonadaceae family [unclassified species and the genus *Barnesiella*] and the *Prevotella* genus. In contrast, DSS treatment was associated with elevated proportions of bacteria from the genera *Enterobacter*, *Bacteroides* and [to a lesser extent] *Lactobacillus* [Figure 7B]. LR12 peptide treatment was linked with relatively greater percentages of Lachnospiraceae [*Clostridium* XIVa and unclassified species] and more importantly, appeared to be able to counter the relative increase in the proportions of *Enterobacter*, *Bacteroides* and *Lactobacillus* genera.

Similarly, in the genetically-deleted TREM-1 mouse model, gut microbial richness and diversity were less affected by DSS treatment than in WT animals [Figure 8A]. While DSS treatment was associated with increased proportions of bacteria from the *Escherichia/Shigella*, *Parabacteroides* and *Bacteroides* genera in WT mice, dysbiosis of these specific bacterial taxa was again countered in TREM-1 KO mice, together with an increased in relative abundance of *unclassified Lachnospiraceae* and *Barnesiella* [Figure 8B]. Moreover, gut microbiota overall composition from healthy TREM-1 KO mice significantly differed when compared to healthy WT mice [supplementary Figure S5A; Monte-Carlo $p=0.008$]. More specifically, 31 genera significantly differed between WT and TREM-1 KO groups, 12 being over-represented in KO mice [supplementary Figure S5B]. While *Bacteroides* was significantly more abundant in healthy TREM-1 KO mice, *Prevotella* and *Alistipes* were over-represented in healthy WT mice.

4. Discussion

There is a growing body of evidence suggesting that TREM-1 is involved in the pathogenesis of IBD.³⁴⁻³⁶ To the best of our knowledge, the current study is the first to have investigated the relationship between TREM-1, autophagy activity, ER stress and intestinal microbial dysbiosis in experimental colitis. Our data confirmed that inhibition of TREM-1 by an antagonistic peptide substantially attenuates inflammatory responses and counters disease exacerbation in experimental colitis, as observed in previous studies of a 17-aa residues long TREM-1 antagonistic peptide [LP17].^{34,35} In line with these previous studies, our results showed a decrease in inflammatory cell infiltration and IL-6 expression, suggesting that monocytes and macrophages were the main cells responsible for the reduced signs of inflammation in the colon of TREM-1 inhibited mice.^{34,35} Indeed, monocytes and macrophages represent the most important sources of IL-6 at inflammatory sites.⁶¹ Unlike previous reports we used a shorter, potentially less antigenic peptide [LR12] with five aa that were deleted at the C-terminal. TREM-1 belongs to the immunoglobulin superfamily and is part of a gene cluster encoding several TREMs and TREM-like molecules that share structural elements but have a low degree of amino acid homology.⁶² For example, the TREM gene cluster also includes TREM-like transcript-1 [TLT-1]. The latter is abundant but is specific

for the platelet and megakaryocyte lineage. Crystallographic studies have revealed structural similarities between TLT-1 and TREM-1, which suggests that the two proteins interact.⁶³ Indeed, it was recently shown that TLT-1 and a TLT-1-derived peptide [LR12] exhibit anti-inflammatory properties by dampening TREM-1 signaling, and thus behave as naturally occurring TREM-1 inhibitors. Here, based on several independent approaches [western blot analysis of colonic TREM-1 protein expression, ELISA analysis of secretion of sTREM-1 in plasma and quantitative RT-PCR of colonic TREM-1 mRNA expression], we demonstrated the effectiveness of the LR12 peptide in the inhibition of TREM-1 during experimental colitis. LR12's inhibition of TREM-1 signaling derives from the peptide's ability to bind to the TREM-1 ligand.⁶⁴ The same study also demonstrated that LR12 peptide modulates the inflammatory cascade triggered by infection *in vivo*, and thus inhibits hyper-responsiveness, organ damage, and death during experimental sepsis in mice.⁶⁴ However, blocking TREM-1 signaling by daily administration of TREM-1 antagonistic peptide in chronic disease models may fail to allow for the possibility that the as-yet unidentified TREM-1 ligand may signal through alternative receptors. Several potential ligands of TREM-1 have been investigated in various diseases,⁶⁵ but to our best knowledge there is no specific ligand of this receptor. To avoid this drawback and to confirm our results for LR12 peptide treatment, we performed the same experiments in *Trem-1* KO mice. Similar results were observed in the two different mouse models - thus emphasizing the LR12 peptide's therapeutic potential in IBD.

Autophagy plays a compensatory role to the UPR to reduce ER stress induced in the pathogenesis of IBD.^{10,17,18} Defects in autophagy [due to autophagy gene mutations and/or microbial antagonism] may trigger the pathogenesis of IBD, impair the antibacterial response and thus weaken the host's ability to control bacterial infection and chronic inflammation.^{3,66,67} In line with these data, our results show that macroautophagy and CMA are strongly impaired in experimental colitis. The observed increase in the expression of mTOR [a protein known to downregulate autophagy] in this setting suggests that the impairment in autophagy may be due to defective initiation. We noted a decrease in the expression levels of [i] macroautophagy proteins [such as ATG13 and ATG1/ULK-1] involved in the initiation of autophagosome formation, [ii] macroautophagy proteins [such as ATG16L1 and MAP1LC3-I/II] involved in membrane elongation and expansion of the forming autophagosome, and [iii] proteins involved in CMA [such as HSPA8 and HSP90AA1]. These results strongly support our initial hypothesis. Our results indicate that inhibition of TREM-1, either pharmacologically by LR12 peptide or genetically with TREM-1 KO mice, can promote the activity of both macroautophagy and CMA in experimental colitis. The lower mTOR expression level in LR12-treated and *Trem-1* KO colitic mice, suggests that TREM-1 inhibition may enhance the onset of autophagy by promoting mTOR downregulation. Indeed, we observed increases in the expression of proteins involved in both macroautophagy [ATG13, ATG1/ULK-1, ATG16L1, ATG5, and MAP1LC3-I/II] and CMA [HSPA8 and HSP90AA1]. As previously observed for other PRRs [NLRs and TLRs],⁶⁸ our results demonstrate that TREM-1 is involved in autophagy. TREM-1 expression and activity are closely linked with the activities of both TLRs and NLRs. It has been shown that TLR activation leads to upregulation of TREM-1 expression in a MyD88-dependent manner.^{37,38}

