

Human gut symbiont *Roseburia hominis* promotes and regulates innate immunity

Patterson A., Mulder I., Travis A., Lan A., Cerf-Bensussan N., Gaboriau-Routhiau V., Garden K., Logan E., Delday M., Coutts A., Monnais E., Ferraria V., Inoue R., Grant G., Aminov R.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© 2017 Patterson, Mulder, Travis, Lan, Cerf-Bensussan, Gaboriau-Routhiau, Garden, Logan, Delday, Coutts, Monnais, Ferraria, Inoue, Grant and Aminov. Objective: *Roseburia hominis* is a flagellated gut anaerobic bacterium belonging to the Lachnospiraceae family within the Firmicutes phylum. A significant decrease of *R. hominis* colonization in the gut of ulcerative colitis patients has recently been demonstrated. In this work, we have investigated the mechanisms of *R. hominis*-host cross talk using both murine and in vitro models. Design: The complete genome sequence of *R. hominis* A2-183 was determined. C3H/HeN germ-free mice were mono-colonized with *R. hominis*, and the host-microbe interaction was studied using histology, transcriptome analyses and FACS. Further investigations were performed in vitro and using the TLR5KO and DSS-colitis murine models. Results: In the bacterium, *R. hominis*, host gut colonization upregulated genes involved in conjugation/mobilization, metabolism, motility, and chemotaxis. In the host cells, bacterial colonization upregulated genes related to antimicrobial peptides, gut barrier function, toll-like receptors (TLR) signaling, and T cell biology. CD4 + CD25 + FoxP3 + T cell numbers increased in the lamina propria of both mono-associated and conventional mice treated with *R. hominis*. Treatment with the *R. hominis* bacterium provided protection against DSS-induced colitis. The role of flagellin in host-bacterium interaction was also investigated. Conclusion: Mono-association of mice with *R. hominis* bacteria results in specific bidirectional gene expression patterns. A set of genes thought to be important for host colonization are induced in *R. hominis*, while the host cells respond by strengthening gut barrier function and enhancing Treg population expansion, possibly via TLR5-flagellin signaling. Our data reveal the immunomodulatory properties of *R. hominis* that could be useful for the control and treatment of gut inflammation.

<http://dx.doi.org/10.3389/fimmu.2017.01166>

Keywords

Flagellin, Immune tolerance, Inflammatory bowel disease, Roseburia, T lymphocytes, TLR5

References

- [1] Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, et al. Diversity of the human intestinal microbial flora. *Science* (2005) 308(5728):1635-8. doi:10.1126/science.1110591

