The composite human microbiome of Western populations has probably changed over the past century, brought on by new environmental triggers that often have a negative impact on human health. Here we show that consumption of a diet high in saturated (milk-derived) fat, but not polyunsaturated (safflower oil) fat, changes the conditions for microbial assemblage and promotes the expansion of a low-abundance, sulphite-reducing pathobiont, Bilophila wadsworthia. This was associated with a pro-inflammatory T helper type 1 (Th1) immune response and increased incidence of colitis in genetically susceptible Il10−/−, but not wild-type mice. These effects are mediated by milk-derived-fat-promoted taurine conjugation of hepatic bile acids, which increases the availability of organic sulphur used by sulphite-reducing microorganisms like B. wadsworthia. When mice were fed a low-fat diet supplemented with taurocholic acid, but not with glycocholic acid, for example, a bloom of B. wadsworthia and development of colitis were observed in Il10−/− mice. Together these data show that dietary fats, by promoting changes in host bile acid composition, can markedly alter promoting changes in host bile acid composition, resulting in dysbiosis that can perturb immune homeostasis. The data provide a plausible mechanistic basis by which Western-type diets high in certain saturated fats might increase the prevalence of complex immune-mediated diseases like inflammatory bowel disease in genetically susceptible hosts.

Inflammatory bowel diseases (IBD) and other immune-related human disorders are relatively ‘new’ diseases in that their incidence has increased considerably over the past half century, matching developments in cultural westernization. The rapidity of these developments are probably not caused by genetic drift, but by exposure to non-genetic factors introduced through changes in the diet and lifestyle of genetically susceptible individuals, triggering aberrant host responses that lead to IBD. In this study, we examine whether certain dietary fats present in Western diets are capable of precipitating colonic inflammation through their actions on the enteric microbiota of genetically susceptible hosts.

The effects of three different diets (Supplementary Table 1) on the enteric microbiota of specific-pathogen-free (SPF) C57BL/6 mice are shown in Fig. 1a and Supplementary Table 2. With the exception of the low-fat purified mouse diet (LF), the high-fat diets were isocaloric and differed only in the type of dietary fat used, which was held constant at 37% of total calories and closely mimics Western consumption. These fats also represent sources used in numerous processed dietary interventions for IBD patients. Il10−/− mice were fed diet groups as shown in Supplementary Table 1 for 24 weeks. General characteristics of the five diet groups are shown in Supplementary Table 2.

**Figure 1** | Saturated MF-induced colitis is associated with bloom of B. wadsworthia in Il10−/− mice. a–g. Samples from SPF C57BL/6 (a, g) (n = 6 per group) and SPF Il10−/− mice (b–g) fed MF, PUFA or LF for 24 weeks (n = 20 per group). a, Phyla representation shown for LF, PUFA and MF with means ± s.e.m. *P < 0.05 compared to LF, †P < 0.05 compared to PUFA and LF. b, Gross incidence of colitis. c, Representative colon lengths (top) and haematoxylin and eosin staining of distal colon (bottom). Scale bars, 400 μm. d, Blinded histological colitis scores. e, Distal colonic mucosal cytokines determined by ELISA. f, Principal coordinates analysis (PCoA) plot of the UNIFRAC metric matrix. PC, principal coordinate. g, qPCR of caecal content dsrA (normalized to LF diet).
and confectionary foods. Twenty-one-day exposure to the three study
diets resulted in significant differences in the structure of the enteric
microbiota as assessed by both Sanger-based and 454-based DNA
sequencing of 16S ribosomal RNA (rRNA) libraries from caecal con-
tents and stool. Both high-fat diets reduced the richness of the micro-
biota compared with LF (Supplementary Fig. 1). LF promoted
Firmicutes, but also resulted in a lower abundance of most other phyla,
whereas polyunsaturated (saflower oil) fat (PUFA) and saturated
(milk-derived) fat diets (MF) resulted in a higher abundance of
Bacteroidetes and a lower abundance of Firmicutes. Interestingly, these
changes differed from those induced by lard-based, saturated fats6,7
(Supplementary Fig. 2). Whereas MF and PUFA had similar effects
on Bacteroidetes and Firmicutes, a significant bloom of a member of
the Deltaproteobacteria, B. wadsworthia, was consistently observed
only with MF. B. wadsworthia is a sulphite-reducing, immunogenic
microbe that is difficult to detect in healthy individuals, but emerges
under pathological conditions such as appendicitis and other intestinal
inflammatory disorders8–16.

