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Early-life antibiotic treatment enhances the pathogenicity of CD4+ T cells during intestinal inflammation

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ABSTRACT

The incidence of inflammatory bowel diseases (IBDs) has steadily increased in recent decades—a phenomenon that cannot be explained by genetic mutations alone. Other factors, including the composition of the intestinal microbiome, are potentially important contributors to the increased occurrence of this group of diseases. Previous reports have shown a correlation between early-life antibiotic (Abx) treatment and an increased incidence of IBD. In this report, we investigated the effects of early-life Abx treatments on the pathogenicity of CD4+ T cells using an experimental T cell transfer model of IBD. Our results show that CD4+ T cells isolated from adult mice that had been treated with Abx during gestation and in early life induced a faster onset of IBD in *Rag1*-deficient mice compared with CD4+ T cells of untreated mice. Ex vivo functional analyses of IBD-inducing CD4+ T cells did not show significant differences in their immunologic potential ex vivo, despite their in vivo phenotype. However, genome-wide gene-expression analysis revealed that these cells displayed dysregulated expression of genes associated with cell-cycle regulation, metabolism, and cellular stress. Analysis of Abx-treated CD4+ T cell donors showed systemically elevated levels of the stress hormone corticosterone throughout life compared with untreated donors. The cohousing of Abx-treated mice with untreated mice decreased serum corticosterone, and a consequent transfer of the cells from cohoused mice into *Rag1*-deficient mice restored the onset and severity of disease to that of untreated animals. Thus, our results suggest that early-life Abx treatment results in a stress response with high levels of corticosterone that influences CD4+ T cell function. *J. Leukoc. Biol.* 101: 893–900; 2017.

Abbreviations: Abx = antibiotics, APC = allophycocyanin, DEX = dexamethasone, Foxp3 = forkhead box p3, GWAS = genome-wide association studies, IBD = inflammatory bowel disease, PCA = principal component analysis, PI = propidium iodide, qPCR = quantitative PCR, Rag1 = recombination activating gene 1, RNA-Seq = RNA sequencing, T_{reg} = regulatory T cell

Introduction

The incidence of IBDs has significantly increased over the last century and now affects close to 1% of the population in some countries [1]. IBDs are thought to occur as a result of a complex interplay between host genetics and environmental factors, such as the composition of the intestinal microbiota, which in turn, leads to a dysregulated intestinal immune response. Although GWAS have identified >200 genes that affect the onset and severity of IBDs [2], the precise molecular mechanisms leading to IBDs remain unclear.

Of the many genes identified by GWAS, a significant proportion is associated with immune cell signaling, cytokine production, and T cell activation [2]. In animal models of IBD, CD4+ T cells play a major role in the development of disease. For example, in the absence of regulatory mechanisms, such as loss of T_{regs} or the absence of IL-10, CD4+ T cells are able to react against bacterial antigens and produce high amounts of inflammatory cytokines leading to intestinal inflammation [3]. Changes in the CD4+ T cell compartment by alternative selection, changes in the epigenetic conformation, or changes in the expression levels of critical signaling molecules have been associated with altered behavior of CD4+ T cells during infection, inflammation, or autoimmune diseases [4–6].

Previous work in animal models has shown that the onset of inflammatory diseases later in life correlates to the composition of the microbiota in early life [7], and a study with patients showed a correlation between the development of IBD later in life after exposure to Abx in early life [8]. These studies suggest

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that the interaction between the intestinal microbiome and the developing immune system is critical for shaping the functional capacity of immune cells, such as CD4$^+$ T cells. However, the mechanisms that link changes in the microbiome to changes in immune cell function are not known. In this study, with the use of a mouse model of T cell-dependent intestinal inflammation, we examined the effects of Abx treatment during gestation and in early life on the development of IBD in adult mice. We show that early-life Abx treatment results in increased pathogenicity of CD4$^+$ T cells in a model of intestinal inflammation. This effect is associated with a systemic stress response with high levels of circulating corticosterone. Genome-wide expression analysis of naive CD4$^+$ T cells from Abx-treated mice shows significant changes in genes associated with stress responses, cellular metabolism, cell cycle, and cell death. Furthermore, our results show that this effect is transient, as cohousing of Abx-treated mice with untreated mice after weaning reverses the systemic stress response and reduces the pathogenicity of CD4$^+$ T cells in vivo. Thus, we conclude that early-life Abx treatment results in a stress response, high levels of corticosterone, and dysfunctional CD4$^+$ T cell responses that are reversible later in life. These results provide a link between the regulation of the intestinal microbiome and the developing immune system and identify potential targets for treating IBD.

MATERIALS AND METHODS

Mice and Abx treatments

Foxp3 reporter mice on C57BL/6 background (C.Cg-*Foxp3*$^{tm2Tch/J}$), wild-type C57BL/6, and *Rag1*-deficient mice were bred and kept at the animal facility of the Biomedical Research Centre (University of British Columbia, Vancouver, BC, Canada), according to approved protocols. Parent animals were treated with Abx in the drinking water (autoclaved and supplemented with 0.5 g/l of each ampicillin, streptomycin, vancomycin, and metronidazole containing 4 g/l Splenda, 7 d before mating and throughout their lives). Pups of each group (Abx and control) were weaned after 21 d and returned to regular drinking water (without Abx or Splenda) until analysis at wk 8–10. As indicated for some experiments, mice were weaned from Abx-treated parent animals, kept on regular water, and cohoused, 7 d after weaning, with untreated, control mice until analysis. Serum and tissue samples were collected immediately after death, snap frozen in liquid nitrogen, and then stored at −80°C until later analysis of steroids. The term, "early-life Abx treatment" refers to Abx exposure during gestation and until weaning.