Following LPS stimulation of neutrophils, TREM-1 was found to be recruited to macrophage-lipid rafts and co-localized with TLR4.⁶⁹ Simultaneous activation of TREM-1 and TLR4 leads to synergistic production of pro-inflammatory mediators.⁷⁰ On the other hand, very little is known concerning TREM-1 and NLRs interactions. Previous studies reported that TREM-1 has a synergistic effect on the production of pro-inflammatory mediators induced by NOD1 and NOD2 ligands.^{39,40} Mechanistically, TREM-1 activation can lead to enhanced NOD2 expression, NF- κ B activation and cytokine production such as IL-1 β and IL-6.³⁹ These literature data showed that TREM-1 is strongly linked to other PRRs involved in autophagy, thus strongly supporting our findings.

Evidence of unresolved ER stress and an activated UPR in intestinal epithelial cells [IECs] has been reported in both forms of IBD [UC and CD].^{7,71} Our present results show that the UPR is strongly increased in DSS-induced experimental colitis. The observed increase in the activities of PERK and IRE-1 α and in the expression level of ATF-6 α [the three canonical sensors of ER stress] in this setting suggests that the ER stress occurs in colitic mice. It is well known that the UPR in response to ER stress is a major inducer of autophagy.^{9,13} On the contrary, our data indicate that both macroautophagy and CMA are impaired in these colitic mice. However, not only ER stress may induce autophagy, but *vice versa*, impaired autophagy can also promote ER stress.¹⁴ Yang *et al.* have reported that suppression of ATG7 [a protein involved in autophagosome formation] in the liver leads to increased ER stress [with its downstream consequences], while restoration of ATG7 expression dampens ER stress.¹⁴ The increased UPR observed in colitic mice is probably the consequence of colitis-induced ER stress but may also be caused by the impaired autophagy in these mice. The effect of TREM-1 inhibition on the ER stress has never been investigated. Our results indicate that inhibition of TREM-1, either pharmacologically by LR12 peptide or genetically in TREM-1 KO mice, can decrease the activities of PERK and IRE-1 α and also the expression level of ATF-6 α , suggests that TREM-1 inhibition may reduce the ER stress in DSS-induced experimental colitis. Interestingly, our results show that the impaired activity of both macroautophagy and CMA were restored in LR12-treated and TREM-1 KO colitic mice. In agreement with our hypothesis, impaired autophagy promotes the ER stress [with its downstream consequences], but the restoration of the autophagy activity by TREM-1 inhibition compensate the UPR to reduce ER stress in colitic mice. These observations provide strong support with regard to the previous studies, which described that the autophagy plays an important compensatory role in the context of ER stress and also that both autophagy and the UPR are deeply involved in innate immune mechanisms to maintain mucosal homeostasis. Collectively, these findings appear particularly relevant for host-microbiota interactions at the epithelial surface of the intestine during the pathogenesis of IBD.^{10,15–18}

Previous studies have shown that the presence of a functional autophagy pathway in the intestinal epithelium is essential for counteracting intestinal dysbiosis and bacterial infection. This is because autophagy controls the secretion of antimicrobial proteins and limits their dissemination.^{25,72} The healthy gastrointestinal microbiome is dominated by the phyla Firmicutes and Bacteroidetes, and, to a lesser degree, by the phyla Proteobacteria and

Actinobacteria.⁷³ Bacterial biodiversity is low in both CD and UC, each featuring distinct microbial perturbations and sites of tissue damage.²⁴ Bacterial dysbiosis is characterized by low biodiversity, abnormally low numbers of certain Firmicutes and abnormally high numbers of mucosa-adherent Proteobacteria.²²⁻²⁴ It is well known that different commensal bacteria induce distinct types of colitis in *IL-10*-KO mice.^{60,74} A mono-association study [in which various bacterial strains were inoculated singly into germ-free *IL-10*-KO mice] demonstrated that [i] *E. coli* induced cecal inflammation, [ii] *Enterococcus faecalis* induced distal colitis, [iii] *Pseudomonas fluorescens* did not cause colitis, and [iv] the rodent gut commensal *Helicobacter hepaticus* exacerbated colitis in this model.^{60,74} Hence changes in the composition of the gut microbiota can cause distinct intestinal immune responses - even in a host with a uniform genetic background. This suggests that dysbiosis can modulate the immune response in the gut. Here, we confirmed that DSS-induced experimental colitis is associated with low bacterial diversity and a shift towards a higher proportion of *Enterobacter*. Our data also indicate that inhibition of TREM-1, either pharmacologically by LR12 peptide or genetically in TREM-1 KO mice, might prevent the intestinal microbiota changes associated with experimental colitis, both by maintaining bacterial richness and limiting the number of *Enterobacter* to levels observed in non-colitic mice. This outcome may result from the enhanced autophagy activity derived from the inhibition of TREM-1 in colitic mice.

