Development of Heptylmannoside-Based Glycoconjugate Antiadhesive Compounds against Adherent-Invasive Escherichia coli Bacteria Associated with Crohn’s Disease

Adeline Sivignon, Xibo Yan, Dimitri Alvarez Dorta, Richard Bonnet, Julie Bouckaert, Etienne Fleury, Julien Bernard, Sébastien Gouin, Arlette Darfeuille-Michaud, Nicolas Barnich

To cite this version:

Adeline Sivignon, Xibo Yan, Dimitri Alvarez Dorta, Richard Bonnet, Julie Bouckaert, et al.. Development of Heptylmannoside-Based Glycoconjugate Antiadhesive Compounds against Adherent-Invasive Escherichia coli Bacteria Associated with Crohn’s Disease. mBio, American Society for Microbiology, 2015, 6 (6), pp.e01298-15. 10.1128/mBio.01298-15. hal-01265411
Development of Heptylmannoside-Based Glycoconjugate Antiadhesive Compounds against Adherent-Invasive Escherichia coli Bacteria Associated with Crohn’s Disease

Adeline Sivignon,a,b Xibo Yan,c,d,e Dimitri Alvarez Dorta,f Richard Bonnet,a,b,g Julie Bouckaert,h Etienne Fleury,c,d,e Julien Bernard,c,d,e Sébastien G. Goun,f Arlette Darfeuille-Michaud,a,b,h Nicolas Barnich,a,b

Clermont Université, UMR 1071 INSERM/Université d’Auvergne, Clermont-Ferrand, France; INRA, Unité Sous Contrat 2018, Clermont-Ferrand, France; Université de Lyon, Lyon, France; INSA- Lyon, Ingénierie des Matériaux Polymères (IMP), Villeurbanne, France; CNRS, UMR 5223, Ingénierie des Matériaux Polymères, Villeurbanne, France; LUNAM Université, Chimie et Interdisciplinarité, Synthèse, Analyse, Modélisation (CEASIM), UMR CNRS 6230, UFR des Sciences et des Techniques, Nantes, France; Centre Hospitaller Universitaire, Clermont-Ferrand, France; Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), UMR8576, CNRS, Université Lille 1, Lille, France.

ABSTRACT The ileal lesions of Crohn’s disease (CD) patients are colonized by adherent-invasive Escherichia coli (AIEC) bacteria. These bacteria adhere to mannose residues expressed by CEACAM6 on host cells in a type 1 pili-dependent manner. In this study, we investigated different antagonists of FimH, the adhesin of type 1 pili, for their ability to block AIEC adhesion to intestinal epithelial cells (IEC). Monovalent and multivalent derivatives of n-heptyl α-D-mannoside (HM), a nanomolar antagonist of FimH, were tested in vitro in IEC infected with the AIEC LF82 strain and in vivo by oral administration to CEACAM6-expressing mice infected with LF82 bacteria. In vitro, multivalent derivatives were more potent than the monovalent derivatives, with a gain of efficacy superior to their valencies, probably owing to their ability to form bacterial aggregates. Of note, HM and the multi-HM glycoconjugates exhibited lower efficacy in vivo in decreasing LF82 gut colonization. Interestingly, HM analogues functionalized with an isopropylamide (1A-HM) or β-cyclodextrin pharmacophore at the end of the heptyl tail (1CD-HM) exerted beneficial effects in vivo. These two compounds strongly decreased the amount of LF82 bacteria in the feces of mice and that of bacteria associated with the gut mucosa when administered orally at a dose of 10 mg/kg of body weight after infection. Importantly, signs of colitis and intestinal inflammation induced by LF82 infection were also prevented. These results highlight the potential of the antiadhesive compounds to treat CD patients abnormally colonized by AIEC bacteria and point to an alternative to the current approach focusing on blocking proinflammatory mediators.

