Interleukin 15 (IL-15) promotes intestinal dysbiosis with butyrate deficiency associated with increases susceptibility to colitis

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ABSTRACT

Dysbiosis resulting in gut-microbiome alterations with reduced butyrate production are thought to disrupt intestinal immune homeostasis and promote complex immune disorders. However, whether and how dysbiosis develops before the onset of overt pathology remains poorly defined. Interleukin-15 (IL-15) is upregulated in distressed tissue and its overexpression is thought to predispose susceptible individuals to and have a role in the pathogenesis of celiac disease and inflammatory bowel disease (IBD). Although the immunological roles of IL-15 have been largely studied, its potential impact on the microbiota remains unexplored. Analysis of 16S ribosomal RNA-based inventories of bacterial communities in mice overexpressing IL-15 in the intestinal epithelium (villin-IL-15 transgenic (v-IL-15tg) mice) shows distinct changes in the composition of the intestinal bacteria. Although some alterations are specific to individual intestinal compartments, others are found across the ileum, cecum and feces. In particular, IL-15 overexpression restructures the composition of the microbiota with a decrease in butyrate-producing bacteria that is associated with a reduction in luminal butyrate levels across all intestinal compartments. Fecal microbiota transplant experiments of wild-type and v-IL-15tg microbiota into germ-free mice further indicate that diminishing butyrate concentration observed in the intestinal lumen of v-IL-15tg mice is the result of intrinsic alterations in the microbiota induced by IL-15. This reconfiguration of the microbiota is associated with increased susceptibility to dextran sodium sulfate-induced colitis. Altogether, this study reveals that IL-15 impacts butyrate-producing bacteria and lowers butyrate levels in the absence of overt pathology, which represent events that precede and promote intestinal inflammatory diseases.

Overexpression of IL-15 in the intestinal epithelium promotes colitis

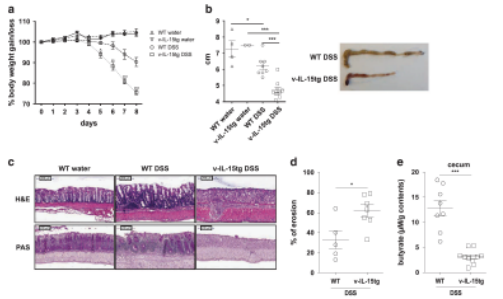

Fig. 1: WT littermate control and v-IL-15tg mice were either control treated or received 2% DSS into drinking water for 7 days and were then switched to water until mice were killed at day 8. (a) Time course of percent body weight changes. (b) Analysis of colon length at day 8 (left panel) and representative picture of colon (right panel) from DSS-treated WT littermate control and v-IL-15tg mice. (c) Histology (hematoxylin and eosin-stained (H&E), upper panel; periodic acid–Schiff (PAS) staining lower panel) of colon from control and DSS-treated mice. (d) Areas of erosion were quantified by a gastrointestinal pathologist and displayed as percentage of erosion relative to the total mucosal surface area. Data are from two indep. exp. (WT (n = 5), v-IL-15tg (n = 7)). (e) Levels of butyrate were measured by GC-MS in cecal contents of DSS-treated v-IL-15tg mice or WT littermate controls and are displayed as μM per g luminal contents. (a–c) (WT water (n = 4), v-IL-15tg water (n = 2), WT DSS (n = 8), v-IL-15tg DSS (n = 10). (a–d) Data are from two indep. Exp. Error bars, mean ± s.e.m.; *P<0.05; **P<0.01; ***P<0.001.

