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Adenomatous polyps are driven by microbe-instigated focal inflammation and are controlled by IL-10 producing T-cells

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Abstract

Interleukin-10 (IL-10) is elevated in cancer and is thought to contribute to immune tolerance and tumor growth. Defying these expectations, the adoptive transfer of IL-10 expressing T-cells to mice with polyposis attenuates microbial-induced inflammation and suppresses polyposis. To gain better insights into how IL-10 impacts polyposis, we genetically ablated IL-10 in T-cells in $APC^{\Delta 468}$ mice and compared the effects of treatment with broad-spectrum antibiotics. We found that T-cells and Tregs were a major cellular source of IL-10 in both the healthy and polyp-bearing colon. Notably, T-cell-specific ablation of IL-10 produced pathologies that were identical to mice with a systemic deficiency in IL-10, in both cases increasing the numbers and growth of colon polyps. Eosinophils were found to densely infiltrate colon polyps, which were enriched similarly for microbiota associated previously with colon cancer. In mice receiving broad-spectrum antibiotics, we observed reductions in microbiota, inflammation, and polyposis. Together our findings establish that colon polyposis is driven by high densities of microbes that accumulate within polyps and trigger local inflammatory responses. Inflammation, local microbe densities, and polyp growth are suppressed by IL-10 derived specifically from T-cells and Tregs.

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Disclosures

The authors disclose no potential conflicts of interest.

Additional supplemental materials including data statistics are available online.

Keywords

interleukin-10; polyposis; microbiota; inflammation; colon

Introduction

Inflammation is a cardinal feature of polyposis in mice as well as colon cancer in humans. Our earlier studies show that reduced expression of interleukin-10 (IL-10) by regulatory T-cells (Tregs) contributes to the dysregulation of inflammation in polyposis and colon cancer [1,2]. Many different types of cells are known for producing IL-10, including activated macrophages, dendritic cells, B-cells, mast cells, and intestinal epithelial cells [3–6]. Expression of IL-10 by macrophages is thought to limit inflammatory responses in the mouse intestine by ensuring expression of Foxp3 by $CD4^{+}CD25^{+}$ Tregs [7], although Tregs have also been reported to develop in the absence of IL-10[8], and Tregs have IL-10–dependent, but also IL-10–independent, suppressive effects (reviewed in [9]). IL-10-deficiency does not hamper T-cell suppressive properties of Tregs but does eliminate their ability to suppress intestinal inflammation [10–12]. In addition to Tregs, IL-10-expressing regulatory type 1 (Tr1) cells are potent suppressors of immune effector cells [13]. It is not known how expression of IL-10 by T-cells contributes to tissue levels of IL-10 or to increases in IL-10 during colon cancer. Furthermore, it is not known to what extent the natural history of tumor growth in cancer is affected by IL-10 or by T-cells expressing IL-10.

Human familial adenomatous polyposis syndrome is caused by the inheritance of a defective allele of the adenomatous polyposis coli (*APC*) gene [14]. Random loss of heterozygosity of this locus with age initiates formation of numerous polyps in the colon and predisposes to colon cancer; the same defect is found in up to 90% of individuals with sporadic colorectal cancer (CRC)[15]. Microbial-induced inflammation is an inherent causative component of colon cancer [16] and inflammatory bowel diseases (IBD) in humans [17,18]. Earlier studies show that polyposis in the multiple intestinal adenoma $APC^{Min/+}$ and in $APC^{\Delta 468}$ mouse models is driven by inflammation [19–21], however the source of this inflammation is not clear. While germ-free $APC^{Min/+}$ mice are not protected against polyposis [22], colonic polyposis appears to be microbial-dependent and we earlier documented that oral delivery of beneficial commensal bacteria prevents polyp growth in the colon [23]. Furthermore, microbes are causatively associated with colitis and colitis-induced cancer in mice [18,24].

To determine the role of T-cell-derived IL-10 in polyposis and colon cancer, we generated $APC^{\Delta 468}$ mice that expressed IL-10 and also expressed a Treg reporter ($IL\text{-}10^{Thy1.1}xFoxp3^{GFP}$), or had a selective deficiency for IL-10 only in T-cells ($CD4^{Cre}IL\text{-}10^{fl/fl}$), or were completely devoid of IL-10 ($IL\text{-}10^{-/-}$). We compared T-cell and innate immune responses of these animals to responses of IL-10-competent $APC^{\Delta 468}$ mice. We found that T-cells and Tregs were the primary if not exclusive source of IL-10 in the colon in health and disease, and that T-cell deficiency in IL-10 increased inflammation and polyposis. We provide evidence that IL-10 producing T-cells control microbial densities within colonic polyps and limit inflammation and growth of adenomatous polyps in the

colon. Thus, IL-10 expressing T-cells are a suitable therapeutic target for polyposis and potentially colon cancer.

Materials and Methods

Animals

B6 (C57BL/6J), $CD4^{Cre}$, and $IL10^{-/-}$ mice were purchased from Jackson Laboratories. The $APC^{\Delta 468}$ mouse model of polyposis has been reported earlier and extensively characterized for intra-polyp inflammatory reactions and the role of inflammation in polyp growth [1,2,19–21,25]. IL-$10^{fl/fl}$ mice were generated by Roers and Müller [26]. IL-$10^{Thy1.1}$x$Foxp3^{GFP}$ reporter mice were generated by Weaver [8]. All animal work was approved and conducted according to the guidelines of the Animal Care and Use Committee of Northwestern University.