MF did not affect wild-type mice, but increased the onset and
incidence of colitis in Il10−/− mice, driving it from a spontaneous rate
of 25–30% (on LF) to over 60% in a 6-month period (Fig. 1b). In
contrast, the incidence of colitis in Il10−/− mice fed PUFA was no

Figure 2 | B. wadsworthia mono-association in GF Il10−/− mice can only be
established with consumption of MF diet, resulting in a T½1 immune
response and development of colitis. a–c: Samples from GF Il10−/−
mice ± mono-association with 10⁸ c.f.u. B. wadsworthia (Bw) maintained on
LF, PUFA or MF for 5 weeks (n = 5 per group). a, c.f.u. counts of cultured
caecal-derived B. wadsworthia. b, Blinded histological colitis scores. c, IFN-γ
production by CD4⁺ T cells in MLNs. d, IFN-γ production by MLNs from GF
C57BL/6 and Il10−/− mice colonized with either B. wadsworthia or L. murinus
(Lacto) and restimulated ex vivo with pure culture lysate from the respective
bacterium. e, In vitro CD4⁺ T-cell differentiation assay. Left, IL-12p40
produced by dendritic cells challenged with pure lysates from B. wadsworthia,
L. murinus or Alistipes (Alist). Unstim., unstimulated. Data represent pooled
values from MLN dendritic cells and splenic dendritic cells in the presence of
retinoic acid/TGF-β. Right, IFN-γ production by CD4⁺ T cells stimulated with
supernatants from the bacteria-challenged dendritic cells. Data shown
represent one out of two assays, performed in triplicate.
different than those fed LF. The colitis seen in mice fed MF was also more severe and extensive (Fig. 1c). These changes paralleled differences in histological colitis scores (Fig. 1d). Inflammatory mucosal cytokine levels from the distal colon were significantly higher compared to LF and in most cases, PUFA (Fig. 1e). LF, PUFA and MF elicited effects on the total enteric microbiota of mice that were similar to those observed in wild-type mice (shown for LF and MF, Fig. 1). Similarly, B. wadsworthia, as detected by quantitative polymerase chain reaction (qPCR) of the dissimilatory sulphite reductase gene, was unique to sulphite-reducing bacteria of which B. wadsworthia is the most prominent in our model, was found at equal relative abundance in mice on MF, independent of genotype (Fig. 1g). The bloom of B. wadsworthia induced by MF was also observed in dextran sodium sulphate (DSS)-treated SPF C57BL/6 mice, in which the onset and severity of colitis were more severe than that seen in LF- and PUFA-fed mice (Supplementary Fig. 3). Altogether these observations suggest that the bloom of sulphite-reducing Deltaproteobacteria, particularly B. wadsworthia, is associated with colitis in hosts that are genetically susceptible or have compromised mucosal barrier function.

To explore whether a MF diet was necessary for the survival and proliferation of B. wadsworthia, we mono-associated germ-free (GF) Il10−/− mice with B. wadsworthia that were consuming either LF, PUFA or MF. Five weeks after gavage, colonization of the colon could only be established in mice fed MF (Supplementary Fig. 4, shown for LF and MF), whereas on LF, B. wadsworthia was undetectable. B. wadsworthia identity was confirmed by PCR of the 16S rRNA encoding gene from caecal-derived DNA using universal primers, followed by direct sequencing of the PCR product and cultivation of B. wadsworthia from caecal contents (Fig. 2a). Additionally, qPCR analysis of luminal- versus mucosal-associated B. wadsworthia revealed a nearly 45-fold increase in the latter (Supplementary Fig. 5). Colonization of the colon could only be established in mice fed MF (Supplementary Fig. 4, shown for LF and MF), whereas on LF, B. wadsworthia was undetectable.