T cell transfer colitis

Splenic CD4$^+$ T cells from donor mice (either Abx treated or on regular water) were pre-enriched with the CD4$^+$ T Cell-Negative Isolation Kit (Stemcell Technologies, Vancouver, BC, Canada) and FACS sorted for viable CD4$^+$ CD25$^+$ Foxp3$^-$ CD45RB^{hi} cells. Cells (4×10^5) were injected intraperitoneally into 8- to 10-wk-old *Rag1*-deficient mice. Weight loss of >20% was considered the humane endpoint. Proximal colons were processed for histologic H&E staining and scored for disease (inflammatory cell infiltration, loss of epithelial architecture, thickening of colonic wall). Isolated cells were stimulated with 1 μg/ml αCD3/αCD28 overnight; then stimulated with PMA, ionomycin, and Brefeldin A for 4 h; and intracellularly stained for flow cytometry.

Measurement of commensal bacteria-specific T cells

Single-cell suspensions of spleens from donor mice were stained with CBir1-APC tetramer (kind gift from Dr. Timothy Hand, University of Pittsburgh, Pittsburgh, PA, USA) and analyzed as described previously [9, 10].

RNA isolation and real-time qPCR

RNA was isolated from proximal colons by mechanical disruption and the TRIzol method (Thermo Fisher Scientific, Waltham, MA, USA). RNA was purified from sorted CD4$^+$ T cells using RNeasy Mini Kits (Qiagen, Germantown, MD, USA). Reverse transcription was used to generate cDNA, and qPCR was performed using SYBR Green primer sets. Reactions were run on an ABI 7900 Real-Time PCR System (Thermo Fisher Scientific). Samples were normalized relative to expression of β-actin (*Actb*).

RNA-Seq and bioinformatics

Naive CD4$^+$ T cells, sorted as above, were harvested, and total RNA was extracted using an RNeasy Mini Kit, according to the manufacturer's instructions (Qiagen). Approximately 500 ng RNA was prepared with an mRNA kit (TruSeq Stranded; Illumina, San Diego, CA, USA) and sequenced on a MiSeq paired-end run (75 × 75, v3; Illumina). Samples were aligned to the mm10 transcript reference using TopHat2, and differential expression was assessed using Cufflinks (Illumina). The RNA-Seq datasets described in this article are available at the National Center for Biotechnology Information (accession number GSE92282).

T cell polarization and flow cytometry

CD4$^+$ T cells were isolated with the CD4$^+$ T Cell-Negative Isolation Kit (Stemcell Technologies) and used in T cell polarization assays, as described previously [6]. For screening of the TCR Vβ use in the CD4$^+$ T cell population, we used the mouse TCR Vβ Screening Panel, according to the manufacturer's instructions (BD Biosciences, San Jose, CA, USA).

T cell apoptosis assay

Highly enriched CD4$^+$ T cells were cultured under neutral conditions (1 μg/ml each plate-bound αCD3 and αCD28, 10 ng/ml recombinant mouse IL-2) in the presence of indicated concentrations of the corticosteroid DEX. After 16 h, cells were stained and analyzed for early and late apoptotic cells (Annexin V and PI).

Steroid extraction and immunoassay

Steroids were extracted from serum, thymus, proximal colon, and cecum samples using solid-phase extraction with C_{18} columns, as described elsewhere [11]. Dried steroid extracts were resuspended in assay diluent with 5% ethanol to aid resuspension, and corticosterone concentrations were measured using a sensitive and specific radioimmunoassay kit (07-120103; MP Biomedicals, Santa Ana, CA, USA), as described previously [11]. Recovery of corticosterone was 78% in serum, 113% in thymus, 109% in proximal colon, and 116% in cecum. Reported tissue corticosterone concentrations are corrected for recovery. In some experiments, only serum corticosterone was measured; therefore, these serum samples were not extracted but diluted in assay diluent and directly quantified by radioimmunoassay.

Statistical analysis

Data are presented as means ± SEM. Statistical significance was determined by a 2-tailed Student's *t* test using GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA). Results were considered statistically significant with $P < 0.05$.

RESULTS AND DISCUSSION

Early-life treatment with Abx leads to an earlier onset of experimental IBD

To investigate the hypothesis that gestational and early-life treatment with Abx affects the onset and severity of CD4$^+$ T cell-

induced intestinal inflammation later in life, we compared the potency of transferred, naive CD4$^+$ T cells from unmanipulated or Abx-treated mice to induce intestinal inflammation in recipient Rag1-deficient mice. We transferred naïve CD4$^+$ T cells that were isolated either from >8 wk-old wild-type, untreated mice or mice that were treated with Abx in utero and for the first 3 wk of life (**Fig. 1A**). Strikingly, animals that received CD4$^+$ T cells from Abx-treated mice displayed accelerated weight loss compared with mice that received control CD4$^+$ T cells (Fig. 1B). At d 28 post-transfer, in addition to increased weight loss, we observed significantly increased disease severity (Fig. 1C and D). Analysis of the immune response at d 28 post-transfer revealed a trend toward increased expression of several proinflammatory cytokines in the intestine, which was consistent with the enhanced disease. We found that expression of *Tnfa*, *Ifng*, *Il1b*, and *Il17a* was increased in the gut of mice that received CD4$^+$ T cells isolated from Abx-treated mice (Fig. 1E). Although the differences in cytokine gene expression did not achieve statistical significance, they were consistently higher over all independent experiments. Thus, early-life Abx treatment resulted in more pathologic CD4$^+$ T cells that caused more severe intestinal inflammation.

