Gut microbiota-derived short-chain fatty acids regulate IL-17 production by mouse and human intestinal $\gamma\delta$ T cells

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SUMMARY

Gut interleukin-17A (IL-17)-producing γδ T cells are tissue-resident cells that are involved in both host defense and regulation of intestinal inflammation. However, factors that regulate their functions are poorly understood.

In this study, we find that the gut microbiota represses IL-17 production by cecal γδ T cells. Treatment with vancomycin, a Gram-positive bacterium-targeting antibiotic, leads to decreased production of short-chain fatty acids (SCFAs) by the gut microbiota. Our data reveal that these microbiota-derived metabolites, particularly propionate, reduce IL-17 and IL-22 production by intestinal γδ T cells. Propionate acts directly on γδ T cells to inhibit their production of IL-17 in a histone deacetylase-dependent manner.

Moreover, the production of IL-17 by human IL-17-producing γδ T cells from patients with inflammatory bowel disease (IBD) is regulated by propionate.

These data contribute to a better understanding of the mechanisms regulating gut γδ T cell functions and offer therapeutic perspectives of these cells.

RESULTS

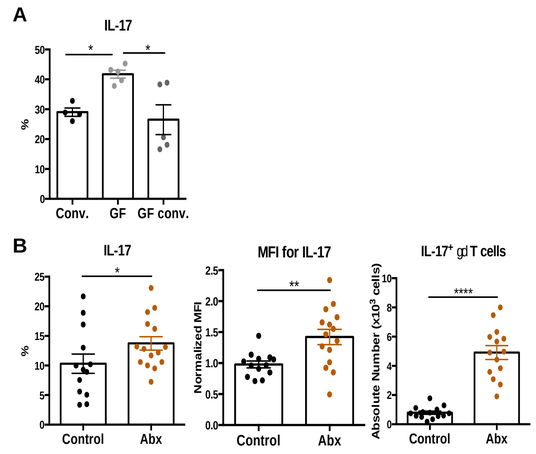


Figure 1. The gut microbiota represses IL-17 production by cecal γδ T cells.

(A) Intracellular analysis of gated γδ T cells from cecum obtained from untreated conventional mice (Conv.), germ-free mice (GF) and germ-free mice colonized with conventional mouse microbiota for 4 weeks (GF conv.); IL-17 production by cecal γδ T cells.

(B) Intracellular analysis of gated γδ T cells from cecum obtained from untreated mice (Control) and broad spectrum antibiotics-treated mice (Abx); IL-17 production (left) and normalized median fluorescence intensity (MFI) for IL-17 (middle) in gated γδ T cells and absolute number of IL-17⁺ γδ T cells from cecum.

In each case, cells were stimulated with PMA + Ionomycin + IL-1β + IL-23 for 3h.

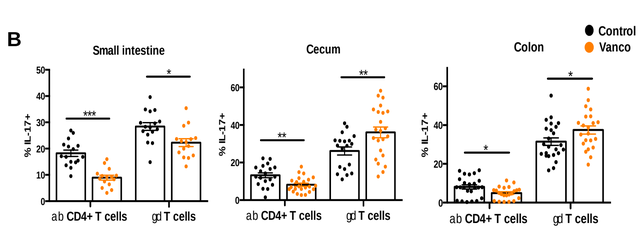


Figure 2. The gut microbiota regulates differentially IL-17 production by γδ T cells from small intestine, cecum and colon.

(A) Intracellular analysis of IL-17 expression by gated γδ T cells from cecum obtained from untreated mice (Control) and vancomycin (Vanco)-treated mice; Absolute number of IL-17⁺ γδ T cells (left) and normalized median fluorescence intensity (MFI) for IL-17 (right) in gated γδ T cells.

(B) Intracellular analysis of IL-17 expression by gated αβ CD4⁺ T cells and γδ T cells from small intestine (left), cecum (middle) and colon (right) obtained from untreated mice (Control, black) and vancomycin-treated mice (Vanco, orange).

In each case, cells were stimulated with PMA + Ionomycin + IL-1β + IL-23 for 3h.