Figure 3 | Induction of TC bile acid following consumption of MF promotes bloom of B. wadsworthia both in vitro and in SPF Il10−/− mice, resulting in colitis. a, TC content of gall bladder aspirates from Il10−/− mice consuming LF, PUFA or MF for 5 weeks. b, Growth curve of B. wadsworthia in media containing gall bladder aspirates. c-g, Samples from SPF Il10−/− mice gavaged with PBS, TC or GC daily for 21 days while maintained on LF diet (n = 8 per group). Phyla representation with bloom of B. wadsworthia in the TC group with means ± s.e.m. *P < 0.05 compared to PBS and GC. d, Relative abundance of data in caecal contents (by qPCR and normalized to LF diet). e, f, Blinded histological colitis scores (e) and haematoxylin and eosin staining of distal colon (f). Scale bars, 400 μm. g, IFN-γ production in MLNs.
from each respective treatment and analysed for IFN-\(\gamma\) and IL-17. MLN and splenic T cells incubated with supernatants from dendritic cells exposed to \textit{B. wadsworthia} produced nearly twofold higher levels of IFN-\(\gamma\) compared to T cells incubated with supernatants of dendritic cells exposed to \textit{Alisipes} or \textit{L. murinus} (Fig. 2c, right). In accordance with in vivo data, T cells did not produce IL-17. Therefore, \textit{B. wadsworthia} seems to activate dendritic cells in a way that selectively induces a T\(_{H1}\)-mediated colitis. Of note, the possibility that other \textit{B. wadsworthia} by-products (for example, H\(_2\)S, secondary bile acids) also stimulate dendritic cells cannot be excluded.

\textit{B. wadsworthia} flourishes in the presence of taurine-conjugated (TC) bile acid (a property from which it got its name), a rich source of organic sulphur, which is used as the terminal electron acceptor of the electron transport chain resulting in the formation of H\(_2\)S as a by-product\(^{17}\). Because of their hydrophobicity, milk fats will promote growth when re-isolated from caecal content of TC-fed GF mice gavage with PBS, GC or TC for 21 days (\(n = 5\) per group). This resulted in a bloom of \textit{B. wadsworthia} with TC (Fig. 3c), nearly identical to that which was observed with consumption of MF. In contrast, GC and phosphate-buffered saline (PBS) had little effect and \textit{B. wadsworthia} remained undetectable (Fig. 3d). The bloom of \textit{B. wadsworthia} observed in TC-gavaged mice was associated with increased incidence and severity of colitis (Fig. 3e, f). T\(_{H1}\) cytokines were increased in both the mucosa (Supplementary Fig. 7) and MLNs (Fig. 3g). In further support of the role of bile in MF-diet-induced pathogenesis, mono-association with \textit{B. wadsworthia} can be established in GF \(11^{0-\gamma-}\) mice when accompanied by TC administration, but not by GC or PBS (Fig. 4a), as demonstrated by the re-isolation of \textit{B. wadsworthia} from the caecal contents of TC-fed mice (note that black colour change indicates H\(_2\)S production, Fig. 4a, left), and confirmed by colony counts (Fig. 4a, right). These mice developed colitis (Fig. 4b and Supplementary Fig. 8a) and again exhibited elevated T\(_{H1}\) mucosal responses (Fig. 4c and Supplementary Fig. 8b). Together these findings indicate that the bloom of \textit{B. wadsworthia} promoted by these dietary factors is selectively associated with T\(_{H1}\) immunity.