Abx treatment does not lead to increased cytokine gene expression in CD4$^+$ T cells

We next sought to understand how early-life Abx treatment led to more pathogenic CD4$^+$ T cells later in life. As we observed a trend toward increased production of proinflammatory cytokines in vivo, we next examined the ability of CD4$^+$ T cells from untreated and Abx-treated mice to produce cytokines under several conditions in vitro by intracellular cytokine staining and flow cytometry. We found equivalent production of IFN-γ, IL-10, IL-13, IL-17A, and TNF-α by CD4$^+$ T cells from both untreated and Abx-treated mice under nonpolarizing or Th1-, Th2-, Th17-, and T_{reg}-differentiating conditions (**Fig. 2A**). Thus, although early-life Abx treatment leads to increased pathogenicity of CD4$^+$ T cells in vivo, it does not affect the ability of CD4$^+$ T cells to produce cytokines in vitro following polyclonal stimulation.

Abx treatment does not affect TCR use or responsiveness

CD4$^+$ T cells that induce IBD are primarily activated by antigens from intestinal microbes [9, 10, 12], and thus, it has been suggested that one of the benefits of Abx treatment in IBD patients is through the reduction of antigen load [13]. As we could not detect a general dysregulation of cytokine production

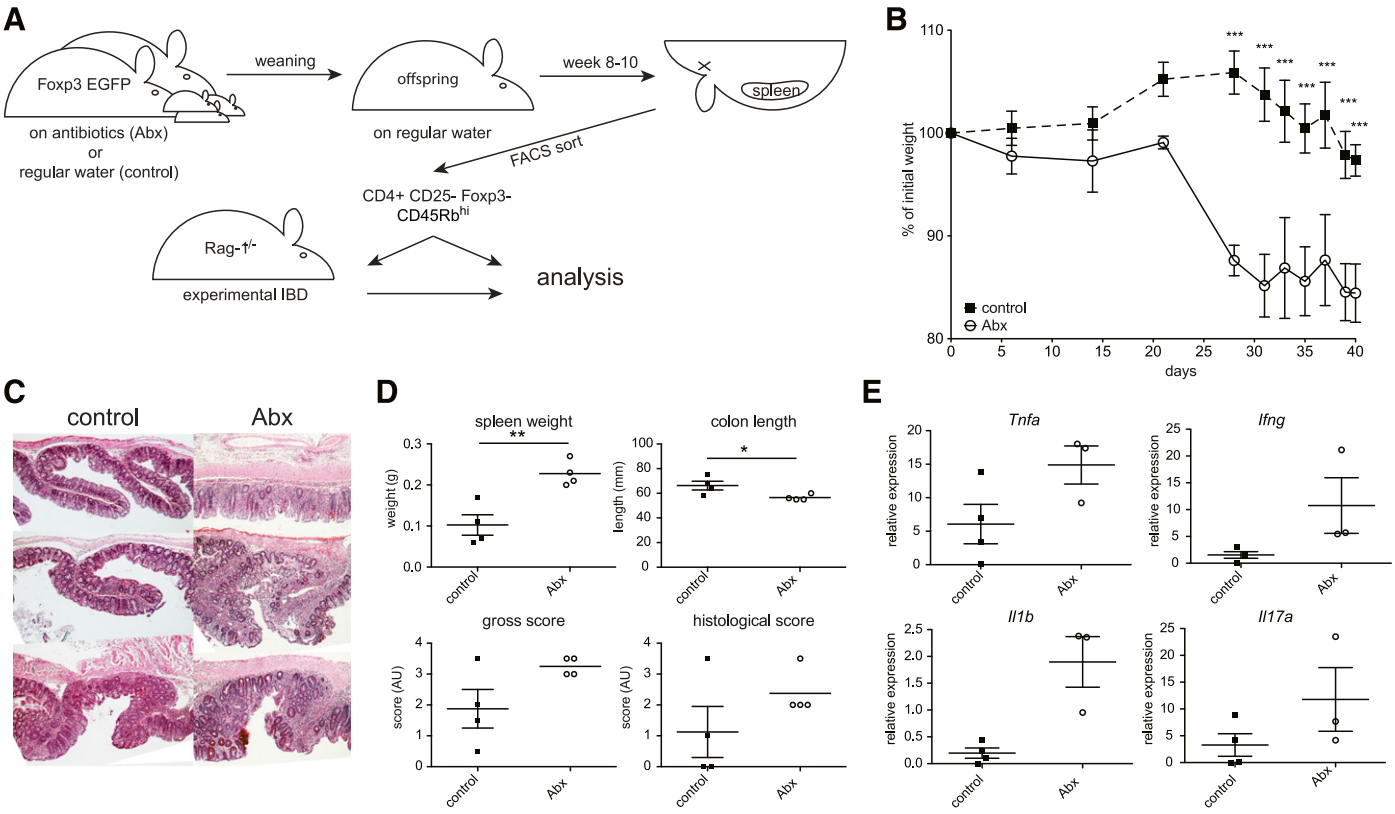


Figure 1. Early-life Abx treatment enhances the pathogenicity of CD4$^+$ T cells. (A) Schematic overview of the experiment. (B) Weight change during the course of experimental IBD after injection of naive T cells from donor mice that were treated with Abx (○) or control (■) mice into recipient *Rag1*-deficient mice. Results are representative of 16 individual experiments (n = 3 for Abx and n = 6 for control; the last weight of deceased animals was continuously taken into account until the end of the experiment). (C) Histologic analysis of the proximal colons from recipient mice at the time point of analysis of mice shown in B. (D) Characterization of experimental IBD disease markers after analysis of the mice at d 28 (n = 4). (E) Gene-expression analysis of proinflammatory cytokines in the proximal colon of mice analyzed at d 28 post-T cell transfer (n = 4). *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Not significant where not indicated. Error bars represent SEM.