RESULTS

Dysregulated expression of IL-15 in the epithelium induces dysbiosis across intestinal compartments

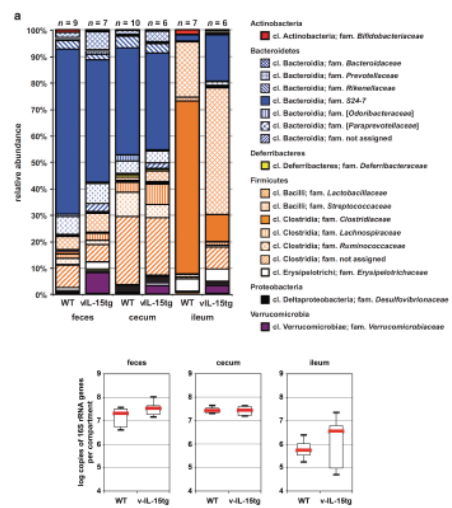

Fig. 2: Dysregulated expression of IL-15 in the epithelium induces dysbiosis across intestinal compartments. (a) Bacterial community composition is organized by site (feces, cecum and ileum) and mouse genotype (side-by-side columns). The phyla identified in the samples are according to the GreenGenes taxonomy. The number of samples (n) per intestinal compartment-genotype is noted at the top. Data are from two independent experiments; WT: feces (n = 9); cecum (n = 10), ileum (n = 7); v-IL-15tg: feces (n = 7), cecum (n = 6), ileum (n = 6). (lower panel) Bacterial load in the feces or gut compartment contents of WT littermate and v-IL-15tg mice. Quantitative PCR (qPCR) targeting the 16S rRNA-encoding gene was used to measure bacterial load in the feces, cecum, and ileum of each animal. No statistical difference was found between mouse genotype.

Butyrate treatment significantly reduces DSS-mediated intestinal inflammation in v-IL-15tg mice

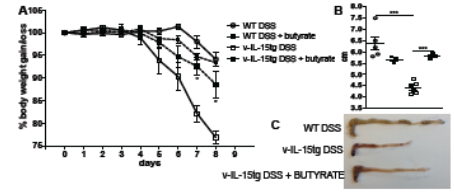

Fig. 6: Butyrate treatment significantly reduces DSS-mediated intestinal inflammation in v-IL-15tg mice. Mice received regular water or DSS water and either PBS or butyrate (100mM) i.p. daily. (a) Weight was recorded daily (b+c) colon length. Data are from two independent experiments.

Analysis of OTUs reveals that IL-15 overexpression influences the abundance of butyrate-producing bacteria

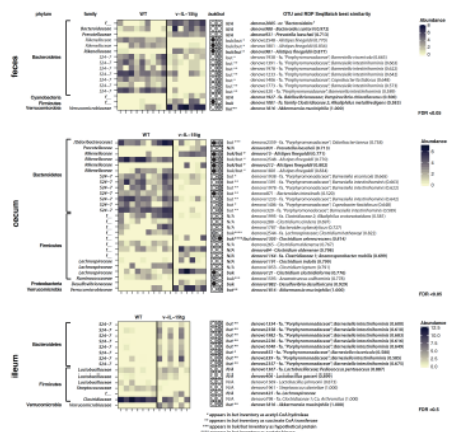

Fig. 3: The figure depicts significant log2-fold changes in the rel. abundance of specific OTUs as heatmaps. On the left are the phylum and family classifications of the OTUs and on the right are the putative genus/species designation based on best similarity matches from the RDP using SeqMatch (numbers in parentheses indicate SeqMatch score (Sab)). OTUs and SeqMatch genus/species assignments in bold italics indicate that the OTU had an increased log2-fold change in the v-IL-15tg mice. The panel to the right of the heatmaps indicate whether butyrate kinase (buk) or butyryl-CoA:acetate CoA-transferase (but) encoding genes have been detected in the genomes of the SeqMatch genus/species assignments based on the buk and but entries in the RDP FunGene Functional Gene Pipeline and Repository. Closed black circle, presence of a buk or but gene in genome: closed gray circle, alternate annotation (defined bottom). Open circle, no match.

