



HAL
open science

Endocannabinoids Attenuate the Virulence of Certain Enteropathogenic Bacteria

Marwan Osman, Nicolas Papon, François-Xavier Weill

► **To cite this version:**

Marwan Osman, Nicolas Papon, François-Xavier Weill. Endocannabinoids Attenuate the Virulence of Certain Enteropathogenic Bacteria. Trends in Microbiology, 2020, 10.1016/j.tim.2020.12.008 . hal-03096638

HAL Id: hal-03096638

<https://univ-angers.hal.science/hal-03096638>

Submitted on 9 Mar 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

1
2 *Spotlight*

3
4 **Endocannabinoids attenuate the virulence**
5 **of certain enteropathogenic bacteria**

6
7 Marwan Osman ¹, Nicolas Papon ^{2,3}, François-Xavier Weill ^{4,*}

8
9 ¹ Laboratoire Microbiologie Santé et Environnement, Doctoral School of Sciences and Technology, Faculty of Public
10 Health, Lebanese University, Tripoli, Lebanon.

11 ² Host-Pathogen Interaction Study Group (GEIHP, EA 3142), UNIV Angers, UNIV Brest, Angers, France.

12 ³ Federative Structure of Research « Cellular Interactions and Therapeutic Applications », SFR 4208 ICAT, Univ Angers,
13 Angers, France.

14 ⁴ Institut Pasteur, Unité des Bactéries Pathogènes Entériques, Paris, France.

15
16
17 * Correspondence: francois-xavier.weill@pasteur.fr (F.X. Weill)

18
19
20
21 **Abstract**

22
23 **The mammalian endocannabinoid system modulates gastrointestinal**
24 **physiology and immunity via lipid hormones (endocannabinoids). Ellermann**
25 ***et al.* have recently revealed the effect of the endocannabinoid, 2-**
26 **arachidonoyl glycerol (2-AG) on intestinal infection. They show that 2-AG**
27 **directly modulates bacterial function by antagonizing the provirulence**
28 **receptor QseC, thereby protecting mice against enteric infection. The**
29 **endocannabinoid system has already been identified as a promising target in**
30 **the treatment of inflammatory bowel diseases, but these new findings raise**
31 **questions about the possible benefits of using cannabinoids to treat**
32 **intestinal bacterial infections.**

33
34
35 **Keywords:** Endocannabinoids, 2-arachidonoyl glycerol, QseC histidine kinase, enteric infections.

36

37 Enteric infections and diarrheal diseases are a pervasive global public health
38 problem [1]. Each year, millions of people suffer from severe intestinal infections,
39 many requiring hospitalization, particularly for children under the age of five years in
40 developing countries. Diarrhea causes more than 2000 infant deaths daily — a number
41 that is higher than that for AIDS, measles, and malaria combined [2].

42 The pathogenicity of certain enteric bacterial pathogens, such as enterohemorrhagic
43 *Escherichia coli* (EHEC) and its murine counterpart *Citrobacter rodentium* (CR), is due
44 to their ability to attach to the colonic epithelium and cause so-called “attaching-and-
45 effacing” (A/E) lesions, effacing the microvilli and triggering the rearrangement of the
46 cytoskeleton to form pedestal-like structures in the enterocytes [3,4]. These pathogens
47 harbor a particular pathogenicity island, the locus of enterocyte effacement (LEE),
48 which contains type III secretion system (T3SS) genes [3,4]. The LEE-encoded T3SS
49 enables A/E bacterial pathogens to inject their effectors into host cells, an essential
50 step for successful gut colonization, with epithelial cell A/E lesions leading to intestinal
51 disease [3,4]. Small molecules in the intestine, such as catecholamines, cysteine,
52 fucose, and indole, have been shown to alter the growth, metabolism, and virulence of
53 EHEC [5].

54 Two host endocannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), have
55 long been known to inhibit peristalsis, control intestinal inflammation, and reduce the
56 severity of diarrhea [6]. The endocannabinoid system is an intercellular communication
57 network of cannabinoid receptors (CB), their cognate endogenous ligands, and
58 regulatory synthesis and degradation enzymes. Novel findings have recently emerged
59 concerning the machinery for mammalian endocannabinoid signaling, which modulates
60 gastrointestinal physiology, immunity, and disease susceptibility [6]. Host
61 endocannabinoid signaling has proved highly effective for the management of many
62 medical conditions, by promoting anti-inflammatory, antinociceptive, and antisecretory
63 effects [7].

64 Ellermann and coworkers [8] recently explored the effects of these compounds on
65 host susceptibility to enteric bacterial infection. They used the murine CR infection
66 model with engineered mice lacking monoacylglycerol (*Mgll*) lipase — the principal
67 enzyme degrading 2-AG — resulting in high levels of 2-AG in various organs, including
68 the colon. Colonic levels of other biologically active lipids (monoacylglycerols and
69 arachidonic acid) capable of modulating gut inflammation were unaltered by this
70 genetic ablation of *Mgll*.