Clearly, future research must further focus on the link between colitis protection and changes in the microbiota, and on the characterization of the mechanisms that lead to increased autophagy activity upon TREM-1 inhibition.

In summary, we first confirmed that the administration of LR12 peptide [known to modulate the TREM-1 pathway] exerts a strong protective effect against DSS-induced colitis in the mouse. Furthermore, we bring evidence that blocking TREM-1 upon the induction of experimental colitis compensates for the defect in autophagy activity and may have prevents dysbiosis of the intestinal microbiota. These results collectively suggest that TREM-1 plays a key role in the control of autophagy activity in acute phase of colitis, with a consequent effect on dysbiosis. These results further argue for a role for TREM-1 in IBD pathogenesis. Altogether, these findings reinforce the idea that TREM-1 may constitute a drug target of choice in the treatment of chronic inflammatory diseases and autophagy disorders. We are confident that this promising strategy will be evaluated in patients with IBD in due course.

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LPB had the initial concept and managed the study. TK, CM, BG and JH collected the data. SG provided the LR12 peptide and *Trem-1* knock-out mice. PL provided the microbiota analyses data and expertise. HBV provided the histological data and histopathological expertise. NCN conducted the statistical analyses. TK and LPB wrote the initial draft of the manuscript. FH, DM, JYJ, SD'A, SD, JLG, SM, and LPB were involved in analysis and interpretation of data, drafting and critical revision of the manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors have no conflict of interest to declare.

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Figures legends

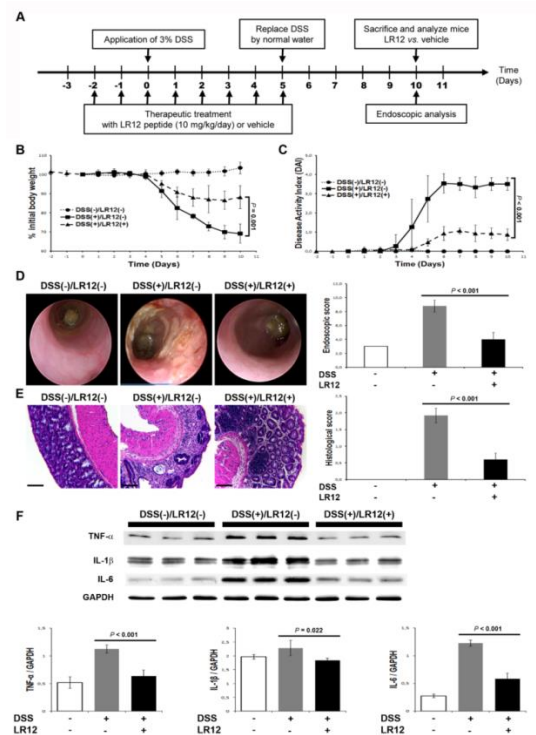


Figure 1.

Inhibition of TREM-1 by LR12 peptide prevents colonic inflammation in experimental colitis. [A] Experimental design for pharmacological inhibition of TREM-1 [by LR12 peptide treatment]. [B] Bodyweight was monitored daily, and bodyweight loss [as a percentage of initial bodyweight] was calculated for each animal in the three groups of mice: [DSS(-)/LR12(-), DSS(+)/LR12(-), and DSS(+)/LR12(+); n=5 for each group]. [C] The disease activity index [DAI] for the three groups of mice was determined as described in the Methods. [D] Representative, colonoscopy images and endoscopy scores on day 10 for the three groups of mice. [E] Representative, HES-stained colonic tissue sections and histological scores on day 10 were presented for the three groups of mice. Scale bar: 200 μ m. [F] Western blot analyzes of TNF- α [25 kDa], IL-1 β [35 kDa] and IL-6 [23 kDa] in colon samples from the three groups of mice on day 10. GAPDH [37 kDa] served as loading control. Densitometric quantification [mean values \pm SD normalized against GAPDH are shown for each protein] evidences an effect of LR12 peptide on inflammation in experimental colitis. All data are quoted as the mean \pm SD. All *p* values are from two-tailed Student's *t* tests.