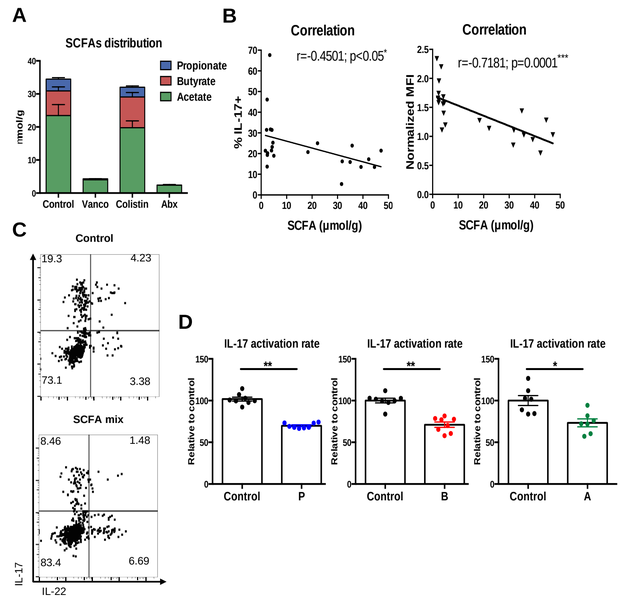


Figure 3. SCFAs inhibit *ex vivo* IL-17 production by γδ T cells.

(A) Concentrations of propionate (blue), butyrate (red) and acetate (green) per gram of cecal content obtained from untreated mice (Control) and mice treated 3 weeks with antibiotics (vancomycin, colistin or 4 Abx).

(B) Spearman correlation between SCFAs and IL-17 production (percentage, left or median fluorescence intensity, right) in gated γδ T cells from cecum obtained as described in A.

(C) Total cecal cells were cultured for 4h with a mix of propionate, butyrate and acetate (SCFA mix) or PBS (Control), activated with PMA + Ionomycin + IL-1β + IL-23 for the last 3h and stained for intracellular IL-17 and IL-22. Representative plots on gated γδ T cells. For all plots, numbers indicate percent of cells in relevant quadrant.

(D) Total cecal cells were cultured for 4h with PBS (Control), or propionate (P), butyrate (B) or acetate (A), activated as in (C) and stained for intracellular IL-17.

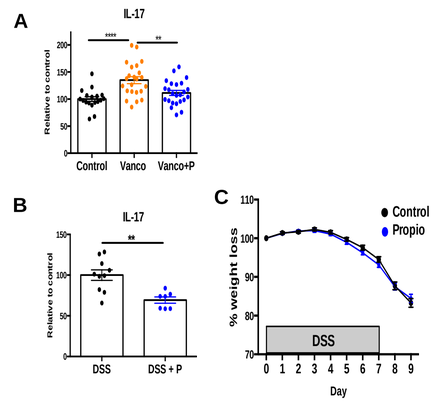


Figure 4. SCFAs inhibit *in vivo* IL-17 production by γδ T cells.

(A) Percentage of IL-17⁺ γδ T cells from mice treated with PBS (Control, black), vancomycin (Vanco) or vancomycin + propionate (Vanco+P);

(B) DSS-exposed mice were treated with PBS (Control, black) or propionate (Propio, blue). Production of IL-17 by cecal γδ T cells on day 9 after initiation of DSS treatment.

(C) Weight loss of DSS-exposed mice were treated with PBS (Control, black) or propionate (Propio, blue).

In each case, cells were stimulated with PMA + Ionomycin + IL-1β + IL-23 for 3h.

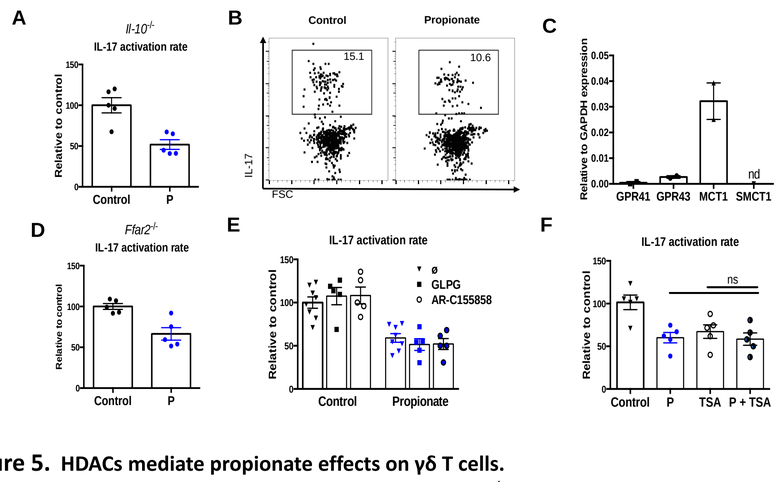


Figure 5. HDACs mediate propionate effects on γδ T cells.