Histology

Paraffin sections (4 μm) were used throughout. Details are in the Supplementary Materials.

Bone marrow reconstitution

4–6 week old mice were lethally irradiated at 1000 rad (split dose). Mice were retro-orbitally (RO) injected with bone marrow cells which had been Lin-depleted. Details can be found in the Supplementary Materials.

Antibiotic treatment

Mice were treated with an antibiotic cocktail as described previously [27]. Details are in the Supplementary Materials.

Microbial DNA extraction

Microbial DNA was extracted from mouse colonic and fecal samples as described previously [28]. Details are in the Supplementary Materials.

16S rRNA-based illumina library preparation, sequencing and data analysis

Microbial DNA was amplified as described previously [29]. Sequencing was performed by the Next Generation Sequencing Core at Argonne National Laboratory using an Illumina MiSeq [29] and sequences were classified, analyzed, and uploaded in the MG-RAST system (http://metagenomics.anl.gov/). (4513044.3 and 4513045.3; project title: T-cell IL-10 regulates inflammation and colorectal cancer-Dennis). Details are in the Supplementary Materials.

Statistical analysis

Box and whisker histogram depicts box with median flanked by upper and lower 25% quartile with whiskers showing the maximum and minimum data points. The statistical analyses were performed with the use of the Prism 4 software. *P* values determined with two-tailed un-paired *t*-test unless where otherwise indicated; $P<0.01$*, $P<0.001$**, $P<0.0001$***. Not statistically significant data, “ns”. For Figure 4B–D, significance was

determined by performing a paired, one sample t-test with the theoretical mean set to a value of 0. Only values above 0.002 (0.2%) abundance were analyzed.

Results

T-cells and Tregs are the major cellular source of IL-10 in the colon during polyposis

We monitored IL-10 expression in the colonic mucosa using transgenic IL-$10^{Thy1.1}$x$Foxp3^{GFP}$ reporter mice [8]. Lethally irradiated Thy1.2 B6 and $APC^{\Delta 468}$ mice were reconstituted with bone marrow from reporter IL-$10^{Thy1.1}$x$Foxp3^{GFP}$ mice. Chimeric mice were sacrificed and colons were excised, frozen, and sectioned for immunofluorescence staining with Thy1.1 antibody and GFP fluorescence (compare Thy1.1 specific antibody to IgG1, κ isotype control in Supplemental Figure 1). Polyp-ridden $APC^{\Delta 468}$ mice had elevated frequencies of IL-10-expressing cells in both the polyp and marginal tissue as compared to B6 (Figure 1A). We found that total $CD4^+$ cells were significantly elevated in polyps of $APC^{\Delta 468}$ mice as compared to healthy B6 colon, and this was largely attributed to $CD4^+Foxp3^+$ cells (Figure 1B&C). Furthermore, a large fraction of both T-cells and Tregs did not express IL-10 in the polyp-ridden colons (Figure 1B&D). Interestingly, $CD4^+$ T-cells and $Foxp3^+$ Tregs remained the major cellular source of IL-10 in the colon in healthy mice and also during polyposis (Figure 1E). We did not detect IL-10 expression in any cells other than $CD4^+$ cells.

T-cell-specific ablation of IL-10 aggravates colonic polyposis

We examined how epithelial mitotic activity and polyposis in colon were altered by T-cell deficiency for IL-10 in $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ mice or complete IL-10 deficiency in IL-$10^{-/-}APC^{\Delta 468}$ mice. As reported previously, $CD4^{Cre}$IL-$10^{fl/fl}$ and IL-$10^{-/-}$ mice developed spontaneous colitis demonstrated by increased epithelial mitotic activity and crypt elongation as compared to healthy B6 mice (Figure 2A, upper panel, &B). Likewise, colons of $CD4^{Cre}$IL-$10^{fl/fl}$ and IL-$10^{-/-}$ mice were elongated as compared to B6 colons (Figure 2A, lower panel). $APC^{\Delta 468}$ mice develop abundant polyposis by four months of age, however colonic mitotic activity and overall colon length were comparable to that of B6 mice (Figure 2A&B). At this age, $APC^{\Delta 468}$ mice with a T-cell deficiency for IL-10 or complete IL-10 deficiency produced comparable pathologies to one another. Epithelial mitotic activity was significantly increased (Figure 2A), producing crypt elongation (Figure 2B) and overall colon elongation (Figure 2A, lower panel) in both groups of mice as compared to IL-10 proficient $APC^{\Delta 468}$ mice.

$APC^{\Delta 468}$ mice with a T-cell or complete IL-10 deficiency increased colonic polyps by roughly 5-fold (Figure 2C&D). Furthermore, the polyps had expanded stroma and were filled throughout with abnormal crypts containing large polyploid nuclei, whereas in IL-10-proficient $APC^{\Delta 468}$ mice, the polyp stroma was less expanded and the abnormal crypts typically also contained hyper-proliferative but otherwise normal appearing crypts at the basal edge of the lesions (Figure 2E). Thus, T-cell IL-10 deficiency produced similar pathologies to complete IL-10 deficiency, in both instances aggravating epithelial mitosis and polyposis in the colon.