IMPORTANCE Current treatments for Crohn’s disease (CD), including immunosuppressive agents, anti-tumor necrosis factor alpha (anti-TNF-α) and anti-integrin antibodies, focus on the symptoms but not on the cause of the disease. Adherent-invasive Escherichia coli (AIEC) bacteria abnormally colonize the ileal mucosa of CD patients via the interaction of the mannose-specific adhesin FimH of type 1 pili with CEACAM6 mannosylated proteins expressed on the epithelial cell surface. Thus, we decided to develop an antiadhesive strategy based on synthetic FimH antagonists specifically targeting AIEC bacteria that would decrease intestinal inflammation. Heptylmannoside (HM)-based glycoconjugates strongly inhibit AIEC adhesion to intestinal epithelial cells in vitro. The antiadhesive effect of two of these compounds of relatively simple chemical structure was also observed in vivo in AIEC-infected CEACAM6-expressing mice and was associated with a reduction in the signs of colitis. These results suggest a new therapeutic approach for CD patients colonized by AIEC bacteria, based on the development of synthetic FimH antagonists.
The FimH adhesin located at the tip of type 1 fimbriae binds to oligomannosides displayed on this glycoprotein. CEACAM6 has been shown to be overexpressed in ileal tissue from CD patients than in ileal tissue from healthy controls, and the level of expression increased after gamma interferon (IFN-γ) or TNF-α stimulation and was upregulated by AIEC themselves (6). In transgenic CEABAC10 mice expressing human CEACAMs, an in vivo model reproducing the high expression of CEACAM6 reported in CD patients, the AIEC reference strain LF82 induced the development of severe clinical symptoms of colitis in a type 1 pili-dependent manner (7, 8). Analysis of the AIEC genome revealed the presence of pathoadaptive mutations in some genes or bacterial DNA sequences that could participate in AIEC pathogenicity in a susceptible host (9, 10). Recently acquired nonsynonymous substitutions have been reported in FimH expressed by AIEC strains, conferring on them greater adhesion ability (11).

Therapeutic strategies to impair AIEC adhesion to the gut mucosa, based on the development of FimH antagonists, should be considered for CD treatment. Synthetic mannosides have been developed for the treatment of urinary tract infections, with promising antiadhesive properties (12–17). One of the most potent antagonists of the FimH adhesin is the n-heptyl α-linked mannose (HM) (18), which reduced the bacterial load in vivo in a murine cystitis model (19). Interestingly, compounds harboring multiple copies of HM exhibited stronger inhibitory properties than expected according to their valency, when assessed against a uropathogenic E. coli strain UTI89 (20, 21). Multivalent HM-based polymers of high valencies also exhibited excellent antiadhesive potencies against AIEC bacteria in vitro and ex vivo (22). This multivalency effect could be explained by the potency of the compounds to form bacterial aggregates (21).

Here we investigated the ability of monovalent HM or HM grafted on multi- and polymeric structures to inhibit AIEC LF82 adhesion to IEC and to decrease LF82 colonization in the CEABAC10 transgenic mouse model. HM was selected as the FimH binding motif because of its nanomolar affinity for the adhesin and its relatively simple chemical structure compared to previously described FimH antagonists. To evaluate possible multivalent effects in vivo, we selected our previously reported β-cyclodextrin-based 7-valent HM (named 7CD-HM [23]) and the polymeric 188-valent HM (named 188P-HM [22]) for the study. To accurately assess the potential effect of multivalency, 1CD-HM and 1A-HM, the two monovalent analogues of 7CD-HM and 188P-HM, were included in the assay. We demonstrated that HM derivatives are effective in inhibiting LF82 adhesion and that multivalency improved these inhibition properties in vitro. Interestingly, the monovalent mannosides 1A-HM and 1CD-HM exerted beneficial antiadhesive effects in vivo in the CEABAC10 mouse model, and AIEC elimination of the gut was accompanied by a decrease in intestinal inflammation. To eliminate AIEC bacteria from the gut and to decrease and/or prevent intestinal inflammation, optimized mannosides selected from this study represent promising lead candidates for the development of an antiadhesive strategy in CD patients.

**RESULTS**

**Monovalent heptamannosides and multivalent heptamannosides exhibited strong antiadhesive effects on AIEC LF82 adhesion to intestinal epithelial cells.** HM, the monovalent derivative named 1A-HM, the β-cyclodextrin grafted with a single copy of HM named 1CD-HM and two multivalent derivatives named 7CD-HM, a β-cyclodextrin grafted with seven copies of HM, and 188P-HM, a polymethacrylamide having (on average) 188 pendant HM ligands, were assessed for their ability to inhibit AIEC LF82 adhesion to IEC T84 cells, expressing a high level of CEACAM6. In the preincubation protocol to estimate their antiadhesive potential in a prophylactic approach, the mannosides and LF82 bacteria were incubated together, before bacterial infection of T84 cells. In the postincubation experiment, the cells were infected with LF82 bacteria for 3 h, and then the inhibitors were added. Results are expressed in percentages of associated bacteria to the cells. The values in both panels are means plus SEM (error bars) of four to six experiments. Values that are significantly different from the control value (100%) are indicated by asterisks as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. LF82 infection in the absence of treatment was normalized at 100%.