IL- 15-induced microbial alterations in GF v- IL-15tg mice 21 days post FMT

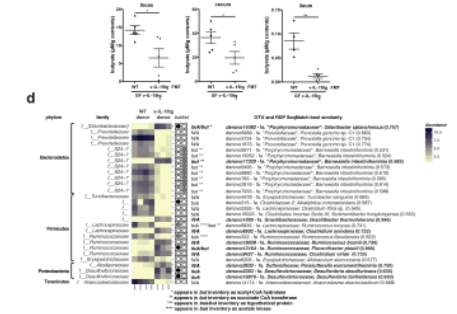

Fig. 4: GF v-IL-15tg recipient mice received SPF WT or v-IL-15tg FMT. Samples were collected 21 days post FMT (a) Graph shows concentrations of butyrate from GF v-IL-15tg recipient mice by GC-MS; (d) The figure depicts significant log2-fold changes in the rel. abundance of specific OTUs between recipients based on donor host genotype in feces.

IL-15-induced microbial alterations are associated with a significant reduction in both, butyrate concentration and butyrate-producing bacteria

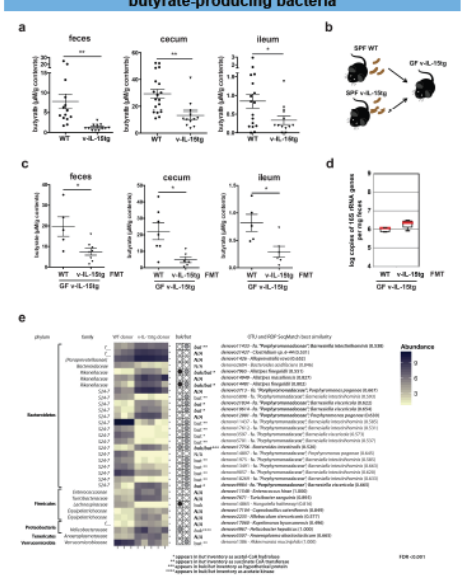

Fig. 5: (a) Levels of butyrate were measured by GC-MS in feces or luminal contents (cecum and ileum) of v-IL-15tg mice or WT littermates. Data are from three indep. Exp. (n=7-14). (b) GF v-IL-15tg mice received fecal microbiota of either SPF WT littermate or v-IL-15tg mice. Panel depicts experimental design for fecal microbial transplantation (FMT) experiment. (c) Graph shows measured concentrations of butyrate in feces or luminal contents from recipient mice by GC-MS at day 7 post FMT (GF v-IL-15tg mice transplanted with WT littermate feces, n=5; GF v-IL-15tg mice transplanted with v-IL-15tg feces, n=8). (d) Quantitative PCR (qPCR) targeting the 16S rRNA-encoding gene was used to measure bacterial load in the feces of mice receiving FMT. No statistical difference was found between both groups. (e) The heatmap depicts significant log2 fold changes in the relative abundance of specific OTUs between recipients based on donor host genotype (WT or v-IL-15tg) in feces (for detailed description of statistics see Figure 4). As in Figure 4, phylum and family classifications of the OTUs are on the left and on the right are the putative genus/species designation based on best similarity matches from the RDP using SeqMatch. OTUs and SeqMatch genus/species assignments in bold italics indicate that the OTU had an increased log2-fold change in the recipient mice transplanted with feces from v-IL-15tg mice; GF v-IL-15tg mice transplanted with WT littermate feces, n=3; GF v-IL-15tg mice transplanted with v-IL-15tg feces, n=5. One representative analysis out of two indep. experiments is shown.

Reference

Marlies Meisel, Toufic Mayassi, Hannah Fehlner-Peach, Jason C. Koval, Sarah L. O'Brien, Reinhard Hinterleitner, Kathryn Lesko, Sangman Kim, Romain Bouziat, Li Chen, Christopher R. Weber, Sarkis K. Mazmanian, Bana Jabri and Dionysios A. Antonopoulos. *Interleukin-15 promotes intestinal dysbiosis with butyrate deficiency associated with increased susceptibility to colitis.* **International Society for Microbial Ecology Journal *ISME 2016, Sept 20***, doi: 10.1038/ismej.2016.114

This study was supported by the *Erwin Schrödinger* Stipendium J3418-B19 FWF