71 In response to CR infection, these *Mgll* knockout (*Mgll* KO) mice displayed (i)
72 attenuated intestinal disease, as shown by gross pathology and histology assessments,

73 (ii) lower levels of colonic expression for several proinflammatory cytokines, and (iii) a
74 lower cecal and colonic burden and less fecal shedding of CR than WT mice on day 10.
75 The use of an *MgII* inhibitor, JZL184, to increase colonic levels of 2-AG in WT mice, also
76 led to a lower CR cecal burden and attenuated cecal pathology. The protective effects
77 of JZL184 were not reversed by blockade of the 2-AG host receptor, CB2, with AM630.
78 Microbiota from *MgII* WT or KO donors transplanted into germ-free WT mice did not
79 modify the outcome of CR infection. These findings suggest that the higher levels of 2-
80 AG lead to a faster clearance of the pathogen and attenuated disease, independently
81 of the CB2 receptor or microbiota.

82 The LEE pathogenicity island is essential for the induction of intestinal disease by
83 A/E pathogens. The authors therefore performed RT-qPCR to assess the expression of
84 the LEE-encoded genes *espA* (*E. coli* secreted protein A) and *tir* (translocated intimin
85 receptor), seven days post-infection in *MgII* WT or KO mice. Both genes were less
86 strongly expressed in CR recovered from *MgII* KO mice before the significant decrease
87 in tissue colonization and fecal shedding observed on day 10. *In vitro* assays using
88 physiological concentrations of 2-AG not affecting the growth of CR or EHEC revealed
89 that 2-AG downregulated many LEE-encoded T3SS genes, including *espA*, in both
90 pathogens. Other cannabinoids, such as anandamide and 1-AG, had no such effect. In
91 epithelial lines, infection with EHEC pretreated with 2-AG resulted in significantly
92 smaller numbers of A/E lesions (pedestals), whereas the inhibition of 2-AG by a
93 diacylglycerol lipase inhibitor (tetrahydrolipstatin) led to an increase in these lesions.
94 The inhibitory effects of 2-AG were not restricted to A/E pathogens, as this molecule
95 also inhibited the T3SS-dependent virulence activity of the prevalent foodborne
96 pathogen, *Salmonella enterica* serotype Typhimurium. It remains to be determined
97 whether 2-AG or other endocannabinoids could also antagonize the virulence programs
98 of other enteric bacterial pathogens, such as *Campylobacter*, *Shigella*, or *Vibrio*
99 *cholerae*.

100 The authors then deciphered the mechanism by which 2-AG exerts its inhibitory
101 effects on A/E pathogens (Figure 1). They first showed that FadL, a long-chain fatty
102 acid transporter located in the bacterial outer membrane is necessary for pathogen
103 sensing of 2-AG, as LEE inhibition by 2-AG was abolished when an isogenic *fadL*
104 mutant of EHEC was used. They then studied the bacterial provirulence receptor,
105 QseC, an inner membrane-bound histidine kinase (found in several pathogens including
106 A/E pathogens and *Salmonella*), which autophosphorylates upon activation by the host
107 neurotransmitters epinephrine (Epi) and norepinephrine (NE). This is followed by
108 transfer of the phosphate to downstream regulators, which then activate
109 transcriptional programs for LEE-encoded virulence including T3SS, thereby

110 exacerbating A/E lesion formation and disease [9]. By assessing the transcription and
111 secretion of LEE-encoded factors, the authors showed that 2-AG inhibited LEE in the
112 parental strains, but not in CR and EHEC *qseC* mutants. In an *in vitro* liposome system,
113 QseC activation (autophosphorylation) was decreased by 2-AG and was not restored by
114 addition of its agonist, epinephrine. Finally, when infecting *MgII* WT and KO mice with
115 the CR *qseC* mutant, no difference in fecal or cecal tissue colonization relative to the
116 parental bacterial strain was observed.

117 This study shows that the host endocannabinoid 2-AG confers protection against
118 bacterial enteric pathogens by specifically antagonizing the bacterial provirulence
119 receptor, QseC. Unlike other endocannabinoids, such as anandamide, 2-AG crosses the
120 outer membrane of pathogens via the fatty acid transporter FadL, and inhibits QseC
121 autophosphorylation. This affects the enteric pathogen transcriptome by significantly
122 decreasing T3SS-dependent virulence functions. This article neatly introduces the
123 potential for broader effects of 2-AG in the modulation of bacterial function, with
124 potential impacts on host-bacterial interactions and the outcome of enteric diseases.
125 This study also paves the way for a new approach (antivirulence strategies) to combat
126 bacterial infections, particularly those caused by antimicrobial drug-resistant bacteria,
127 or EHEC, for which antibiotics may worsen the infection [5]. A QseC inhibitor, LED209,
128 has been shown to decrease virulence of several Gram-negative pathogens *in vitro* and
129 in mice [10,11]. Given that LED209 and 2-AG have different chemical structures, it
130 would be interesting to investigate the interaction of both compounds on QseC. These
131 new findings also raise questions as to whether cannabis compounds or synthetic
132 derivatives can mimic 2-AG to inhibit the induction of virulence among enteric
133 pathogens, and whether they might one day be used to treat intestinal bacterial
134 infections without antibiotics.