![FIG 1 Effects of the monovalent and multivalent HM-based glyocompounds on the ability of the LF82 strain to adhere to intestinal epithelial cells T84. Cells were infected at a multiplicity of infection of 10 bacteria per cell for a 3-h period. Monovalent compounds HM, 1A-HM, and 1CD-HM and multivalent compounds 7CD-HM and 188P-HM were tested at concentrations ranging from 0.001 to 100 μM. (A) Preincubation experiment. The inhibitors were coincubated with AIEC LF82 strain 1 h before bacterial infection of T84 cells. (B) Postincubation experiment. The cells were infected with LF82 bacteria for 3 h, and then the inhibitors were added. Results are expressed in percentages of associated bacteria to the cells. The values in both panels are means plus SEM (error bars) of four to six experiments. Values that are significantly different from the control value (100%) are indicated by asterisks as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001. LF82 infection in the absence of treatment was normalized at 100%.
sion of bacteria to their host receptor from the concentration of 10 μM. A similar decrease in bacterial adhesion was obtained with the monovalent -cyclodextrin-based compound 1CD-HM at the higher concentration of 100 μM. Thus, 1CD-HM was 10-fold less potent in inhibiting bacterial adhesion than HM and 1A-HM. We next determined the factor, a value frequently used to quantify a multivalent effect in glycocluster-lectin interactions. This value can be directly obtained by dividing the HM ligand concentration in solution from the multivalent structure of interest by the concentration of the monovalent HM reference required to give a similar inhibitory effect. As multivalent 7CD-HM and polyvalent 188P-HM were tested at equivalent molarity (Fig. 1), the valencies had to be corrected by a factor of 7 for 7CD-HM and by 188 for 188P-HM to determine the factor. Multivalent derivative 7CD-HM significantly decreased AIEC LF82 adhesion at a concentration 100-fold lower than that of the monovalent analogue 1CD-HM, in the pre- and postincubation protocols, corresponding to a factor of 14. Finally, polymer 188P-HM decreased bacterial adhesion down to 22% at the very low concentration of 1 nM in the preincubation assay, corresponding to a gain of 10,000-fold in comparison with the residual adhesion of 35.3% in the presence of a 10 μM concentration of 1A-HM compound ( factor of 53). This multivalent inhibitory effect was also observed in postincubation experiments, since 188P-HM was able to detach 80% of the adherent bacteria at a concentration of 100 nM. In contrast, a 1,000-fold-higher concentration of 1A-HM is required to obtain a similar effect ( factor of 5). Thus, these results confirmed that clustering HM in multiple copies to a synthetic scaffold significantly enhances the in vitro antiadhesive potential of the FimH antagonists.

HM failed to improve the symptoms of colitis in CEACAM6-expressing mice infected with LF82 bacteria. To mimic a curative treatment, effect of the reference FimH antagonist HM was evaluated in vivo in CEABAC10 transgenic mice by oral administration 2 h and 18 h after LF82 infection of mice at a dose of 10 mg/kg. The body weight and disease activity index (DAI) score of mice treated with HM were similar to those of the nontreated infected mice (Fig. 2A and B). LF82 bacterial clearance from the gastrointestinal tracts of mice was investigated by enumerating bacteria in the feces. In this experimental condition, HM treatment failed to
prevented AIEC LF82 colonization (Fig. 2C). At day 4 postinfection, the colonos were removed from the mice to assess mucosal inflammation and damage. HM treatment did not modulate the myeloperoxidase (MPO) concentration in the intestinal mucosa, did not ameliorate the histological score, and did not decrease production of proinflammatory cytokines (TNF-α and interleukin 1β [IL-1β]) in comparison with nontreated mice, which means that intestinal inflammation was not prevented by the mannoside administration (Fig. 2D to G). The absence of an effect of HM on the symptoms of colitis can be correlated with the lack of antiadhesive effect of this compound in vivo. Thus, we decided to investigate the ability of monovalent and multivalent HM analogues to prevent AIEC colonization in vivo.