T-cell-specific ablation of IL-10 alters inflammation in polyposis-prone mice

In earlier studies we showed that mast cells are essential for polyp growth in the small intestine [20,21]. Surprisingly, IL-10 deficiency reduced mast cell density in the colon, as determined by *in situ* staining with chloracetate esterase (CAE) and antibody to murine mast cell protease-2 mMCP2 staining (Figure 3A&B). By contrast there were significant increases in the densities of eosinophils, stained with a specific antibody to major basic protein (MBP)[30] (Figure 3C&D). MBP is contained in eosinophil granules, and its release causes inflammation, breakdown of epithelial barrier, and tissue damage [31]. Interestingly, MBP^{+} eosinophils preferentially accumulated within the submucosa and colonic polyp stroma (Figure 3C–F).

Polyps and marginal tissues have distinct microbiota

Depending on their composition and abundance, colon-resident microbiota either maintain a balanced healthy inflammation or provoke chronically elevated pathogenic inflammation (reviewed in [32]). To determine the complexity of the bacterial communities inside colonic polyps relative to the marginal tissue and also relative to healthy colon, and link microbiota, inflammation, and polyp growth, we analyzed the phylogenetic diversity of the sampled microbial assemblages by sequencing the V4 region of the bacterial 16S RNA gene, generating an average of 3,000 reads per sample. In order to control for diversity of microbial populations between animals of the different mouse strains, littermate B6, $APC^{\Delta 468}$, and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice were generated by crossing heterozygous $CD4^{Cre/+}IL\text{-}10^{fl/+}APC^{\Delta 468}$ and $IL\text{-}10^{fl/+}$ mice, yielding offspring from a single mother. Though we did not detect a major shift in the bacterial populations at the phylum level (Supplementary Figure 2), this analysis revealed that the composition of resident microbiota at the genus level was disparate when comparing marginal and polyp tissues, as well as polyp versus healthy B6 tissues (Figure 4A–D).

Two genera, *Bacteroides* and *Porphyromonas* of the phylum Bacterioidetes, were consistently and significantly elevated in abundance within colonic polyps as compared to marginal tissues [*P* values of *0.0300* and *0.0024*, respectively] (Figure 4B). In addition, *Bacteroides* and *Rikenella* of the Bacterioidetes phylum, were significantly elevated in abundance within polyps as compared to healthy B6 tissues [*P* values of *0.0143* and *0.0409*, respectively] (Figure 4B). *Prevotella* of the Bacteroidetes phylum also tended towards increased abundance in the polyp as compared to marginal or B6 tissue (Figure 4B). Elevated genera of the phylum Bacteroidetes have been implicated as a risk factor for IBD and CRC in humans [33]. In addition, *Bacteroides* and *Porphyromonas*, as well as *Prevotella*, have been shown to adhere to the colon mucosa, and tumor tissues are enriched for these bacteria in CRC patients [34,35]. Polyp tissue was not enriched overall above marginal tissue or B6 tissue in other bacteria that fell under different phyla, including Proteobacteria, and Firmicutes (Figure 4C&D).

Evidence from animal studies specifically implicates T-cell-derived IL-10 as a requisite, non-redundant mediator of colonic immune homeostasis [11,12,36] essential for control of microbial-driven TH17 inflammation [37] and colitis [38,39]. Past reports have documented that colons of $IL\text{-}10^{-/-}$ mice have a different microbial composition than colons of healthy

wt mice [39]. To investigate how T-cell IL-10-deficiency in polyposis alters microbiota composition of the colon we carried out sequencing of the bacterial DNA from colons of littermate *wt*, $APC^{\Delta 468}$, and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice, and performed principle coordinate analysis (PCoA). We observed a genotype-specific clustering dependent on two components of variation (Figure 5). PC1 (37.24% of variation) strongly separated B6 and $APC^{\Delta 468}$ groups from the $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ group, while PC3 (10.77% of variation) strongly separated the B6 group from the $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ groups (Figure 5). Altogether, these observations suggest that the microbial composition of polyps is distinct from that of the healthy surrounding tissue and that T-cell IL-10 deficiency alters the composition of microbial communities in the colonic mucosa. Next, we examined the role of microbiota in polyposis.

Colonic polyps are highly sensitive to antibiotic treatment

Formation of polyps coincides with loss of epithelial barrier integrity and incursion of microbial products [40], and we reasoned that this may have been the cause of the observed intra-polyp eosinophilic response. Therefore, we treated littermate $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice with a cocktail of broad-spectrum antibiotics: kanamycin, gentamycin, colistin, metronidazole, and vancomycin by intra-peritoneal (*i.p.*) injection. This cocktail produces profound shifts in the composition of the intestinal microbiota and ablates microbial-induced TLR4 signaling in the murine colon [41]. Mice were administered antibiotics starting at the onset of polyposis (9–10 weeks of age) and continuing every other day for a total of three weeks (Figure 6A). After a week of rest, the mice were analyzed for polyp load, inflammatory eosinophilia, and the abundance of specific polyp-associated microbial taxa.