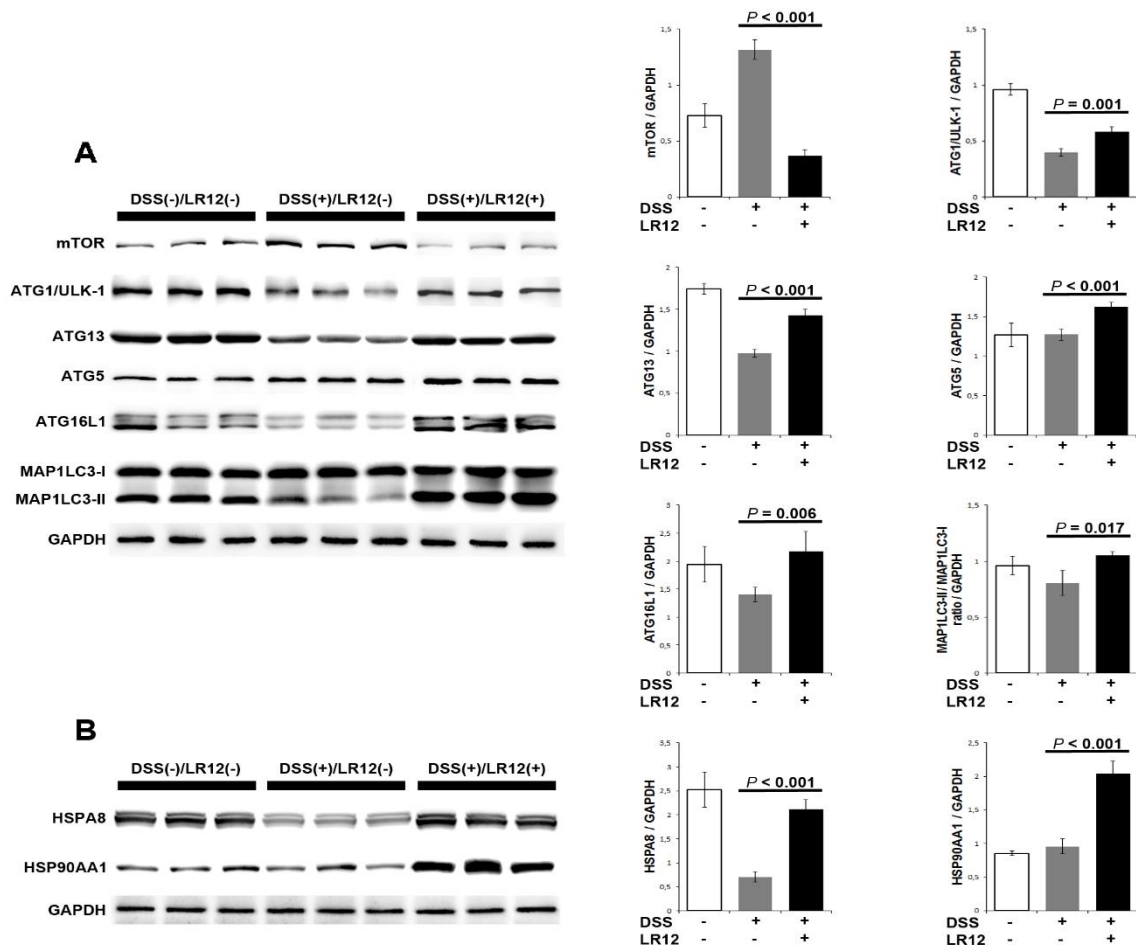


Figure 2.
Inhibition of TREM-1 by LR12 peptide restores impaired autophagy activity during experimental colitis. [A] Western blots of macroautophagy proteins mTOR [240 kDa], ATG1/ULK-1 [130 kDa], ATG13 [45 kDa], ATG5 [55 kDa], ATG16L1 [68 kDa], MAP1LC3-I [16 kDa], and MAP1LC3-II [14 kDa], with densitometric quantification [mean values \pm SD normalized against GAPDH]. [B] Western blots of chaperone-mediated autophagy [CMA] proteins HSPA8 [70 kDa] and HSP90AA1 [90 kDa], with densitometric quantification [mean values \pm SD normalized against GAPDH]. The colonic samples were obtained from three groups of mice [DSS(-)/LR12(-), DSS(+)/LR12(-), and DSS(+)/LR12(+); n=5 for each group] on day 10. GAPDH [37 kDa] served as a loading control. All data are quoted as the mean \pm SD. All *p* values are from two-tailed Student's *t* tests.