**Monovalent heptylmannosides 1A-HM and 1CD-HM exhibit strong antiadhesive properties in LF82-infected CEABAC10 mice.** The bacterial antiadhesive potential of the monovalent mannoside 1A-HM, of the HM-grafted cyclodextrins 1CD-HM and 7CD-HM, and of the HM-grafted polymer 188P-HM, was assessed in AIEC LF82-infected CEABAC10 mice, according to an experimental design similar to that previously used for HM treatment. The monovalent derivatives 1A-HM and 1CD-HM were particularly effective in eliminating AIEC bacteria from the gut, as quantified in the feces and in the ileal and colonic tissues (Fig. 3). Indeed, a significant decrease (around 3 log units) in the amount of LF82 bacteria in the feces was observed at 4 days postinfection in groups of mice treated with the two monovalent compounds 1A-HM and 1CD-HM (Fig. 3A). In these two groups, the number of bacteria associated with the intestinal mucosa was below the detection level, indicating that 1A-HM and 1CD-HM were able to effectively eradicate bacteria from the gut at day 4 postinfection (Fig. 3B and C). 188P-HM treatment also decreased the bacterial load in the feces, but the antiadhesive properties of the polymer were less effective against AIEC LF82 bacteria associated with the intestinal tissues. Finally, no effect was observed on LF82 colonization with the heptavalent cyclodextrin 7CD-HM. Although multivalency gave a strong advantage to HM-based ligands in vitro, in vivo experiments revealed that multivalent compounds were less potent than their monovalent reference compounds in eliminating LF82 bacteria from the guts of CEABAC10 mice in these experimental conditions.

**Synthetic heptylmannosides decreased the severity of colitis in LF82-infected CEABAC10 mice.** The effect of the oral treatment of LF82-infected CEABAC10 mice with the three glycoconjugates showing in vitro antiadhesive properties (1A-HM, 1CD-HM, and 188P-HM) was then assessed on the symptoms of colitis. The DAI scores indicated that the three HM conjugates tested were effective in decreasing the signs of colitis from day 3 postinfection (Fig. 4A). These mannosides decreased neutrophil infiltration in the colon, as demonstrated by MPO quantification in the colonic tissues at day 4 postinfection (Fig. 4B). With regard to proinflammatory cytokines, all compounds succeeded in inhibiting TNF-α secretion induced by AIEC LF82 bacterial infection, with 1A-HM treatment being the most potent (Fig. 4C).

**DISCUSSION**

Mannose-binding type 1 pili are crucial virulence factors for the establishment of AIEC colonization in the guts of patients with CD (23). To select molecules with the best antiadhesive properties, we have used two complementary approaches, in vitro assay of AIEC LF82 infection using IEC and in vivo LF82 infection in CEABAC10 transgenic mice. This method led to the characterization of two candidates of interest, 1A-HM and 1CD-HM, for an antiadhesive therapy in CD patients abnormally colonized by AIEC bacteria.

To date, HM is one of the best monomeric mannose-based inhibitors developed against FimH and has a nanomolar dissociation equilibrium constant for this target (18). The heptyl chain of HM strongly enhances FimH affinity compared to L-mannose, likely due to hydrophobic interactions with two tyrosines and one isoleucine at the entrance of the FimH binding site, the so-called...
tyrosine gate (18). HM was shown to prevent uropathogenic Escherichia coli adhesion to human bladder cells in culture at submicromolar concentrations. However, high concentrations (5 mM) of this compound were needed to reduce in vivo adhesion of these bacteria 10-fold in a model of murine cystitis (19). In the present study, we showed that HM effectively prevented AIEC LF82 adhesion and detached adherent LF82 bacteria from T84 cells at low concentrations of 1 and 10 μM, respectively, but failed to achieve any real effect in vivo in the CEABAC10 mouse model. The lack of antiadhesive effect of HM in vivo could be due to an inadequate pharmacodistribution of the compound, which may not have access to mucosa-associated bacteria in the gut. We hypothesize that the aliphatic chain of HM produces nonspecific interaction in the gut in vivo, thereby impairing its potential beneficial effect. Indeed, alkyl compounds can be used to increase absorption of therapeutic molecules (24) and can increase paracellular permeability of monolayers of Caco-2 cells in vitro (25–27), which suggests that they compromise intestinal barrier integrity.