Antibiotic treatment significantly decreased eosinophilia (Figure 6C) and polyp load (Figure 6D) in the colon of $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice, however did not have any impact on mastocytosis (Figure 6B). To analyze microbial complexity following antibiotic treatment, fecal pellets were collected from mice just prior to antibiotic treatment (*pre*-treatment) and again at the conclusion of the treatment (*post*-treatment). Amplicon analysis of the 16S rRNA V4 region identified the two genera, *Bacteroides* and *Porphyromonas* which were significantly elevated in colonic polyps as compared to healthy marginal tissues; these significantly decreased relative abundance in all antibiotic-treated $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice (Figure 6E&F). By calculating the Shannon index for overall microbial diversity, we were able to demonstrate a drop in the overall microbial diversity in fecal content from animals treated with antibiotics (Figure 6G). These observations indicate that microbiota critically contribute to eosinophilic infiltration of polyps and to polyposis in the colon.

Discussion

We provide evidence that colonic polyposis is driven by intra-polyp inflammatory reactions, which are tuned by microbial communities within the polyps and by infiltrating T-cells. Our findings reveal that T-cells and Tregs are the major cellular source of IL-10 in the colon, and

their expression of IL-10 is critical for the control of intra-polyp inflammation and polyp-growth.

It is surprising but at the same time reassuring that the major source of IL-10 in the colon is T-cells and Tregs. This explains why in the polyposis-prone $APC^{\Delta 468}$ mice T-cell/Treg ablation of IL-10 produced near identical pathologies as complete IL-10-deficiency. When we ablated IL-10 specifically in T-cells, polyp frequency in the colon increased by nearly 5-fold, producing a pathology reminiscent of human FAP. Ubiquitous ablation of IL-10 produced a near identical result, emphasizing the critical role of IL-10-expressing T-cells in limiting both inflammation and polyp growth in the colon.

T-cell IL-10-deficiency in the colon resulted reduced mast cell numbers. IL-10 dependence of mast cells has been reported before [42], and could explain the loss of mast cells from the colon of IL-10-deficient mice. There was a notable increase in the density of polyp-infiltrating eosinophils in colons of IL-10-deficient $APC^{\Delta 468}$ mice. The enrichment of colonic polyps for microbial communities suggested to us that the eosinophils were responding to microbial intrusion. We validated this by showing that both eosinophils and microbes were eliminated by treatment with broad-range antibiotics. Loss of eosinophils coincided with reduction in the number of colonic polyps. These observations suggest that chronic inflammatory reactions to intra-polyp microbes help promote polyp growth.

Our findings extend an earlier report suggesting that adenomas are leaky and serve as ports of entry of microbial products [40] by demonstrating that polyps are focally enriched in pro-inflammatory bacteria. Our finding agrees with recently reported penetration of bacteria into the inner colonic mucosa layers of both mice with colitis and patients with ulcerative colitis [18]. Our findings are also in line with an earlier report that a mutant strain of *Lactobacillus acidophilus* lacking cell surface expression of the TLR5 ligand, lipoteichoic acid, protects against colonic polyposis [23]. In this earlier study we did not see any response by small intestine polyps.

Mucosal immune responses determine the quality and quantity of commensal bacteria [43]. In the chronic situation this leads to selection of pathogenic bacteria [17]. Thus, both epithelial barrier defects and growing tumors can selectively alter the microbial community causing expansion of pathogenic bacteria [16,44] that promote vicious cycles of inflammation in colitis and colon cancer [45–47]. Accordingly, specific microbiota were enriched within the colonic polyps and were of distinct taxa with significant abundance of *Bacteroides* and *Porphyromonas* as compared to those found in healthy gut regions. This finding is in agreement with previous studies that have shown biopsies from patients with colorectal adenomas to have elevated bacterial abundance as compared to healthy colon tissue, and suggests that analysis of specific taxa of human microbiota could be used to potentially identify patients at risk for IBD[48,49] and colonic adenomas [50]. Our observations show that IL-10 producing T-cells and Tregs regulate bacterial-instigated polyp growth and therefore enhancing their activity is a promising strategy for immune therapy of polyposis and colon cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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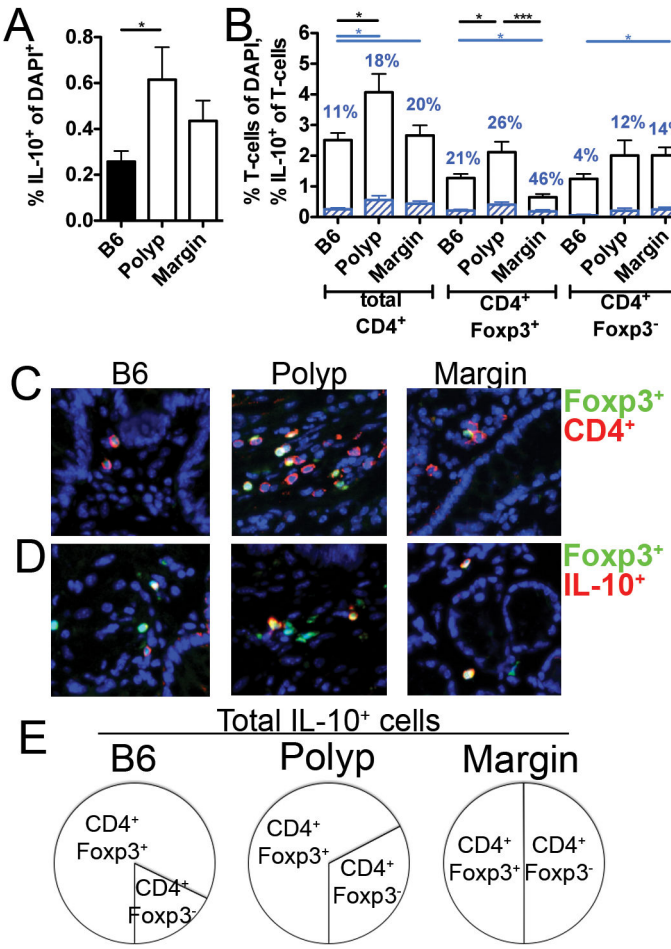