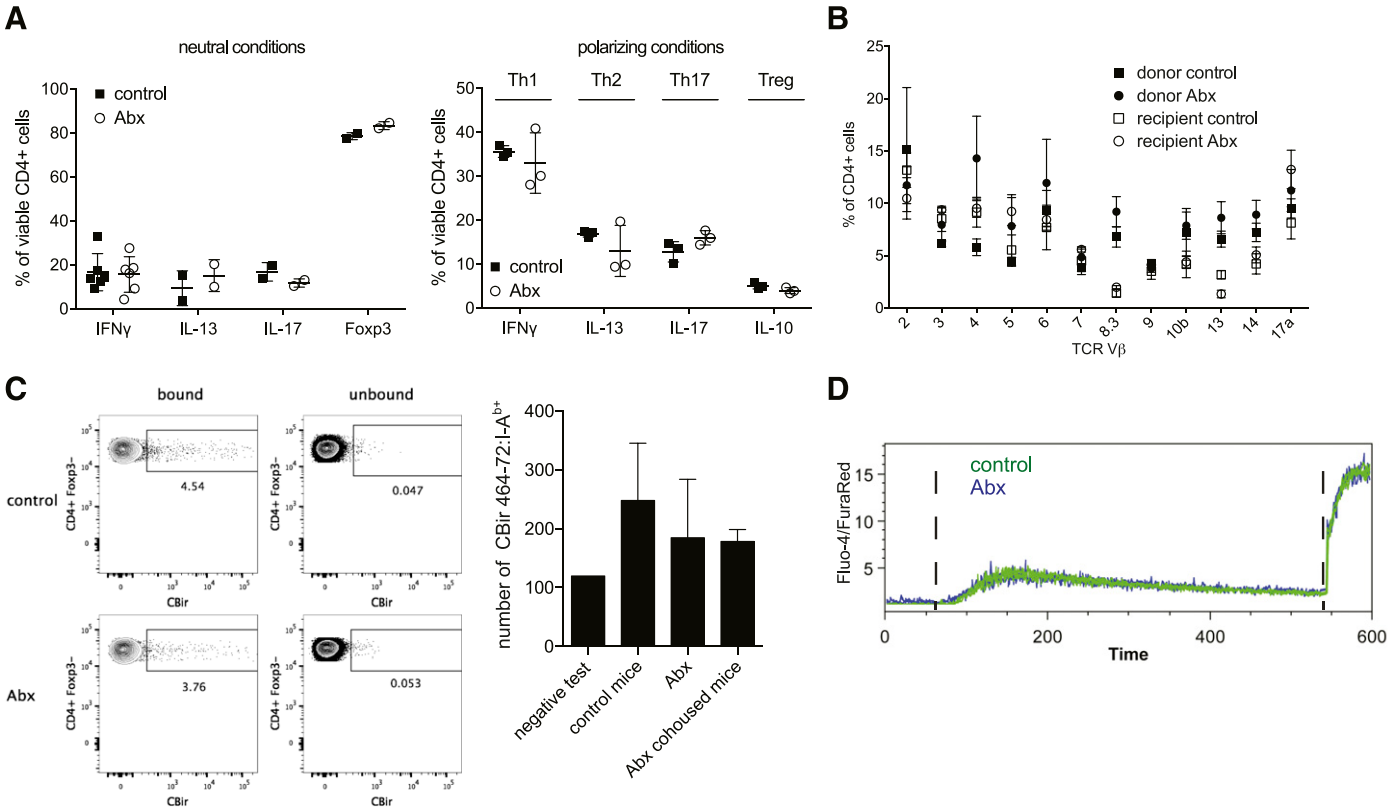


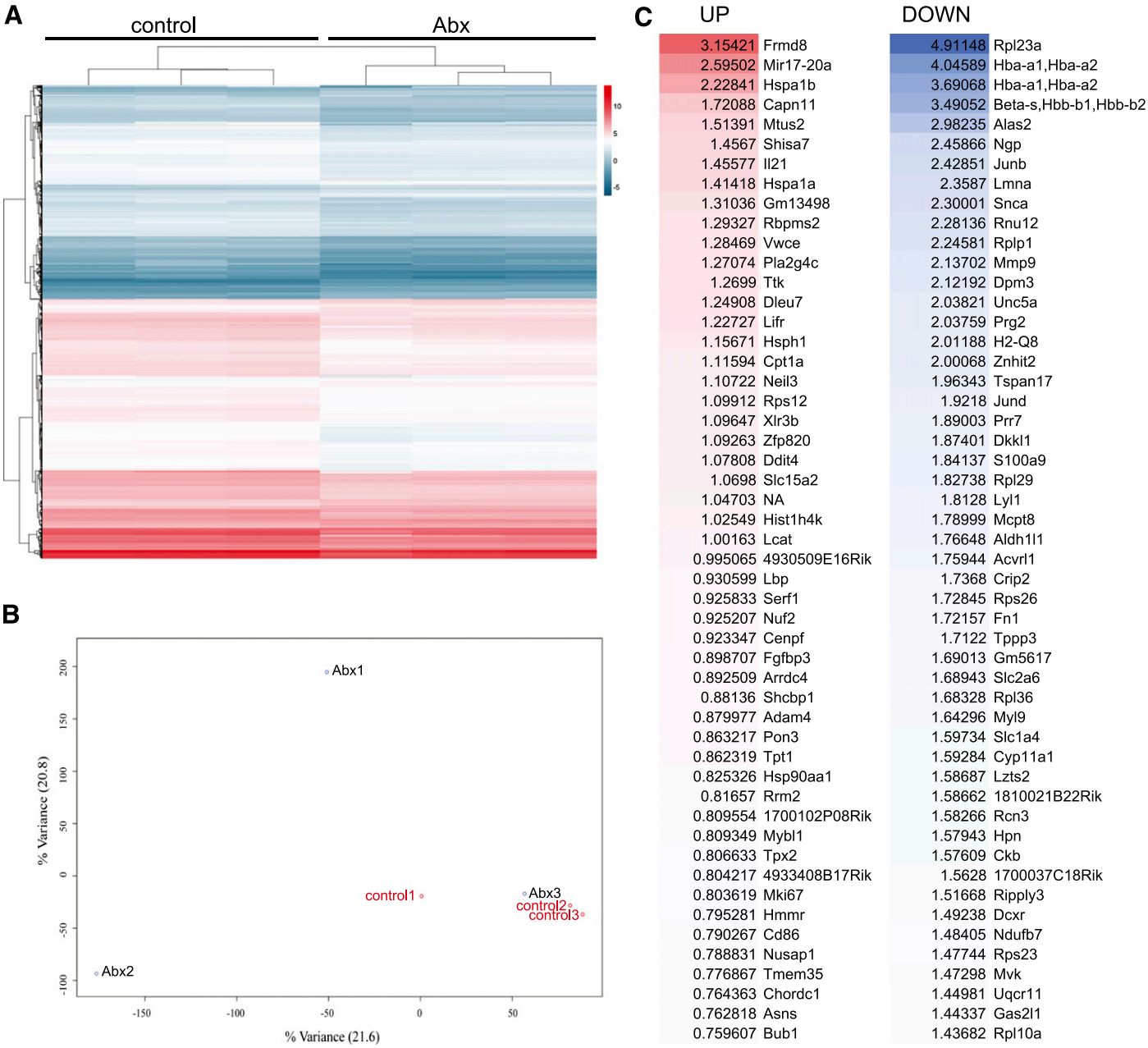
Figure 2. In vitro analysis of CD4⁺ T cells from Abx-treated mice. (A) Cytokine production by CD4⁺ T cells stimulated under nonpolarizing (left) or polarizing (right) conditions at d 3 and 4, respectively. Data shown are signature cytokines and are representative of 2 (neutral conditions) and 3 (polarizing conditions) individual experiments (n = 3–6 mice/group). (B) TCR Vβ analysis of highly enriched CD4⁺ T cells from donor mice, as assessed by flow cytometry (n = 3). (C, left) Representative FACS plot analysis of column-bound and -unbound fractions of the CBir1 (CBir1 tetramer coupled to APC)-specific CD4⁺ T cells from donor mice after enrichment with APC-specific magnetic beads (n = 3). (D) TCR reactivity to αCD3 stimulation of highly enriched CD4⁺ T cells, as assessed by calcium signaling over time by the analysis of the ratio of Fura Red and Fluo-4 (representative n = 3 mice/group shown from 3 individual experiments). Not significant where not indicated. Error bars represent SEM.

in vitro, we hypothesized that the increased disease observed could be a result of a higher frequency of microbiome-specific T cells among the transferred, naive T cell population from Abx-treated mice. However, we found that the expression of TCR Vβ chains on CD4⁺ T cells from untreated and Abx-treated mice was similar, as assessed by flow cytometric analysis (Fig. 2B), suggesting that there was not a skewed T cell population in general. In addition, we observed no difference in the frequency of commensal bacteria-specific CD4⁺ T cells in the spleens of untreated or Abx-treated mice, as measured by binding to the CBir1-specific tetramer (Fig. 2C), which is consistent with results seen in germ-free mice [14]. However, we cannot exclude that transferred CD4⁺ T cells from Abx-treated mice are enriched for a distinct antigen of the microbiome and thus, cause a faster onset of the disease. As CBir1 reactivity is elevated in a subset of Crohn's disease patients [15], our results suggest that increased frequencies of anti-commensal CD4⁺ T cells in patients are a consequence rather than the cause of the disease.