Hence, HM monovalent or multivalent analogues represent an interesting strategy with the shielding of the end of the 7-carbon alkyl tail either with an isopropylamide function or with a common scaffold such as a cyclodextrin or linear polymer. Capping with polar groups could prevent nonspecific hydrophobic interactions with cell membranes while retaining a similar affinity for FimH (28). Furthermore, cyclodextrins are hydrophilic molecules of high molecular weight, and it is unlikely that they are absorbed in the intestine (29). Thus, their use as the organic scaffold for antiadhesive presentation and delivery should represent an advantage for treatment at an ileal site of action, which is the preferential site of AIEC colonization in CD. High synergistic effects were observed in vitro when HM ligands were multi- or polymerized on organic scaffolds. Indeed, HM ligands in 7CD-HM and 188P-HM are more effective than the corresponding monovalent reference compounds 1CD-HM and 1A-HM to prevent and disrupt AIEC bacterial adhesion to IEC in culture. This is in full accordance with our previous in vitro and in vivo results, which showed the greater potential of the multi-HM in disrupting adhesion of the uropathogenic E. coli strain UTI89 to bladder cells (20, 21, 30). This enhancing effect could be due either to a stronger affinity of the polyvalent multi-HM for the FimH protein or to a

FIG 4 Effects of HM-based analogues (oral treatment) in CEABAC10 mice on the severity of colitis and intestinal inflammation. (A) Assessment of the DAI score on days 1, 3, and 4 postinfection (mean plus SEM). (B) MPO concentration in colonic tissues determined by an ELISA. (C) TNF-α cytokines released by proximal colon specimens measured by an ELISA. In panels B and C, each value represents the value for an individual mouse, and each horizontal bar represents the median for a group of mice (seven or eight mice in each group). Values that are significantly different are indicated by asterisks and bars as follows: *, P < 0.05; **, P < 0.01; ***, P < 0.001). NI, not infected (control).
superior avidity of these inhibitors to multiple type 1 pili on the
same bacterial cell, or it may reflect the potency of the compounds
to form bacterial aggregates, as previously shown by dynamic light
scattering and microscopy with E. coli strain UTI89 (22, 30). Bac-
terial clusters are formed by the simultaneous interaction of sev-
eral HM ligands from the compound with FimH adhesins dis-
played by different bacteria, which allows the formation of a cross-
linked network between the bacteria and ligands. Of note, monovalent cyclodextrin 1CD-HM was 10-fold less effective than
HM and 1A-HM in inhibiting LF82 adhesion. This loss of inhib-
itory activity could be explained by the lower intrinsic affinity of
1CD-HM for FimH, as previously shown by microcalorimetry
measurements (30).

The host/bacteria cross talk in host susceptibility to CD can be
mimicked using CEABAC10 transgenic mice expressing human
CEACAM6 receptor (8). In this CEABAC10 mouse model, AIEC
bacteria colonize the gut, inducing colitis in a type 1 plus-
dependent manner (7). Previously, we demonstrated that a pro-
biotic treatment, based on the use of Saccharomyces cerevisiae
yeast in a prophylactic protocol, accelerated AIEC LF82 bacterial erad-
cation from the guts of CEABAC10 mice, preventing the subse-
quent disruption of intestinal barrier function and colitis (31). As
synthetic mannosides have the ability to efficiently disrupt prees-
ablished colonization of AIEC bacteria in the gut of a mouse model
imicking CD susceptibility. Importantly, bacterial clearance led
to a decrease in the inflammatory response. Our study opens new
avenues for personalized therapies in which FimH antagonists
could be a valuable alternative for CD patients abnormally colo-
nized by AIEC bacteria.

MATERIALS AND METHODS
Synthesis of compounds HM (18), 1CD-HM and 7CD-HM (30), and
188P-HM (22) was previously described.

Chemistry. (i) Chemical synthesis of 1A-HM. 1A-HM was prepared
from the previously described 1-azidoheptyl-mannoside, compound 1
(32), by a one-pot Staudinger-amide coupling with isobutyric acid to
form acetyl-protected compound 2. Methanalysis of the acetate groups
under Zemplén conditions led to 1A-HM (Fig. 5), according to the fol-
lowing experimental protocols.

(ii) Compound 2. Compound 1 (50 mg, 0.099 mmol) and isobutyric
acid (17 μL, 0.178 mmol, 1.8 equivalents), were combined with hydroxy-
diazotized triphenylphosphine (47 mg, 0.178 mmol, 1.8 equiva-
lents). The mixture was stirred for 10 min, followed by the
addition of triphenylphosphine (Ph3P) (47 mg, 0.178 mmol, 1.8 equiva-
lents). The mixture was stirred for 1 h at 0°C, then stirred overnight at
room temperature, diluted with water, and extracted twice with ethyl
acetate (EtOAc). The combined organic phases were washed with brine,
dried with MgSO4, filtered, and concentrated under reduced pressure.