Figure 1. IL-10 is produced by T-cells in polyposis

(A) Thy1.2 B6 (N=3) and APC$^{\Delta 468}$ (N=4) mice were reconstituted with IL-10$^{Thy1.1}$ x Foxp3^{GFP} reporter bone marrow. Histogram depicts frequency (%) of IL-10^{+} staining cells as a percent of total cells (DAPI). At least 5 representative regions counted per mouse.

(B) Thy1.2 B6 and APC$^{\Delta 468}$ mice were reconstituted with IL-10$^{Thy1.1}$ x Foxp3^{GFP} reporter bone marrow. Histogram depicts frequency (%) as a percent of total nuclear cells (DAPI) of total CD4^{+}, as well as CD4^{+}Foxp3^{+}, and CD4^{+}Foxp3^{-} cells. Shaded regions of each histogram represent frequency of IL-10-expressing cells in the different T-cell subsets. At least 5 representative regions counted per mouse.

(C) Representative Foxp3^{GFP} and CD4 co-staining in reconstituted B6 and APC$^{\Delta 468}$ mice. CD4 visualized by a Cy5-conjugated secondary antibody.

(D) Representative Foxp3^{GFP} and IL-10 co-staining in reconstituted B6 and APC$^{\Delta 468}$ mice. IL-10 visualized by an anti-Thy1.1-biotin primary antibody and streptavidin-AlexaFluor-594 conjugated flurochrome.

(E) Pie charts depict proportion of IL-10 expressed in either the CD4^{+}Foxp3^{+} or CD4^{+}Foxp3^{-} compartment in B6 or APC$^{\Delta 468}$ mice.

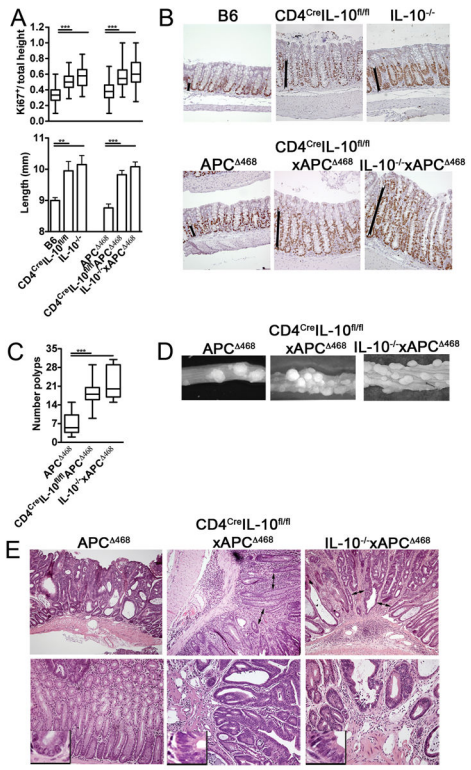

Figure 2. Colonic mitotic activity & polyp load are exacerbated in IL-10-deficiency

(A) Upper histogram depicts quantification of Ki67 staining within healthy appearing tissue of the colon B6, $CD4^{Cre}IL\text{-}10^{fl/fl}$, $IL\text{-}10^{-/-}$, $APC^{\Delta 468}$, $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$, and $IL\text{-}10^{-/-}xAPC^{\Delta 468}$ mice. Data represent the ratio of the height of Ki67-staining cells/total height of each villi per 200X view. Lower histogram (mean ± SEM) depicts colon length of mice in millimeters (mm).

(B) Representative Ki67 staining of colons. Black bar represents average height of $Ki67^{+}$ cells within the villi.

(C) Colonic polyp counts of the various mouse strains.

(D) Photographs of distal colon segments from $APC^{\Delta 468}$, $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$, and $IL\text{-}10^{-/-}$ mice.

(E) Upper panels show representative 100X colonic polyp from an $APC^{\Delta 468}$, $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$, and $IL\text{-}10^{-/-}xAPC^{\Delta 468}$ mice. Double-headed arrows depict width of expanded stromal regions. Lower panels show representative 200X micrographs of colonic polyps from $APC^{\Delta 468}$, $IL\text{-}10^{-/-}xAPC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice, showing crypts at the basal surface of the polyp. Inlay images at 400X, basal crypts of the three mouse strains.