135

136 **References**

137

138 1. GBD 2016 Diarrhoeal Disease Collaborators (2018). Estimates of the global, regional, and national
139 morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global
140 Burden of Disease Study 2016. *Lancet Infect Dis.* 18(11), 1211-28.

141 2. GBD 2016 Causes of Death Collaborators. (2017) Global, regional, and national age-sex specific
142 mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease
143 Study 2016. *Lancet.* 390(10100), 1151-210.

144 3. Croxen, M.A. and Finlay, B.B. (2010) Molecular mechanisms of *Escherichia coli* pathogenicity. *Nat Rev*
145 *Microbiol.* 8, 26-38.

146

147 4. Lai, Y., Rosenshine, I., Leong, J.M., Frankel, G. (2013) Intimate host attachment: enteropathogenic
148 and enterohaemorrhagic *Escherichia coli*. *Cell Microbiol.* 15, 1796-1808.

149 5. Kumar, A. et al. (2019) Taming the Beast: Interplay between Gut Small Molecules and Enteric
150 Pathogens. *Infect Immun.* 87(9).

- 151 6. Cani, P.D. et al. (2016) Endocannabinoids--at the crossroads between the gut microbiota and host
152 metabolism. *Nat Rev Endocrinol.* 12(3), 133-43.
- 153 7. Gotfried, J. et al. (2020) Role of Cannabis and Its Derivatives in Gastrointestinal and Hepatic Disease.
154 *Gastroenterology.* 159(1), 62-80.
- 155 8. Ellermann, M. et al. (2020) Endocannabinoids Inhibit the Induction of Virulence in Enteric Pathogens.
156 *Cell.* 183(3), 650-665.e15.
- 157 9. Njoroge, J. and Sperandio, V. (2012) Enterohemorrhagic *Escherichia coli* virulence regulation by two
158 bacterial adrenergic kinases, QseC and QseE. *Infect Immun.* 80(2), 688-703.
- 159 10. Rasko, D.A. et al. (2008) Targeting QseC signaling and virulence for antibiotic development. *Science.*
160 321(5892),1078-80.
- 161 11. Curtis, M.M. et al. (2014) QseC inhibitors as an antivirulence approach for Gram-negative pathogens.
162 *mBio.* 5(6), e02165.

163
164
165
166

167 **Figure 1. Endocannabinoids inhibit the virulence programs of ECEH. A.** Intestinal epithelial cells
168 provide a physical and biochemical barrier segregating host tissues and commensal bacteria to maintain
169 intestinal homeostasis. The inset depicts the structure of the endocannabinoid 2-arachidonoyl glycerol (2-
170 AG), which is present at higher levels in the small intestine than in the colon. The features shown in the
171 rectangle are presented at higher magnification in panel (B) **B.** Host epinephrine and norepinephrine have
172 been shown to promote (pink arrows) the virulence of enteropathogenic bacteria by increasing the
173 expression of LEE-encoded virulence genes, including T3SS, thereby exacerbating attaching-and-effacing
174 (AE) lesion formation and disease development. This mechanism contributes to the attachment of
175 enteropathogenic bacteria to epithelial cells, whereas 2-AG directly modulates bacterial function (sky blue
176 bars), protecting against infections with these bacteria. **C.** The host endocannabinoid 2-AG confers
177 protection against bacterial enteric pathogens by specifically antagonizing the bacterial provirulence
178 receptor, QseC, promoting the inhibition of pathogen-associated T3SS. Briefly, membrane-bound histidine
179 kinases are the principal sensor proteins involved in cell signaling pathways generically referred to as
180 "two-component systems". In these systems, histidine kinases (here QseC) communicate with cognate
181 response regulators (here KdpE) via phosphorylation events (dark pink balls). Epinephrine and
182 norepinephrine have been shown to induce the phosphorylation of the conserved histidine residue (H) of
183 QseC through the catalytic activity of the H-ATPase domain (HA). The phosphoryl group is then
184 transferred to the KdpE receiver domain (R). Once phosphorylated, KdpE directly activates, through its
185 effector domain (EF), the expression of the LEE-encoded regulator (*ler*), which, in turn, upregulates the
186 expression of LEE genes. Here, Ellermann and colleagues demonstrated that 2-AG crosses the outer
187 membrane of pathogens via the fatty acid transporter FadL, and inhibits QseC autophosphorylation. This
188 affects the enteric pathogen transcriptome, significantly decreasing the expression of the LEE-encoded
189 genes and, subsequently, T3SS-dependent virulence functions.