[a]H = +71 (c = 0.81 in CHCl3); 1H nuclear magnetic resonance
(NMR) (300 MHz, CDCl3): δ = 1.12 (6H, d, J = 6.9 Hz, 2 × CH3-
isobutyric), 1.28 to 1.59 (1H, m), 2.02 (3H, s, AcO), 2.08 (3H, s, AcO),
2.13 (3H, s, AcO), 2.31 (1H, m, CH, isobutyric), 2.5 (2H, m, H-7),
3.41 (1H, m, H-1a), 3.65 (1H, m, H-1b), 3.85 (1H, m, H-1’a), 3.91 (1H,
H-1’b), 5.20 (1H, dd, J = 1.7 Hz, J = 9.6 Hz), 5.24 (1H, dd, J = 2.5 Hz,
J = 2.1 Hz, H-5), 4.10 (1H, dd, J = 12.2 Hz, J = 5.3 Hz, H-6b), 4.25 (1H,
dd, J = 12.2 Hz, J = 5.3 Hz, H-6a), 5.24 (1H, dd, J = 5.3 Hz, H-2), 5.7 (1H,
dd, J = 1.7 Hz, H-1), 5.20 (1H, dd, J = 3.3 Hz, J = 1.7 Hz, H-2), 5.24 (1H,
dd, J = 10.1 Hz, H-4).

FIG 5 Structures of HM-based mannosides assessed for antiadhesive properties against the AIEC reference strain LF82. HM, 1A-HM (this study), 1CD-HM and
7CD-HM, and 188P-HM.
sphere containing 5% CO₂ at 37°C in the culture medium recommended by the American Type Culture Collection (ATCC) and was maintained in an atmosphere of 10% CO₂ in air. AIEC LF82 bacteria at an MOI of 10 for 3 h, then monolayers were extended by washing with PBS for an additional 3-h period at final concentrations between 0.001 and 100 CFU LF82 bacteria (day 0 of the experiment), 2 h after oral administration of cimetidine at 50 mg/kg of body weight to ablate gastric secretion. Mammaries were orally administered in a volume of 0.2 mL of PBS, 2 h and 18 h after LF82 infection at a dose of 10 mg/kg.

Colonization was assessed in fresh fecal samples collected at day 4 postinfection and suspended in sterile saline solution (NaCl [9%]). Samples were diluted and plated onto LB agar containing 100 μg/mL ampicillin and 20 μg/mL erythromycin to select and enumerate LF82 bacteria. The bacterial colonization was expressed in the number of CFU per gram of feces. The severity of colitis was assessed by the disease activity index (DAI) score, which ranges from 0 (healthy) to 12 (high activity of colitis) (see Table S1 in the supplemental material).

Mice were anesthetized with isoflurane and then euthanized by cervical dislocation. LF82 bacteria associated with the colonic or ileal tissues were quantified on a 0.5-cm sample of tissue. Intestinal tissue was cut longitudinally, then washed in PBS, and homogenized in 1 mL of PBS. Samples were diluted and plated onto LB agar containing 100 μg/mL ampicillin and 20 μg/mL erythromycin. The amount of bacteria adherent to the intestine was expressed in the number of CFU per gram of tissue. For histological examinations, 4-μm sections of paraffin-embedded samples of colonic tissues were stained with hematoxylin–eosin–safranin (HES). Mucosal injuries were graded for the extent and depth of inflammation and the extent of crypt damage. A histological score was determined by veterinary histopathologists (ONIRIS, Nantes, France) in a blind manner.

**Statistical analysis.** Data are expressed as means ± standard errors of the means (SEM) or as medians. Data were compared by Student's t test analysis or nonparametric one-way analysis of variance Mann-Whitney test, when appropriate. Differences were significant when the P value was <0.05. Statistical analyses were performed with GraphPad Prism 6.0 (GraphPad Software, San Diego, CA) software package for personal computers (PC).

**SUPPLEMENTAL MATERIAL**


Table S1, DOCX file, 0.02 MB.

Table S2, DOCX file, 0.04 MB.

**ACKNOWLEDGMENTS**

We thank Abdelkrim Alloui for animal care (animal facilities, Clermont-Ferrand, France) and the CICS platform (Université Auvergne) for technical assistance with tissue preparation for microscopy analysis. We thank Laetitia Dorso from LUNAM University, Oniris, PASAP (Plateforme d’Analyses et de Services en Anatomie Pathologique), CRIP (Centre de Recherche et d’Investigation Préclinique), Nantes, France for histological examinations. We thank Jeffrey Watts for help revising the English in the manuscript.

This study was supported by the Ministère de la Recherche and de la Technologie, INSERM (UMR INSERM Université d’Auvergne U1071),