Another explanation for the heightened disease induced by CD4⁺ T cells from Abx-treated mice is increased TCR responsiveness, which could possibly lead to a faster or stronger T cell response and enhanced pathogenicity. To address this, we examined calcium flux following TCR stimulation using a fluorescence-based assay (Fig. 2D). Following stimulation of CD4⁺ T cells with antibodies against CD3, we observed that the calcium-sensitive Fluo-4/Fura Red signal was indistinguishable between T cells that were isolated from Abx-treated and those of control mice. Thus, although Abx treatment leads to T cells with increased pathogenic potential, Abx treatment does not affect TCR diversity or responsiveness.

Unbiased analysis of the donor T cells reveals a differential gene-expression profile

We next performed an unbiased gene-expression analysis (RNA-Seq) of naïve CD4⁺ T cells isolated from untreated and Abx-treated mice. RNA-Seq analysis of the naive CD4⁺ T cells revealed an aberrant RNA expression pattern in T cells from Abx-treated mice (**Fig. 3A**). Strikingly, PCA revealed that although the naïve CD4⁺ T cells from untreated mice clustered together, CD4⁺ T cells from Abx-treated mice did not cluster (Fig. 3B). This could suggest that there is not a stereotypical response to Abx treatment and that CD4⁺ T cells from individual animals might respond distinctly to a loss of commensal microbes. Nevertheless, we did observe some consistent changes in gene expression between the samples (Fig. 3C). Gene-set enrichment analysis revealed that changes in gene expression were most significantly



UP		DOWN	
3.15421	Frmd8	4.91148	Rpl23a
2.59502	Mir17-20a	4.04589	Hba-a1,Hba-a2
2.22841	Hspa1b	3.69068	Hba-a1,Hba-a2
1.72088	Capn11	3.49052	Beta-s,Hbb-b1,Hbb-b2
1.51391	Mtus2	2.98235	Alas2
1.4567	Shisa7	2.45866	Ngp
1.45577	Il21	2.42851	Junb
1.41418	Hspa1a	2.3587	Lmna
1.31036	Gm13498	2.30001	Snca
1.29327	Rbpms2	2.28136	Rnu12
1.28469	Vwce	2.24581	Rplp1
1.27074	Pla2g4c	2.13702	Mmp9
1.2699	Ttk	2.12192	Dpm3
1.24908	Dleu7	2.03821	Unc5a
1.22727	Lifr	2.03759	Prg2
1.15671	Hsph1	2.01188	H2-Q8
1.11594	Cpt1a	2.00068	Znhit2
1.10722	Neil3	1.96343	Tspan17
1.09912	Rps12	1.9218	Jund
1.09647	Xlr3b	1.89003	Prr7
1.09263	Zfp820	1.87401	Dkkl1
1.07808	Ddit4	1.84137	S100a9
1.0698	Slc15a2	1.82738	Rpl29
1.04703	NA	1.8128	Lyl1
1.02549	Hist1h4k	1.78999	Mcpt8
1.00163	Lcat	1.76648	Aldh1l1
0.995065	4930509E16Rik	1.75944	Acvrl1
0.930599	Lbp	1.7368	Crip2
0.925833	Serf1	1.72845	Rps26
0.925207	Nuf2	1.72157	Fn1
0.923347	Cenpf	1.7122	Tppp3
0.898707	Fgfbp3	1.69013	Gm5617
0.892509	Arrdc4	1.68943	Slc2a6
0.88136	Shcbp1	1.68328	Rpl36
0.879977	Adam4	1.64296	Myl9
0.863217	Pon3	1.59734	Slc1a4
0.862319	Tpt1	1.59284	Cyp11a1
0.825326	Hsp90aa1	1.58687	Lzts2
0.81657	Rrm2	1.58662	1810021B22Rik
0.809554	1700102P08Rik	1.58266	Rcn3
0.809349	Mybl1	1.57943	Hpn
0.806633	Tpx2	1.57609	Ckb
0.804217	4933408B17Rik	1.5628	1700037C18Rik
0.803619	Mki67	1.51668	Ripply3
0.795281	Hmmr	1.49238	Dcxr
0.790267	Cd86	1.48405	Ndufb7
0.788831	Nusap1	1.47744	Rps23
0.776867	Tmem35	1.47298	Mvk
0.764363	Chordc1	1.44981	Uqcr11
0.762818	Asns	1.44337	Gas2l1
0.759607	Bub1	1.43682	Rpl10a

Figure 3. Genome-wide analysis of gene expression in naïve CD4$^+$ T cells from untreated and Abx-treated mice. (A) Heat map of gene expression in naive CD4$^+$ T cells from untreated or Abx-treated mice ($n = 3$/group). (B) PCA of gene-expression results. (C) List of top 50 up- and down-regulated genes in Abx-treated CD4$^+$ T cells. Numbers represent log2-fold change.

associated with cellular metabolism, stress response, cell-cycle regulation, and cell death (**Table 1**). Thus, Abx treatment in early life led to changes in gene expression in naïve CD4$^+$ T cells from adult mice.