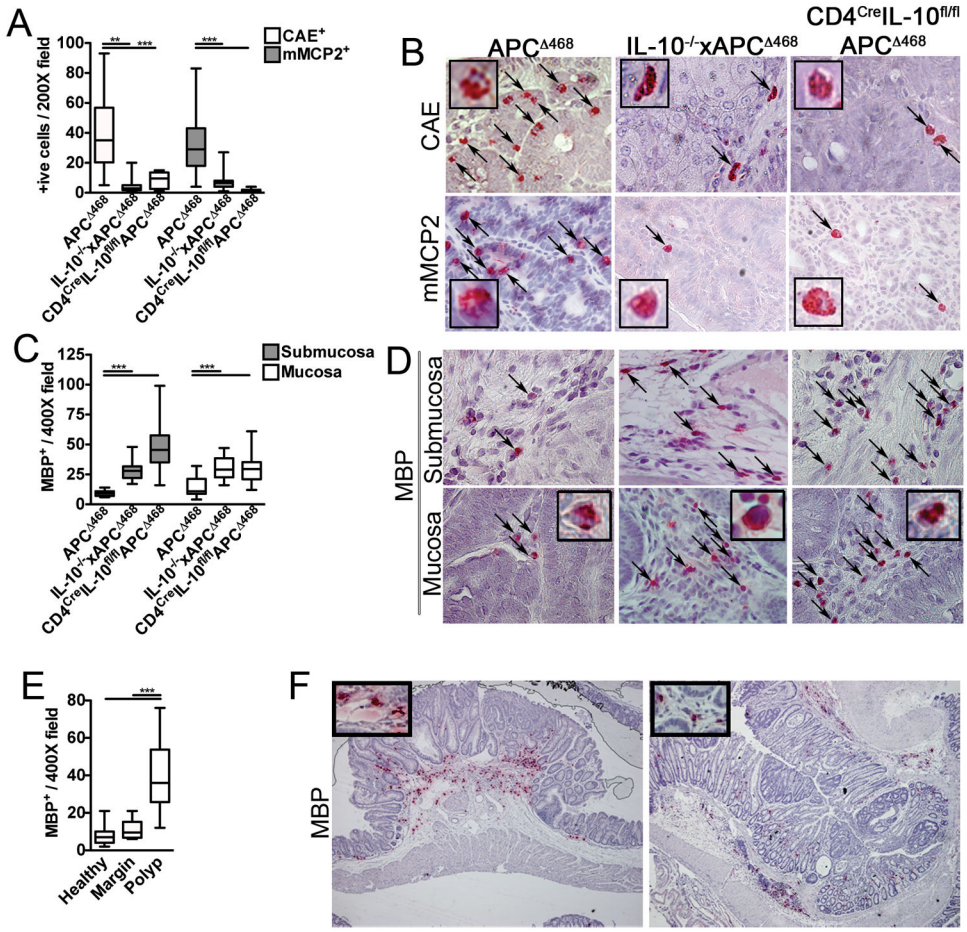

Figure 3. Colonic infiltration of eosinophils and mast cells

(A) Quantification of colon mast cells by CAE (grey bars) and mMCP2 (open bars) for $APC^{\Delta 468}$, IL-$10^{-/-}$x$APC^{\Delta 468}$, and $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ mice.

(B) Representative 400X micrographs of CAE staining (upper panels) or mMCP2 staining (lower panels) of $APC^{\Delta 468}$, IL-$10^{-/-}$x$APC^{\Delta 468}$, and $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ colonic polyps. Arrows point to mast cells. Inlay at 1000X.

(C) Quantification of colon eosinophils by MBP staining in the submucosa (grey bars) and mucosa (open bars) for $APC^{\Delta 468}$, IL-$10^{-/-}$x$APC^{\Delta 468}$, and $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ mice.

(D) Representative 400X micrographs of MBP staining in the submucosa (upper panels) or mucosa (lower panels) in $APC^{\Delta 468}$, IL-$10^{-/-}$x$APC^{\Delta 468}$, and $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ colonic polyps. Arrows point to eosinophils. Inlay at 1000X.

(E) Quantification of eosinophils by MBP staining in $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ mice in the healthy, marginal, and polyp tissue in the colon.

(F) Representative micrographs of $CD4^{Cre}$IL-$10^{fl/fl}APC^{\Delta 468}$ polyps stained with the eosinophil-specific marker MBP. Colonic polyps (at 50X) with inlay (at 400X) showing clusters of MBP^{+} cells.