We find the dependence of \textit{B. wadsworthia} on diet-induced taurocholic acid intriguing and possibly representative of how certain gut microbes use bile to their advantage. Bile formation is unique to vertebrates, providing the host with the ability to digest and utilize a far greater variety of dietary substrates. Bile also has potent antimicrobial properties that can contribute to the selection or exclusion of many potential gut microbiota. However, several intestinal pathogens, including protozoa such as \textit{Giardia}, \textit{Microsporidia} and \textit{Cryptosporidia}, and bacteria such as \textit{B. wadsworthia}, \textit{H. hepaticus} and \textit{Listeria monocytogenes}, are not only bile-resistant, but highly favoured in the presence of bile\(^ {21,22}\), possibly through suppression of symbiotic, commensal microorganisms, allowing pathobionts and pathogens an opportunity to

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Mono-association with \textit{B. wadsworthia} in GF \(11^{0-\gamma-}\) mice is successful only if accompanied by TC gavage. a–c, Samples from GF \(11^{0-\gamma-}\) mice fed LF ± mono-association with \textit{B. wadsworthia} (Bw) followed by daily gavage with PBS, GC or TC for 21 days (\(n = 5\) per group). a, Robust \textit{B. wadsworthia} growth when re-isolated from caecal content of TC-fed GF mice (black film in TC plate indicates H\(_2\)S production), and c.f.u. counts of caecal content. b, Blinded histological colitis scores. c, IFN-\(\gamma\) production in MLN CD\(^4\) T cells determined by intracellular staining. d, Proposed experimental model.}
\end{figure}
establish a niche in the intestine. Once established, the by-products of these bacteria, whether H2S or secondary bile acids, can serve as gut mucosal ‘barrier breakers’, allowing for increased immune-cell infiltration and thus acting synergistically with the bacterial antigen-specific immune response to induce tissue damage. In genetically susceptible hosts, this development has the capacity to tip a compensated state of immune balance in favour of chronic disease.

METHODS SUMMARY

Extraction of bacterial DNA, clone library preparation and sequencing were conducted as previously described.32,33. Amplicon libraries were also used to target the V3–V4 region of the 16S rRNA encoding gene for deeper sequence surveys of the microbial communities. B. wadsworthia cultures were maintained in liquid and solid growth media as previously described.4. GF mice were mono-associated by oral gavage with 107 c.f.u. at a single time point. Bile acids were orally gavaged at 1 g kg−1 body weight in 100 μl PBS. Immunoculation of B. wadsworthia liquid cultures with bile aspirate was performed by collecting gall bladder contents from mice adapted to MF, PUFA or LF and anaerobically adding them to B. wadsworthia in tauroine-free media. Optical density (OD) was then measured every hour for 24 h. Re-isolation of B. wadsworthia from host was achieved by streaking 1:10 serial dilutions of 5 g caecal contents on BBE agar plates, which were allowed to grow for 9 days.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 19 October 2011; accepted 10 May 2012.

Published online 13 June 2012.