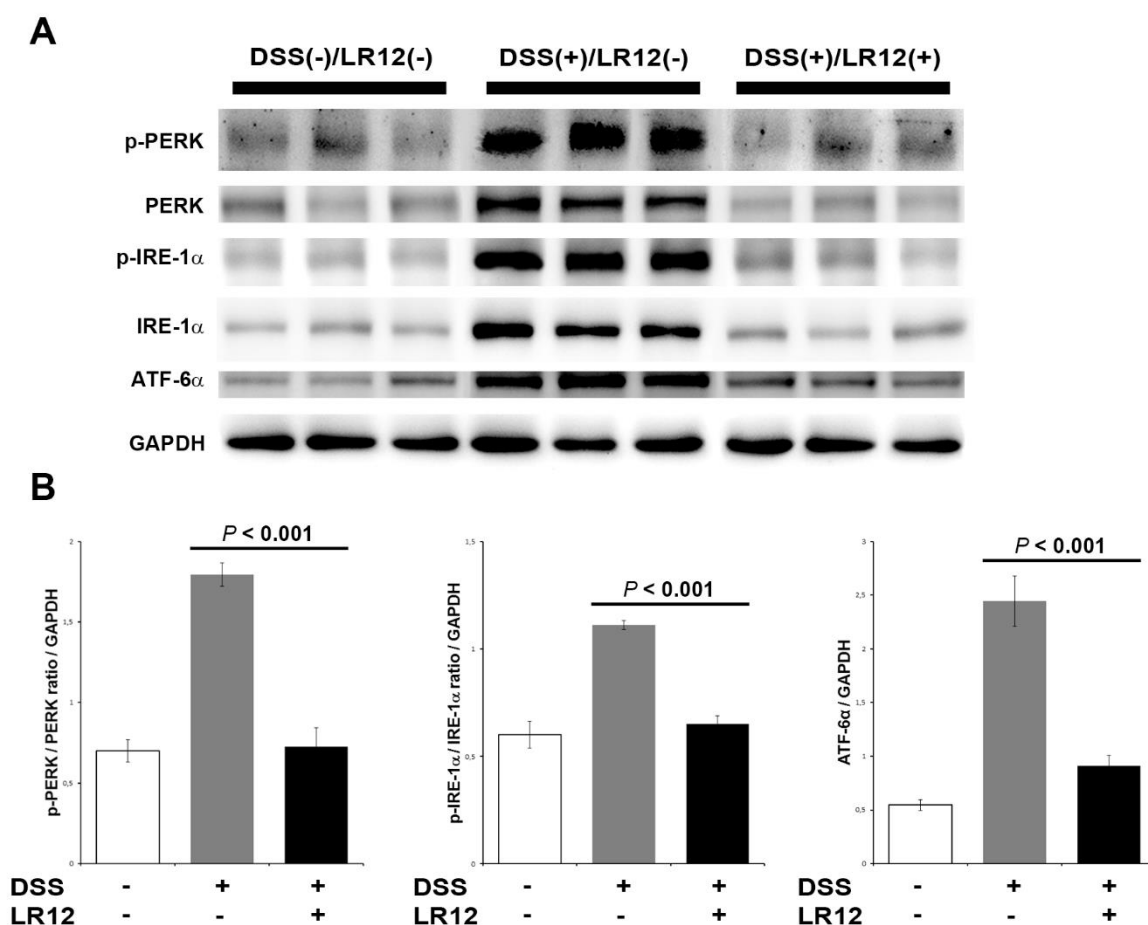


Figure 3.

Inhibition of TREM-1 by LR12 peptide reduces endoplasmic reticulum [ER] stress and induced unfolded protein response [UPR] during experimental colitis. [A] Western blot analyzes of PERK [140 kDa], phosphorylated-PERK [p-PERK] [170 kDa], IRE-1 α [130 kDa], phosphorylated-IRE-1 α [p-IRE-1 α] [110 kDa] and ATF-6 α [90 kDa] in colon samples from the three groups of mice [DSS(-)/LR12(-), DSS(+)/LR12(-), and DSS(+)/LR12(+); n=5 for each group] on day 10. GAPDH [37 kDa] served as loading control. [B] Densitometric quantification of PERK activity [p-PERK/PERK ratio], IRE-1 α activity [p-IRE-1 α /IRE-1 α ratio], and ATF-6 α expression [mean values \pm SD normalized against GAPDH are shown for each protein] evidences an effect of LR12 peptide on ER stress and UPR elements in DSS-induced experimental colitis. All data are quoted as the mean \pm SD. All *p* values are from two-tailed Student's *t* tests.

