LETTER

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Precision editing of the gut microbiota ameliorates colitis

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Inflammatory diseases of the gastrointestinal tract are frequently associated with dysbiosis[1–8], characterized by changes in gut microbial communities that include an expansion of facultative anaerobic bacteria of the Enterobacteriaceae family (phylum Proteobacteria). Here we show that a dysbiotic expansion of Enterobacteriaceae during gut inflammation could be prevented by tungstate treatment, which selectively inhibited molybdenum-cofactor-dependent microbial respiratory pathways that are operational only during episodes of inflammation. By contrast, we found that tungstate treatment caused minimal changes in the microbiota composition under homeostatic conditions. Notably, tungstate-mediated microbiota editing reduced the severity of intestinal inflammation in mouse models of colitis. We conclude that precision editing of the microbiota composition by tungstate treatment ameliorates the adverse effects of dysbiosis in the inflamed gut.

In genetically susceptible rodents, a dysbiotic microbiota is vertically transmissible; the affected offspring are more likely to develop intestinal inflammation[3,9,10], suggesting that components of the microbiota can instigate host responses in a disease-prone setting. The close association between mucosal inflammation and gut-microbiota dysbiosis poses a challenge to establishing causality between these two events. Using metagenomic sequencing, we recently identified molybdenum-cofactor-dependent metabolic pathways as a signature of inflammation-associated dysbiosis[11]. Molybdenum-cofactor-dependent anaerobic respiratory enzymes and formate dehydrogenases contribute independently to the bloom of model Enterobacteriaceae such as *Escherichia coli*[11,12]. We reasoned that identification of molybdenum-cofactor-dependent processes as drivers of dysbiosis would allow us to devise a strategy to manipulate microbiota metabolism and composition during gut inflammation. Selective editing of the microbiota would enable investigation of potential consequences of dysbiosis, such as exacerbation of mucosal inflammation.

Tungsten (W) can replace molybdenum in the molybdopterin cofactor, rendering this cofactor inactive in Enterobacteriaceae[13]. Supplementation of growth media with sodium tungstate does not have a general effect on growth of Enterobacteriaceae under standard aerobic laboratory conditions, but it abolishes anaerobic nitrate-reductase activity[13] in commensal *E. coli*, *Proteus* spp., and *Enterobacter cloacae* (Fig. 1a–c, Extended Data Fig. 1a). To test whether tungstate supplementation could negate the fitness advantage conferred by anaerobic respiration and formate oxidation *in vitro*, we analysed anaerobic growth of wild-type *E. coli* strains (K-12 and Nissle 1917) and isogenic molybdenum-cofactor biosynthesis-deficient mutants (Δ*moaA*) in mucin broth supplemented with sodium tungstate (Fig. 1d, e, Extended Data Fig. 1b). In the presence of an electron acceptor such as nitrate, or an electron donor such as formate, the wild-type strains outcompeted the isogenic *moaA* mutants, but this fitness advantage was abrogated by the addition of tungstate (Fig. 1d, e, Extended Data Fig. 1b).

To investigate whether tungstate could inhibit molybdenum-cofactor-dependent processes in the mammalian gut, we used a mouse model of chemically induced colitis (dextran sulfate sodium (DSS)-induced colitis) in conjunction with experimentally introduced *E. coli* indicator strains. Groups of DSS- and mock-treated C57BL/6 mice were inoculated orally with an equal mixture of the *E. coli* K-12 wild-type strain and the Δ*moaA* mutant after the onset of inflammation. Colonization of the caecum and colon lumen was assessed five days after inoculation (Fig. 1f, g, Extended Data Fig. 2a). Prior to inoculation with *E. coli* K-12, we were unable to isolate any endogenous Enterobacteriaceae family members from these animals. Consistent with previous results[12], the K-12 wild-type strain outcompeted the Δ*moaA* mutant in the caecal and colonic content of DSS-treated mice (Fig. 1f). Administration of tungstate in the DSS-induced-colitis model abrogated the fitness advantage conferred by molybdenum-cofactor-dependent enzymes (Fig. 1f) and decreased overall numbers of *E. coli* K-12 in the gut lumen by several orders of magnitude (Fig. 1g). Similar observations were made using the human *E. coli* strain Nissle 1917 (Fig. 1h, Extended Data Fig. 3a, b) and a mouse *E. cloacae* strain (Fig. 1i, Extended Data Fig. 4a, b). Furthermore, the adherent-invasive *E. coli* (AIEC) strain NRG857c, originally isolated from a patient with inflammatory bowel disease, outcompeted the isogenic Δ*moaA* mutant in the intestinal content of DSS-treated mice (Fig. 1j). Tungstate administration negated the fitness advantage conferred by molybdenum-cofactor biosynthesis and reduced NRG857c colonization (Fig. 1j, Extended Data Figs 1c, 4c). Similarly, tungstate treatment decreased intestinal colonization by the mouse AIEC strain NC101 in a piroxicam-accelerated *Il10*$^{-/-}$ mouse model of colitis (Fig. 1k). Taken together, these experiments based on bacterial model organisms indicate that orally administered tungstate inhibits the molybdenum-cofactor-dependent bloom of Enterobacteriaceae in mouse models of colitis.

Next, we investigated the effect of tungstate treatment on the microbiota. C57BL/6 mice that naturally harboured endogenous Enterobacteriaceae were treated with DSS, DSS plus tungstate, tungstate alone or mock treatment. After nine days, DNA extracted from the caecal content was analysed by shotgun-metagenomic sequencing and 16S profiling (Fig. 2, Extended Data Fig. 2b). Intestinal inflammation was accompanied by changes in the predicted coding capacity of

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