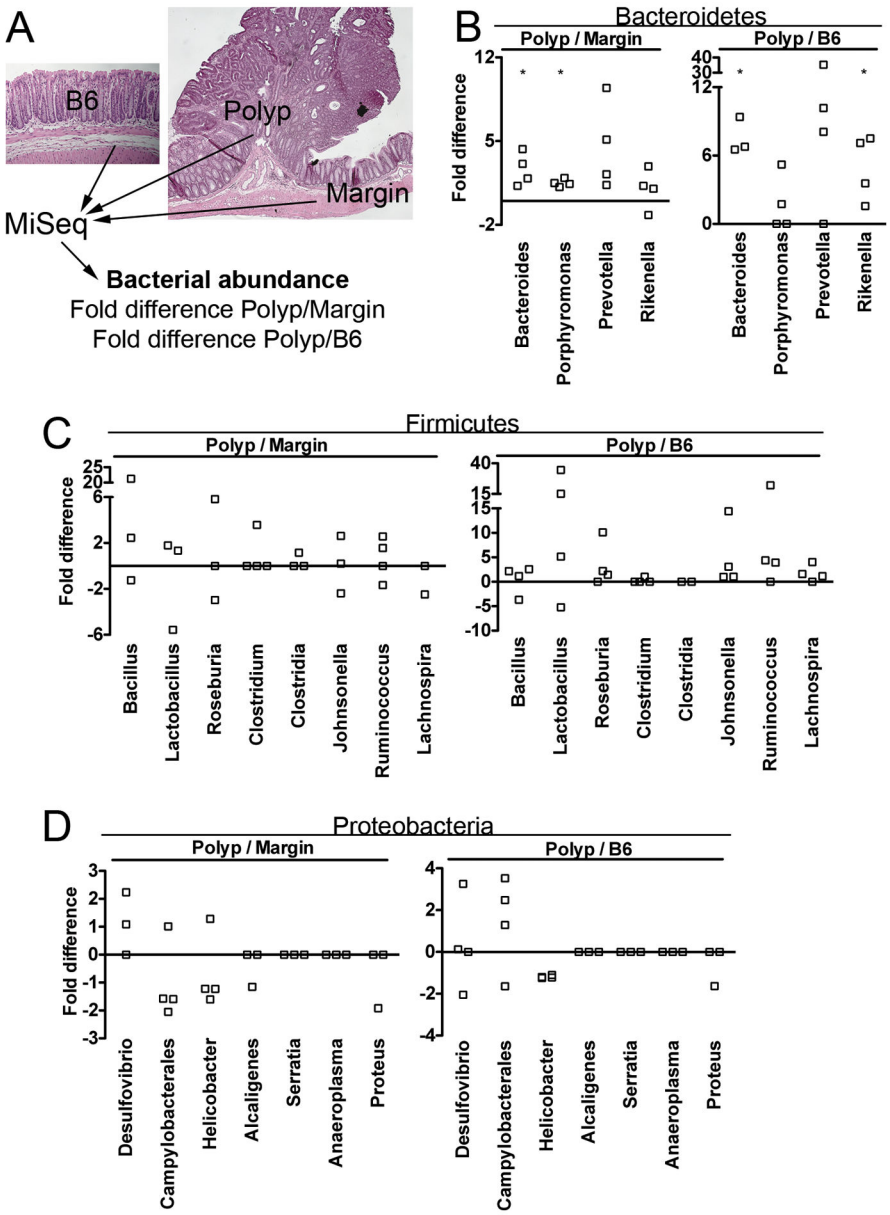

Figure 4. Colonic microbiota is enriched in polyps as compared to healthy marginal tissues

(A) Scheme showing that B6 colonic tissue, as well as polyp and marginal tissue from polyp-bearing mice, was harvested and bacteria sequenced by MiSeq for relative bacterial abundance in the various tissues. Fold difference between polyp and margin, or polyp and healthy B6 tissues was calculated and graphed in (B–D).

Relative fold change of microbes in the polyp versus marginal tissue of polyp-bearing mice, or polyp tissues versus B6 tissues, of the phyla (B) Bacteroidetes, (C) Firmicutes, and (D) Proteobacteria. N=4 mice (2 $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ and 2 $APC^{\Delta 468}$ mice). Paired polyp and margin data points were used to calculate the relative fold-increase of polyp to margin, or margin to polyp. Significance was determined by performing a paired, one sample t-test

with the theoretical mean set to a value of 0, with $P<0.05$ noted by *. Only values above 0.0020 (0.2%) relative abundance were analyzed.