Cohousing of untreated and Abx-treated mice abrogates pathology

We next examined if the changes in the pathogenicity of CD4$^+$ T cells associated with Abx treatment in early life were stable or plastic. We directly assessed this by cohousing Abx-treated mice with untreated mice, 1 wk after weaning, and analyzed the cohoused mice at wk 8 postweaning. We found that cohousing the Abx-treated mice with conventionally treated mice reverted the faster onset of experimental IBD caused by CD4$^+$ T cells from Abx-treated mice (**Fig. 4A** and **B**), suggesting that the Abx-associated changes in CD4$^+$ T cell function were not fixed. We also observed trends toward reductions in expression of *Cd4*, *Tnfa*, and *Ifng* in the proximal colons of cohoused mice compared with Abx-treated mice with similar expression levels compared with control mice (Fig. 4C). Thus, these results suggest that the increased pathogenicity of CD4$^+$ T cells mediated by early-life Abx treatment was

TABLE 1. Gene set enrichment analysis of differentially expressed genes between naive CD4$^+$ T cells isolated from untreated and Abx-treated mice

Gene set	# of Genes	*P*	FDR
Stress			
Response to stress	16	1.19×10^{-8}	9.80×10^{-7}
Metabolic process			
Nucleic acids	32	8.52×10^{-14}	7.03×10^{-11}
Biopolymer	35	2.39×10^{-12}	9.84×10^{-10}
Cellular protein	25	9.4×10^{-10}	2.35×10^{-7}
Macromolecules	25	1.21×10^{-9}	2.35×10^{-7}
Protein	26	1.43×10^{-9}	2.35×10^{-7}
RNA	19	9.23×10^{-8}	4.76×10^{-6}
DNA	11	1.12×10^{-7}	5.42×10^{-6}
Regulation	18	1.70×10^{-7}	7.79×10^{-6}
Cell cycle			
Mitotic cell cycle	10	7.96×10^{-9}	9.24×10^{-7}
Cell cycle	13	1.19×10^{-8}	9.80×10^{-7}
Phase	10	2.18×10^{-8}	1.50×10^{-6}
Process	10	7.24×10^{-8}	3.98×10^{-6}
M Phase	7	2.22×10^{-6}	7.97×10^{-5}
Mitosis	6	4.35×10^{-6}	1.44×10^{-4}
Checkpoint	5	4.90×10^{-6}	1.55×10^{-4}
Regulation	8	5.09×10^{-6}	1.58×10^{-4}
Cell death			
Apoptosis	15	8.69×10^{-9}	9.24×10^{-7}
Regulation of apoptosis	10	1.23×10^{-5}	3.06×10^{-4}
Negative regulation of apoptosis	7	1.36×10^{-5}	3.20×10^{-4}
Programmed cell death	15	8.96×10^{-9}	9.24×10^{-7}
Regulation of programmed cell death	10	1.26×10^{-5}	3.06×10^{-4}
Negative regulation of programmed cell death	7	1.42×10^{-5}	3.25×10^{-4}

Gene set enrichment analysis on the top 100 up-regulated and 100 down-regulated genes was carried out using Molecular Signatures Database (MSigDB; software.broadinstitute.org/gsea/msigdb). FDR, False-discovery rate.

not stable and could be modified by re-exposure to a wild-type microbiome.

Abx-treated mice show higher levels of serum corticosterone

Our gene-expression analysis identified several gene sets that were dysregulated following Abx treatment, including stress, metabolism, proliferation, and death. As stress hormones, such as the glucocorticoid corticosterone, have been shown to affect all of these aspects of cell function [16], we first addressed whether the Abx-treated mice showed higher glucocorticoid levels compared with untreated mice. Indeed, corticosterone levels were higher in the serum of 3- and 6-wk-old Abx-treated mice and were still increased at the time point of analysis and transfer at 8 wk (Fig. 4D). In addition, we found heightened levels of corticosterone in the thymus, proximal colon, and cecum (Fig. 4E). The increased concentrations of corticosterone in the periphery of the Abx-treated mice did not have results in any increased sensitivity or resistance against corticosterone-induced apoptosis ex vivo (Fig. 4F). Thus, in addition to our results showing equivalent production of cytokines (Fig. 2A), these results suggest that the heightened levels of corticosterone in Abx-treated mice have minimal bystander-immunosuppressive effects on CD4$^+$ T cells. Taken together, Abx treatment in early life leads to increased levels of circulating corticosterone that is maintained during aging. Importantly, the cohousing of untreated and Abx-treated mice resulted in a normalization of corticosterone levels (Fig. 4D). As we observed a reduction in disease in cohoused mice (Fig. 4A), these results suggest that early-life Abx treatment leads to a stress response associated with high levels of corticosterone, which impacts upon CD4$^+$ T cell function and not resistance to corticosterone-induced apoptosis.

We show that CD4$^+$ T cells from Abx-treated mice have an altered gene-expression profile that suggests a stressed phenotype. However, this phenotype could be reverted by cohousing Abx-treated mice with untreated mice. From these results, we conclude that an early-life Abx treatment has the potential to change the behavior of naive CD4$^+$ T cells to induce a faster onset of experimental IBD. This finding is in line with previous reports of changes in the phenotypical behavior of CD4$^+$ T cells after early-life Abx treatment in a direct [17] or indirect way [18] or of gnotobiotic mice [19]. However, results from these studies demonstrate that Abx-induced changes are not permanent and can be modified, suggesting that intervention to manipulate the microbiome may provide a mechanism to affect the development of IBD later in life.