- [2] Macpherson AJ, Hunziker L, McCoy K, Lamarre A. IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect* (2001) 3(12):1021-35. doi:10.1016/S1286-4579(01)01460-5
- [3] Macpherson AJ, Martinic MM, Harris N. The functions of mucosal T cells in containing the indigenous commensal flora of the intestine. *Cell Mol Life Sci* (2002) 59(12):2088-96. doi:10.1007/s000180200009
- [4] Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* (2005) 122(1):107-18. doi:10.1016/j.cell.2005.05.007
- [5] Macpherson AJ. IgA adaptation to the presence of commensal bacteria in the intestine. *Curr Top Microbiol Immunol* (2006) 308:117-36. doi:10.1007/3-540-30657-9_5
- [6] Chung H, Kasper DL. Microbiota-stimulated immune mechanisms to maintain gut homeostasis. *Curr Opin Immunol* (2010) 22(4):455-60. doi:10.1016/j.coi.2010.06.008
- [7] Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* (2009) 31(4):677-89. doi:10.1016/j.immuni.2009.08.020
- [8] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* (2009) 139(3):485-98. doi:10.1016/j.cell.2009.09.033
- [9] Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, et al. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. *Immunity* (2011) 34(5):794-806. doi:10.1016/j.immuni.2011.03.021
- [10] Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous *Clostridium* species. *Science* (2011) 331(6015):337-41. doi:10.1126/science.1198469
- [11] Nutsch KM, Hsieh CS. T cell tolerance and immunity to commensal bacteria. *Curr Opin Immunol* (2012) 24(4):385-91. doi:10.1016/j.coi.2012.04.009
- [12] Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, et al. The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* (2011) 332(6032):974-7. doi:10.1126/science.1206095
- [13] Frank DN, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci U S A* (2007) 104(34):13780-5. doi:10.1073/pnas.0706625104
- [14] Scanlan PD, Shanahan F, Marchesi JR. Culture-independent analysis of desulfovibrios in the human distal colon of healthy, colorectal cancer and polypectomized individuals. *FEMS Microbiol Ecol* (2009) 69(2):213-21. doi:10.1111/j.1574-6941.2009.00709.x
- [15] Kang S, Denman SE, Morrison M, Yu Z, Dore J, Leclerc M, et al. Dysbiosis of fecal microbiota in Crohn's disease patients as revealed by a custom phylogenetic microarray. *Inflamm Bowel Dis* (2010) 16(12):2034-42. doi:10.1002/ibd.21319
- [16] Machiels K, Joossens M, Sabino J, De Preter V, Arijs I, Eeckhaut V, et al. A decrease of the butyrate-producing species *Roseburia hominis* and *Faecalibacterium prausnitzii* defines dysbiosis in patients with ulcerative colitis. *Gut* (2014) 63(8):1275-83. doi:10.1136/gutjnl-2013-304833
- [17] Gevers D, Kugathasan S, Denson LA, Vazquez-Baeza Y, Van Treuren W, Ren B, et al. The treatment-naive microbiome in new-onset Crohn's disease. *Cell Host Microbe* (2014) 15(3):382-92. doi:10.1016/j.chom.2014.02.005
- [18] Hedin CR, McCarthy NE, Louis P, Farquharson FM, McCartney S, Taylor K, et al. Altered intestinal microbiota and blood T cell phenotype are shared by patients with Crohn's disease and their unaffected siblings. *Gut* (2014) 63(10):1578-86. doi:10.1136/gutjnl-2013-306226
- [19] Tilg H, Danese S. *Roseburia hominis*: a novel guilty player in ulcerative colitis pathogenesis? *Gut* (2014) 63(8):1204-5. doi:10.1136/gutjnl-2013-305799
- [20] Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, Gratadoux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn's disease patients. *Proc Natl Acad Sci U S A* (2008) 105(43):16731-6. doi:10.1073/pnas.0804812105
- [21] Travis AJ, Kelly D, Flint HJ, Aminov RI. Complete genome sequence of the human gut symbiont *Roseburia hominis*. *Genome Announc* (2015) 3(6):e1286-1215. doi:10.1128/genomeA.01286-15
- [22] Duncan SH, Aminov RI, Scott KP, Louis P, Stanton TB, Flint HJ. Proposal of *Roseburia faecis* sp. nov., *Roseburia hominis* sp. nov. and *Roseburia inulinivorans* sp. nov., based on isolates from human faeces. *Int J Syst Evol Microbiol* (2006) 56(Pt 10):2437-41. doi:10.1099/ijs.0.64098-0
- [23] Flores-Langarica A, Marshall JL, Hitchcock J, Cook C, Jobanputra J, Bobat S, et al. Systemic flagellin immunization stimulates mucosal CD103+ dendritic cells and drives Foxp3+ regulatory T cell and IgA responses in the mesenteric lymph node. *J Immunol* (2012) 189(12):5745-54. doi:10.4049/jimmunol.1202283
- [24] Zhan T, Cao C, Li L, Gu N, Civin CI, Zhan X. MIM regulates the trafficking of bone marrow cells via modulating surface expression of CXCR4. *Leukemia* (2016) 30(6):1327-34. doi:10.1038/leu.2016.39