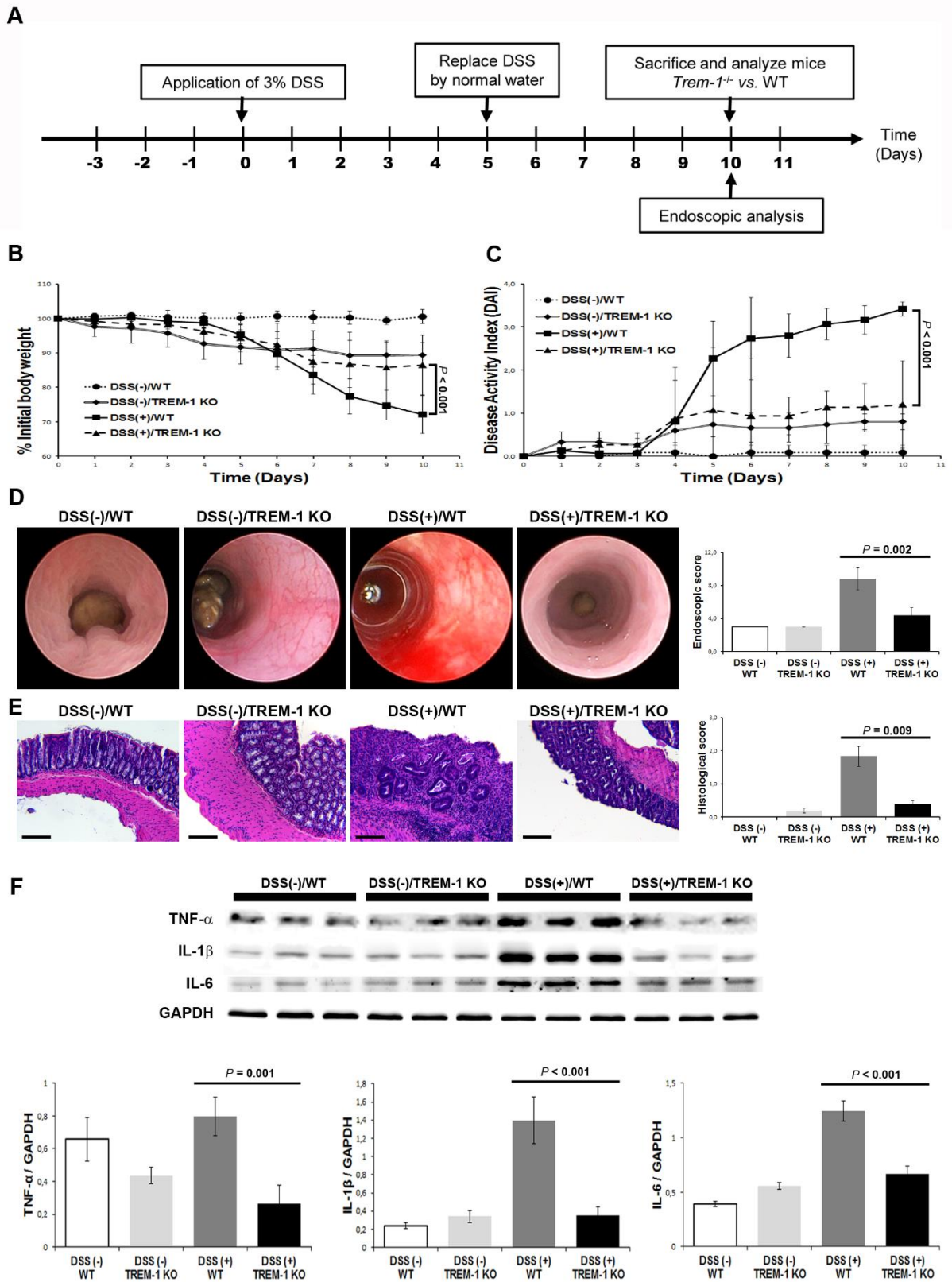


Figure 4. Deletion of TREM-1 in mice prevents colonic inflammation in the DSS-induced model of acute colitis. [A] Experimental design for genetical inhibition of TREM-1 [with *Trem-1* KO

mice]. **[B]** Bodyweight was monitored daily, and weight loss [as a percent of initial bodyweight] for each mouse was calculated in the four groups: DSS(-)/WT, DSS(-)/TREM-1 KO, DSS(+)/WT, and DSS(+)/TREM-1 KO; $n=5$ for each group. **[C]** The disease activity index [DAI] for the four groups of mice was determined as described in Methods. **[D]** Representative colonoscopy images and endoscopy scores on day 10, for the four groups of mice. **[E]** Representative HES-stained colonic tissue sections and histological score on day 10 for the four groups of mice. Scale bar: 200 μm . **[F]** Western blot analyses of TNF- α [25 kDa], IL-1 β [35 kDa] and IL-6 [23 kDa] in colon samples from the four groups of mice on day 10. GAPDH [37 kDa] served as a loading control. Densitometric quantification [mean values \pm SD, normalized against GAPDH] of each protein revealed the effects of a lack of TREM-1 on inflammation in experimental colitis. All data are quoted as the mean \pm SD. The reported p values are from two-tailed Student t tests or [when a one-way ANOVA was significant] *post hoc* tests.

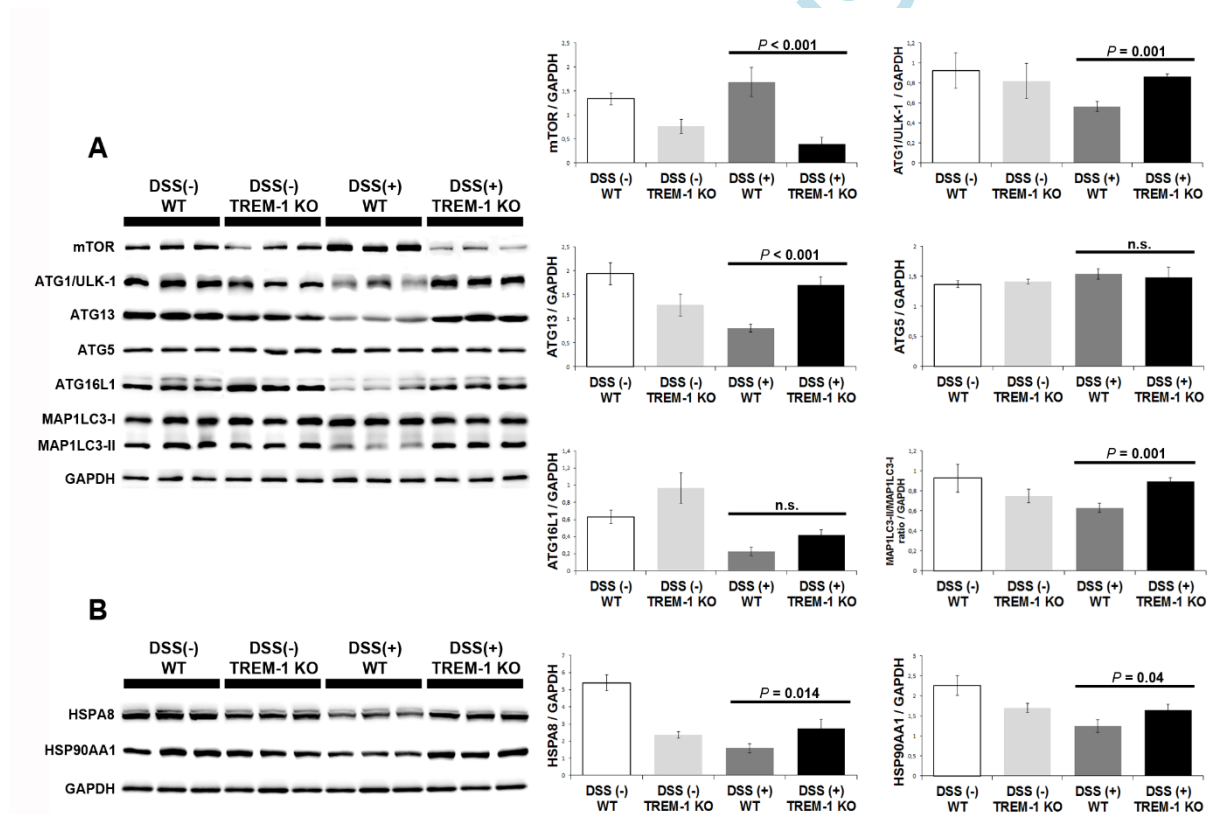


Figure 5.