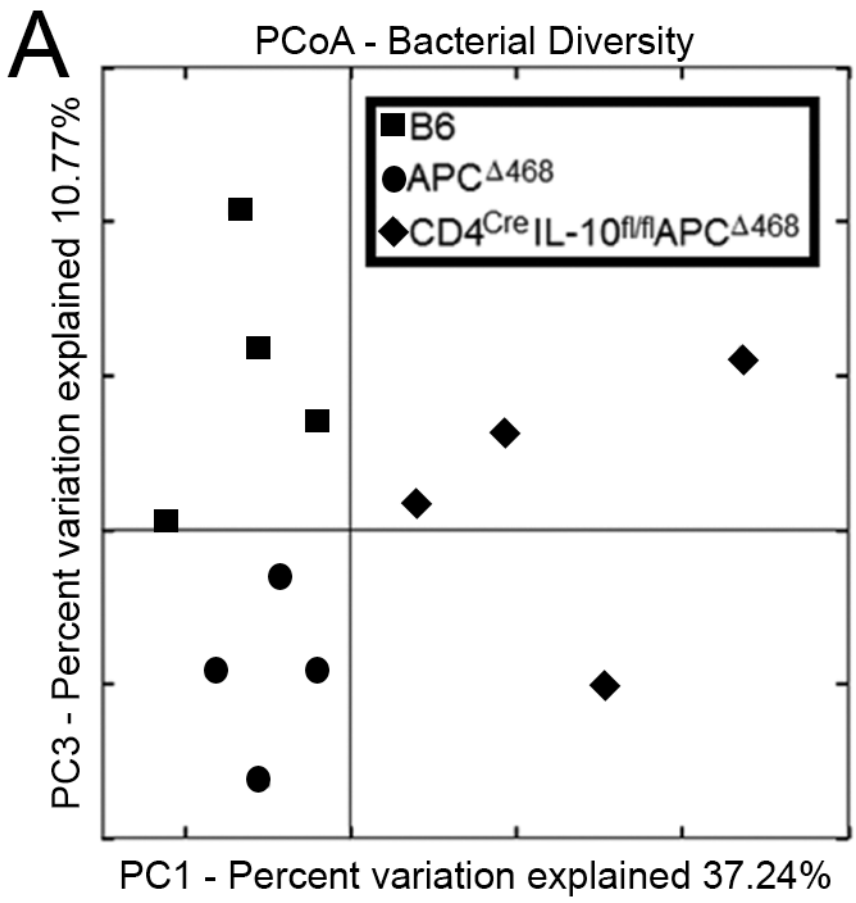

Figure 5. Bacterial composition differs between B6, APC$^{\Delta 468}$, and CD4^{Cre}IL-10$^{fl/fl}$APC$^{\Delta 468}$ colonic mucosa

PCoA plot of bacterial composition of healthy B6 colon (squares), and marginal-to-the-polyp healthy appearing colonic tissue from APC$^{\Delta 468}$ (circles) and CD4^{Cre}IL-10$^{fl/fl}$APC$^{\Delta 468}$ (diamonds) mice. N=4 for each strain. PC3 on the y-axis with a 10.77-percent variation explained. PC1 on the x-axis with a 37.24-percent variation explained.

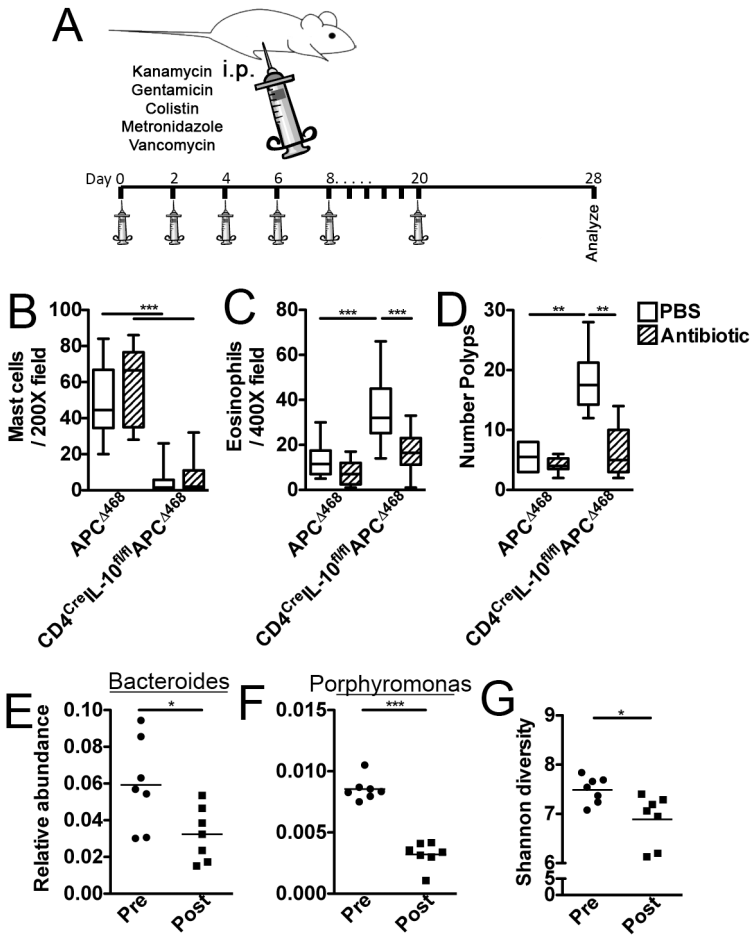

Figure 6. Antibiotic treatment alters microbiota and inflammation-associated colonic polyposis in IL-10-deficiency

(A) Scheme for mice undergoing antibiotic treatment. At 2.5-months of age, $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice received an injection of a 200ul cocktail of antibiotics by intra-peritoneal (i.p.) injection. This cocktail included kanamycin (4mg/ml), gentamycin (0.35mg/ml), colisitin (8500U/ml), metronidazole (2.12mg/ml), and vancomycin (0.45mg/ml). Mice were treated every other day for a total of 3 weeks, then they were allowed 1 week of rest prior to sacrifice and analysis.

(B) Number of mast cells by CAE staining of $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice following antibiotic treatment (per polyp completely filling a 200X field).

(C) Number of eosinophils by eosin staining of $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice following antibiotic treatment (per polyp completely filling a 400X field).

(D) Number of polyps counted in the colon following antibiotic treatment of $APC^{\Delta 468}$ and $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice.

Relative abundance of *Bacteroides* (E) and *Porphyromonas* (F) in fecal pellets collected before (pre) and after (post) antibiotic treatment from polyp-bearing animals (N=3 $APC^{\Delta 468}$ and N=4 $CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice). Bar depicts average abundance score per data set.

(G) Shannon diversities of microbial populations in fecal pellets collected before (pre) and after (post) antibiotic treatment from polyp-bearing animals (N=3 $APC^{\Delta 468}$ and N=4

$CD4^{Cre}IL\text{-}10^{fl/fl}APC^{\Delta 468}$ mice). Histogram plot shows diversity index of seven different polyp-bearing mice. Bar depicts average abundance score per data set.