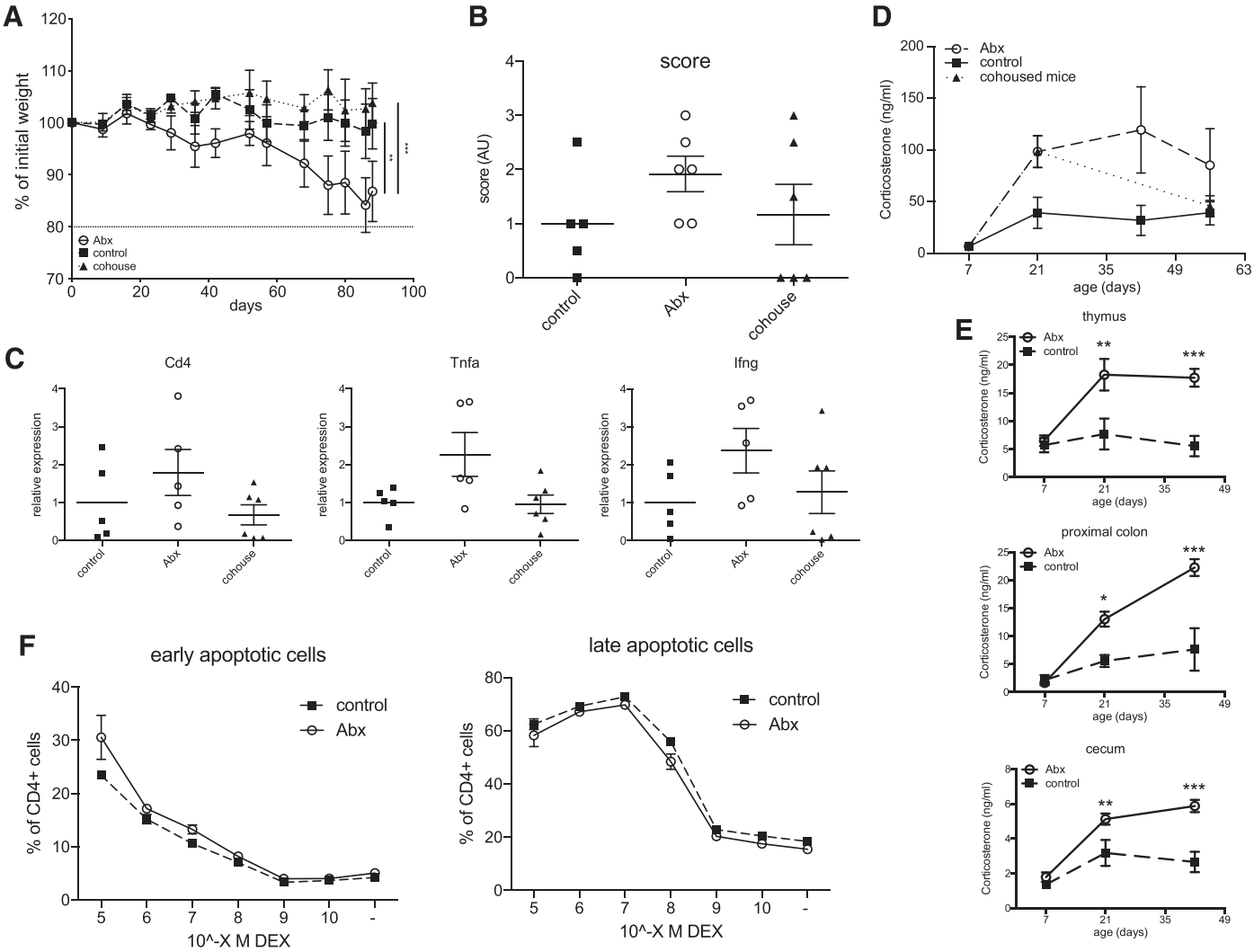


Figure 4. The cohousing of Abx-treated mice with untreated mice leads to reduced pathogenicity of CD4$^+$ T cells. (A) Weight changes of mice with experimental IBD after transfer of splenic, naive CD4$^+$ T cells from Abx-treated, control, and cohoused mice (n = 6/group). (B) Disease score of mice in A. (C) Expression analysis of the indicated genes in the proximal colon of mice in A. (D) Serum corticosterone levels of mice that were treated with Abx in utero and for the first 3 wk of life (Abx), control mice on regular water, and Abx-treated mice that were cohoused after 28 d with control mice (cohoused mice; n = 3–12/time point). (E) Corticosterone levels in the thymus, proximal colon, and cecum (same mice as in D). (F) Analysis of corticosterone-induced apoptosis of CD4$^+$ T cells. CD4$^+$ T cells from Abx and control mice were incubated with indicated concentrations of the corticosteroid DEX and analyzed after 16 h for early (Annexin V$^+$, PI$^-$) and late (Annexin V$^+$, PI$^+$) apoptotic cells (n = 3). Data shown are representative of 2 individual experiments. *$P \leq 0.05$, **$P \leq 0.01$, ***$P \leq 0.001$. Error bars represent SEM.

AUTHORSHIP

S.S., T.S.M., A.M., M.D.T., K.K.S., G.P.-W., D.D.D.C., M.L., C.H.A., and C.Z. designed experiments and analyzed results. S.S., T.S.M., A.M., M.D.T., F.A., and A.C. performed experiments. S.S. and C.Z. wrote the manuscript. S.S., T.S.M., A.M., M.D.T., K.K.S., M.L., C.H.A., and C.Z. edited the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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Early-life antibiotic treatment enhances the pathogenicity of CD4$^+$ T cells during intestinal inflammation

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