TREM-1 deletion restores impaired autophagy activity during experimental colitis. **[A]** Western blots of macroautophagy proteins mTOR [240 kDa], ATG1/ULK-1 [130 kDa], ATG13 [45 kDa], ATG5 [55 kDa], ATG16L1 [68 kDa], MAP1LC3-I [16 kDa], and MAP1LC3-II [14 kDa], with densitometric quantification analysis [mean \pm SD values, normalized against GAPDH] for each protein. **[B]** Western blots of chaperone-mediated autophagy [CMA] proteins HSPA8 [70 kDa] and HSP90AA1 [90 kDa], with densitometric quantification analysis [mean \pm SD values, normalized against GAPDH] for each protein. The

colonic samples were obtained from four groups of mice [DSS(-)/WT, DSS(-)/TREM-1 KO, DSS(+)/WT and DSS(+)/TREM-1 KO; n=5 for each group] on day 10. GAPDH [37 kDa] served as a loading control. All data are quoted as the mean \pm SD. The reported *p* values are from *post hoc* tests [when the one-way ANOVA was significant].

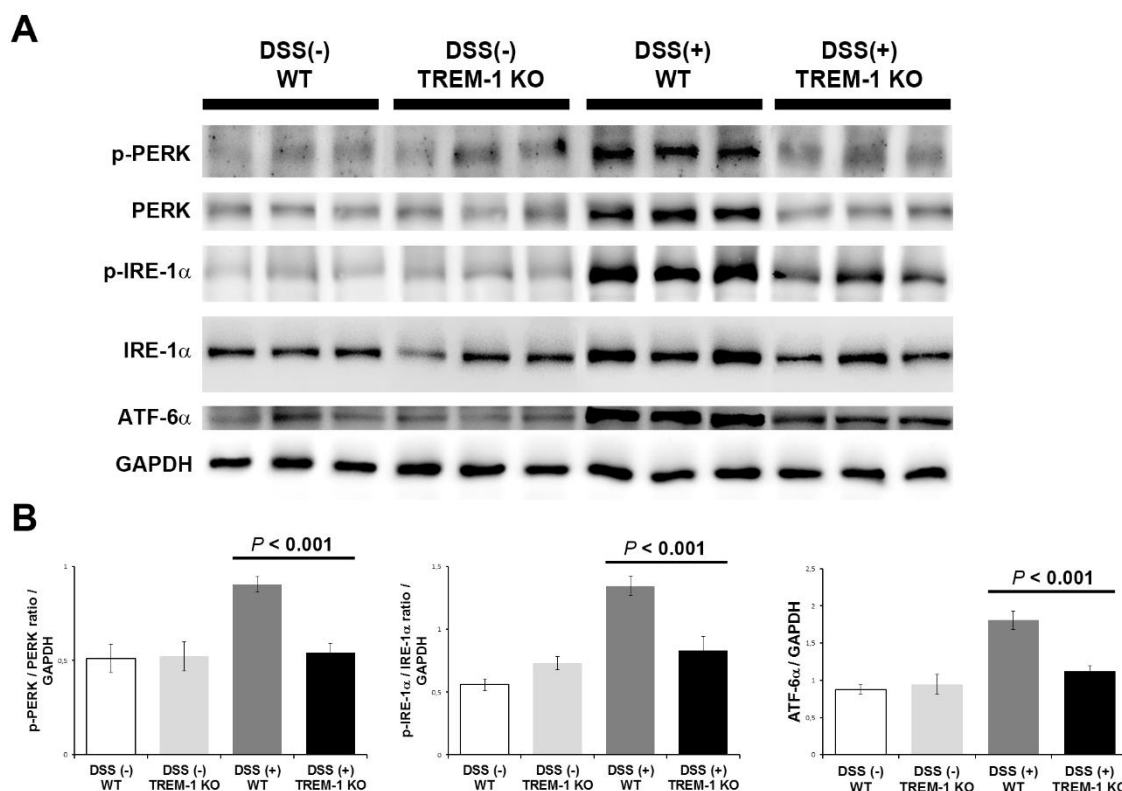


Figure 6.

Deletion of TREM-1 in mice reduces endoplasmic reticulum [ER] stress and induced unfolded protein response [UPR] in the DSS-induced model of acute colitis. [A] Western blot analyzes of PERK [140 kDa], phosphorylated-PERK [p-PERK] [170 kDa], IRE-1 α [130 kDa], phosphorylated-IRE-1 α [p-IRE-1 α] [110 kDa] and ATF-6 α [90 kDa] in colon samples from four groups of mice [DSS(-)/WT, DSS(-)/TREM-1 KO, DSS(+)/WT and DSS(+)/TREM-1 KO; n=5 for each group] on day 10. GAPDH [37 kDa] served as loading control. [B] Densitometric quantification of PERK activity [p-PERK/PERK ratio], IRE-1 α activity [p-IRE-1 α /IRE-1 α ratio], and ATF-6 α expression [mean values \pm SD normalized against GAPDH are shown for each protein] revealed the effects of a lack of TREM-1 on ER stress and UPR elements in experimental colitis. All data are quoted as the mean \pm SD. The reported *p* values are from two-tailed Student *t* tests or [when a one-way ANOVA was significant] *post hoc* tests.