- [25] Berg DJ, Davidson N, Kuhn R, Muller W, Menon S, Holland G, et al. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J Clin Invest* (1996) 98(4):1010-20. doi:10.1172/JCI118861
- [26] Monteleone I, Platt AM, Jaensson E, Agace WW, Mowat AM. IL-10-dependent partial refractoriness to toll-like receptor stimulation modulates gut mucosal dendritic cell function. *Eur J Immunol* (2008) 38(6):1533-47. doi:10.1002/eji.200737909
- [27] Neville BA, Sheridan PO, Harris HM, Coughlan S, Flint HJ, Duncan SH, et al. Pro-inflammatory flagellin proteins of prevalent motile commensal bacteria are variably abundant in the intestinal microbiome of elderly humans. *PLoS One* (2013) 8(7):e68919. doi:10.1371/journal.pone.0068919
- [28] Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* (2010) 464(7285):59-65. doi:10.1038/nature08821
- [29] Scott KP, Martin JC, Chassard C, Clerget M, Potrykus J, Campbell G, et al. Substrate-driven gene expression in *Roseburia inulinivorans*: importance of inducible enzymes in the utilization of inulin and starch. *Proc Natl Acad Sci U S A* (2011) 108(Suppl 1):4672-9. doi:10.1073/pnas.1000091107
- [30] Ukena SN, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, et al. Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. *PLoS One* (2007) 2(12):e1308. doi:10.1371/journal.pone.0001308
- [31] Ulluwishewa D, Anderson RC, McNabb WC, Moughan PJ, Wells JM, Roy NC. Regulation of tight junction permeability by intestinal bacteria and dietary components. *J Nutr* (2011) 141(5):769-76. doi:10.3945/jn.110.135657
- [32] Narushima S, Sugiura Y, Oshima K, Atarashi K, Hattori M, Suematsu M, et al. Characterization of the 17 strains of regulatory T cell-inducing human-derived *Clostridia*. *Gut Microbes* (2014) 5(3):333-9. doi:10.4161/gmic.28572
- [33] Aminov RI, Walker AW, Duncan SH, Harmsen HJ, Welling GW, Flint HJ. Molecular diversity, cultivation, and improved detection by fluorescent in situ hybridization of a dominant group of human gut bacteria related to *Roseburia* spp. or *Eubacterium rectale*. *Appl Environ Microbiol* (2006) 72(9):6371-6. doi:10.1128/AEM.00701-06
- [34] Van den Abbeele P, Belzer C, Goossens M, Kleerebezem M, De Vos WM, Thas O, et al. Butyrate-producing *Clostridium* cluster XIVa species specifically colonize mucins in an in vitro gut model. *ISME J* (2013) 7(5):949-61. doi:10.1038/ismej.2012.158
- [35] Juge N. Microbial adhesins to gastrointestinal mucus. *Trends Microbiol* (2012) 20:30-9. doi:10.1016/j.tim.2011.10.001
- [36] Choi YJ, Im E, Chung HK, Pothoulakis C, Rhee SH. TRIF mediates toll-like receptor 5-induced signaling in intestinal epithelial cells. *J Biol Chem* (2010) 285(48):37570-8. doi:10.1074/jbc.M110.158394
- [37] Singh V, Yeoh BS, Carvalho F, Gewirtz AT, Vijay-Kumar M. Proneness of TLR5-deficient mice to develop colitis is microbiota dependent. *Gut Microbes* (2015) 6(4):279-83. doi:10.1080/19490976.2015.1060390
- [38] Prakash T, Oshima K, Morita H, Fukuda S, Imaoka A, Kumar N, et al. Complete genome sequences of rat and mouse segmented filamentous bacteria, a potent inducer of Th17 cell differentiation. *Cell Host Microbe* (2011) 10(3):273-84. doi:10.1016/j.chom.2011.08.007
- [39] Sczesnak A, Segata N, Qin X, Gevers D, Petrosino JF, Huttenhower C, et al. The genome of Th17 cell-inducing segmented filamentous bacteria reveals extensive auxotrophy and adaptations to the intestinal environment. *Cell Host Microbe* (2011) 10(3):260-72. doi:10.1016/j.chom.2011.08.005
- [40] Yoon SI, Kurnasov O, Natarajan V, Hong M, Gudkov AV, Osterman AL, et al. Structural basis of TLR5-flagellin recognition and signaling. *Science* (2012) 335(6070):859-64. doi:10.1126/science.1215584
- [41] Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. Human CD4+ T cells express TLR5 and its ligand flagellin enhances the suppressive capacity and expression of FOXP3 in CD4+CD25+ T regulatory cells. *J Immunol* (2005) 175(12):8051-9. doi:10.4049/jimmunol.175.12.8051
- [42] Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, et al. Interleukin 23 production by intestinal CD103(+)CD11b(+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. *Immunity* (2012) 36(2):276-87. doi:10.1016/j.immuni.2011.12.011
- [43] Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science* (2013) 341(6145):569-73. doi:10.1126/science.1241165
- [44] Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* (2013) 504(7480):446-50. doi:10.1038/nature12721