(A) IL-17 production by cecal γδ T cells obtained from Il10$^{-/-}$ mice treated 4h with PBS (Control) or propionate, and stimulated with PMA + Ionomycin + IL-1β + IL-23 for the last 3h.

(B) Sorted γδ T cells from peripheral lymph nodes were cultured for 18h with PBS (Control) or propionate, and stimulated as in A. Representative plots on gated γδ T cells (n = 4). For all plots, number indicates percent of IL-17⁺ cells.

(C) GPR41, GPR43, MCT1 and SMCT1 mRNA quantification by qPCR in sorted γδ T cells from peripheral lymph nodes (nd = not detected).

(D) IL-17 production by cecal γδ T cells obtained from Ffar2$^{-/-}$ mice treated 4h with PBS (Control) or propionate, and stimulated as in A.

(E and F) IL-17 activation rate relative to control on gated γδ T cells from cecum cultured for 4h with propionate (P) and/or GLPG, AR-C155858 (E) or TSA (F), and stimulated as in A.

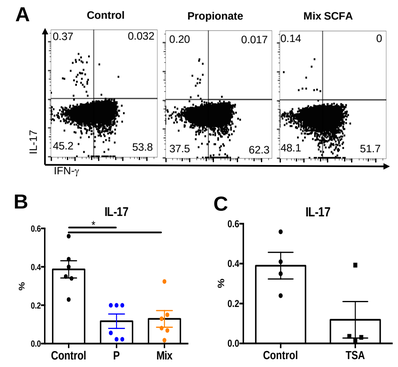


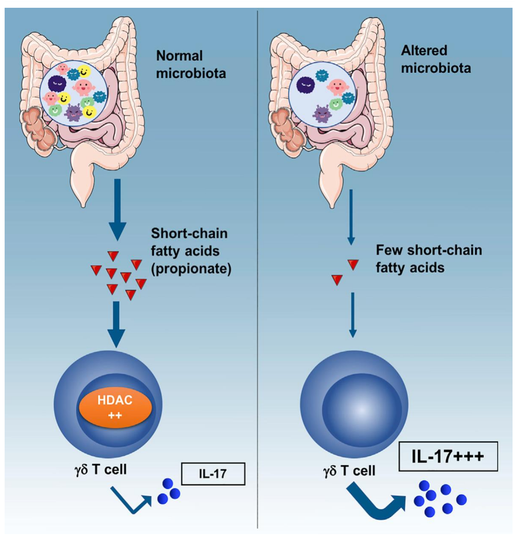
Figure 6. SCFAs inhibit IL-17 production by human γδ T cells.

(A) Intracellular analysis of IL-17 expression by gated γδ T cells from PBMC of IBD patients, untreated (Control), or treated 18h with propionate (P) or a mix of SCFAs (Mix), and stimulated with PMA + Ionomycin for the last 5h. For all plots, numbers indicate percent of cells in relevant quadrant.

(B) Collective analysis of IL-17 expression by gated γδ T cells from IBD patients, treated as described in A; data are expressed as box plots.

(C) IL-17 activation production by gated γδ T cells from IBD patients, treated 18h with PBS (Control) or TSA and activated as in A.

GRAPHICAL ABSTRACT



CONCLUSION

- **The gut microbiota represses IL-17 production by mouse cecal γδ T cells.**
- **Short-chain fatty acids particularly propionate repress IL-17- and IL-22-producing mouse γδ T cells.**
- **Propionate acts on mouse γδ T cell functionalities by inhibiting histone deacetylase.**
- **Propionate represses IL-17 production by human γδ T cells from patients with IBD.**

PUBLICATION

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