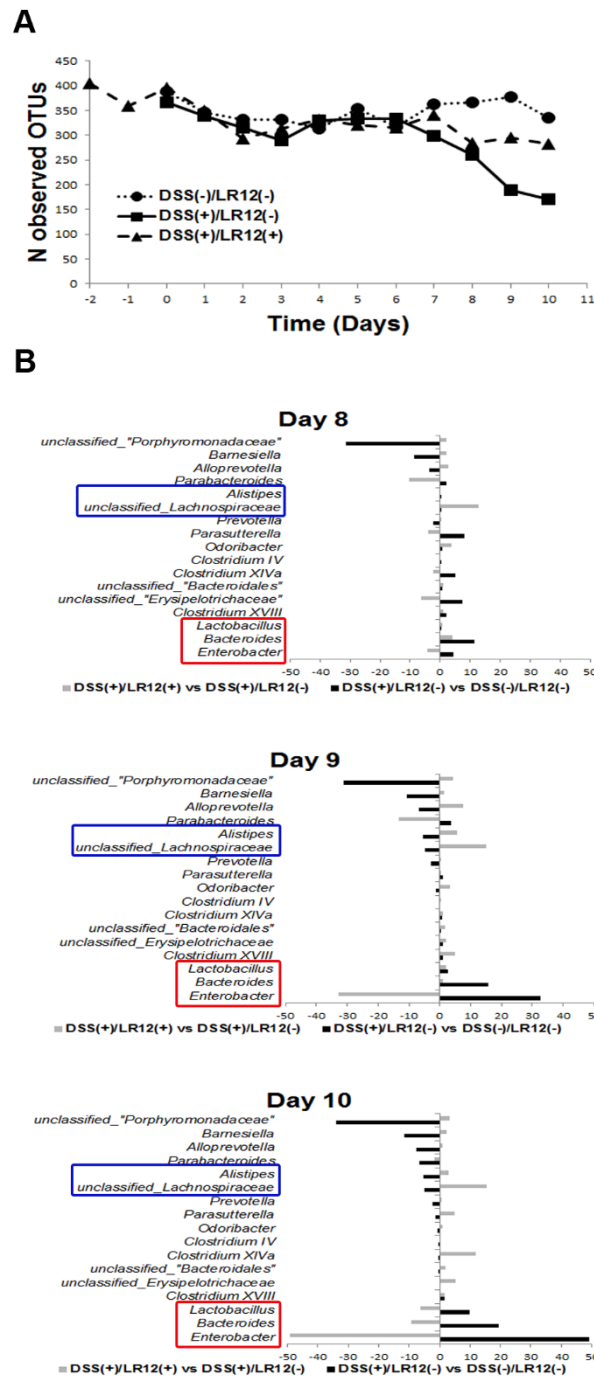


Figure 7. LR12 peptide treatment prevents disease-related changes in intestinal microbiota during DSS-induced acute colitis. [A] Operational taxonomic units [OTUs] richness of gut microbiota in the murine colon was monitored daily in three groups of mice: DSS(-)/LR12(-), DSS(+)/LR12(-), and DSS(+)/LR12(+). [B] Taxonomic composition of the bacterial community at the genus level in fecal pellets from the three groups of mice on days 8, 9 and 10. For each genus, differences were calculated as follows: [percentage in the DSS(+)/LR12(-) group] – [percentage in the DSS(-)/LR12(-) group] and [percentage in the DSS(+)/LR12(+) group] – [percentage in the DSS(+)/LR12(-) group].

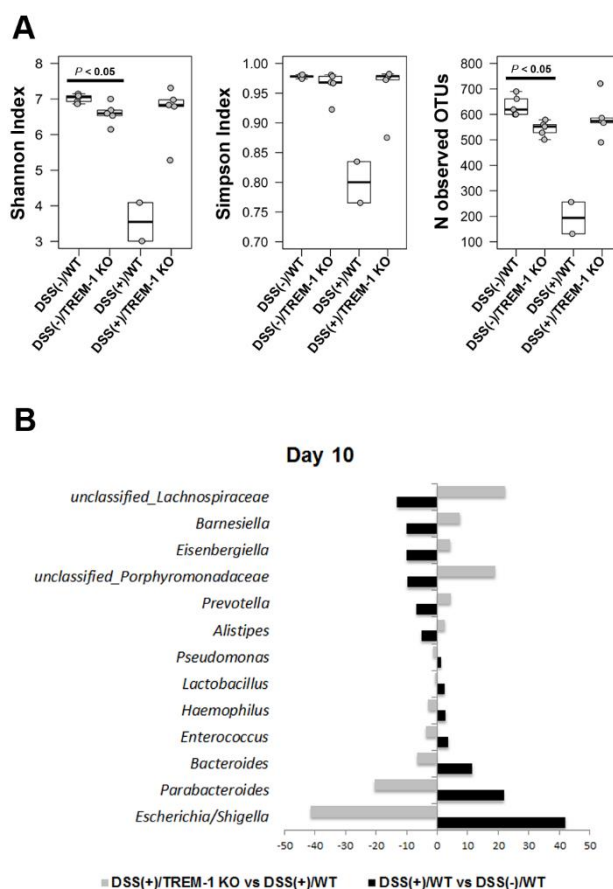


Figure 8.

Deletion of TREM-1 in mice prevents disease-related changes in intestinal microbiota during DSS-induced acute colitis. [A] Diversity and richness of gut microbiota in the murine colon was assessed by Shannon Index, Simpson index and number of observed operational taxonomic units [OTUs] in four groups of mice at Day 10: DSS(-)/WT, DSS(-)/TREM-1 KO, DSS(+)/WT and DSS(+)/TREM-1 KO. [B] Taxonomic composition of the bacterial community at the genus level in fecal pellets from the four groups of mice at day 10. For each genus, differences were calculated as follows: [percentage in the DSS(+)/WT group] – [percentage in the DSS(-)/WT group] and [percentage in the DSS(-)/TREM-1 KO] – [percentage in the DSS(+)/WT group]. The reported p values are from two-tailed Student t tests.