1. Walter, J. & Ley, R. The human gut microbiome: ecology and recent evolutionary changes. Annu. Rev. Microbiol. 65, 411–429 (2011).
2. Baron, E. J. Bilophila wadsworthia: a unique Gram-negative anaerobic rod. Anaerobe 3, 83–86 (1997).
3. Molodecky N. A. et al. Increasing incidence and prevalence of the inflammatory bowel diseases with time, based on systematic review. Gastroenterology 142, 46–54 (2012).
4. Maslowski, K. M. & Mackay, C. R. Diet, gut microbiota and immune responses. Nature Immunol. 12, 5–9 (2011).
5. Nhanes. Trends in intake of energy and macronutrients—Units States 1971–2000 http://www.cdc.gov/nmmwr/preview/nmwrhtml/mm5304a3.htm (2004).
6. Turnbaugh, P., Bäckhed, F., Fulton, L. & Gordon, J. I. Diet-induced obesity is linked to bile-tolerant Helicobacter species in patients with chronic liver diseases, a randomized population group, and healthy blood donors. Clin. Vaccine Immunol. 9, 1160–1164 (2002).
7. Dussurget, O., Cabanes, D., Dehoux, P. & Lecuit, M. Listeria monocytogenes bile salt hydrolase is a PrAα-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Mol. Microbiol. 4, 1098–1106 (2002).
8. Wang, Y. et al. Bile-tolerant Helicobacter bacteria and hydrogen sulphide in the etiology of ulcerative colitis. Br. J. Surg. 96, 151–158 (2009).
9. Beech, I. B. & Zinkewich, V. Screening of sulfate-reducing bacteria in colonoxy samples from healthy and colitic human gut mucosa. FEMS Microbiol. Ecol. 2, 147–155 (2000).
10. Loubinoux, J., Bronowicz, J.-P., Peireira, I. A. C., Mougnet, J. L. & Le Faou, A. E. Sulphate reducing bacteria in human faeces and their association with inflammatory diseases. FEMS Microbiol. Ecol. 40, 107–112 (2002).
11. Rowan, E. D., Docherty, N. G., Coffey, J. C. & O’Connell, P. R. Sulphate-reducing bacteria and hydrogen sulphide in the etiology of ulcerative colitis. Br. J. Surg. 96, 151–158 (2009).
12. Beech, I. B. & Zinkewich, V. Screening of sulfate-reducing bacteria in colonoxy samples from healthy and colitic human gut mucosa. FEMS Microbiol. Ecol. 2, 147–155 (2000).
13. Gibson, G. R., Cummings, J. H. & Macfarlane, G. T. Growth and activities of sulfate-reducing bacteria in gut contents of healthy subjects and patients with ulcerative colitis. FEMS Microbiol. Ecol. 86, 103–112 (1991).
14. Scanlan, P. D., Shanahan, F. & Marchesi, J. Culture-independent analysis of desulfovibrios in the human distal colon of healthy, colorectal cancer and poympolytomed individuals. FEMS Microbiol. Ecol. 69, 213–221 (2009).
15. Arze, A., Mercure, F., Treviran, R., Menozzi, G. & Botta, G. Recovery of Bilophila wadsworthia from clinical specimens in Italy. Anaerobe 3, 219–224 (1997).
16. Baron, E. J. et al. Bilophila wadsworthia isolates from clinical specimens. J. Clin. Microbiol. 30, 1862–1887 (1992).
17. Laue, H., Denger, K. & Cook, A. M. Taurine reduction in anaerobic respiration of Bilophila wadsworthia RZATAU. Appl. Environ. Microbiol. 63, 2016–2021 (1997).
18. Lindstedt, S., Avigan, J., Goodman, D. S., Svjalj, J. & Steinberg, D. The effects of dietary fat on the turnover of cholic acid and on the composition of the biliary bile acids in man. J. Clin. Invest. 44, 1754–1765 (1965).
19. Rueda, A. et al. Conjugated bile acids and intestinal flora during the preeminent stage in goat: influence of a lamb milk replacer. Arch. Physiol. Biochem. 104, 246–251 (1996).
20. Graham, T. O., Van Thiel, D. H., Little, J. M. & Lester, R. Synthesis of taurocholate by rat fetal liver in organ culture: effects of cortisol in vitro. Am. J. Physiol. 237, E177–E184 (1979).
21. Ananieva, O., Nilsson, I., Vorobjova, T., Uibo, R. & Wadstro¨m, T. Immune responses to bile-tolerant Helicobacter species in patients with chronic liver diseases, a randomized population group, and healthy blood donors. Clin. Vaccine Immunol. 9, 1160–1164 (2002).
22. Dussurget, O., Cabanes, D., Dehoux, P. & Lecuit, M. Listeria monocytogenes bile salt hydrolase is a PrAα-regulated virulence factor involved in the intestinal and hepatic phases of listeriosis. Mol. Microbiol. 4, 1098–1106 (2002).
23. Wang, Y. et al. Local mucosa-associated microbiota determine physiological expression of TLR2 and TLR4 in murine colon. PLoS ONE 5, e13607 (2010).
24. Wang, Y. et al. 16S rRNA gene-based analysis of fecal microbiota from preterm infants with and without necrotizing enterocolitis. J. Clin. Microbiol. 43, 944–954 (2009).
25. Berg, D. J. et al. Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4+ Treg-like responses. J. Clin. Invest. 4, 1010–1020 (2006).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements This work was supported by the National Center for Research Resources and the NIDDK, NIGMS and NCCAM of the National Institutes of Health through grant number DK-62065 (E.B.C.), DK47722 (E.B.C.), U19DK083993 (E.B.C.), F31AT006703 (S.D.). Also, the Gastrointestinal Research Foundation, Crohns and Colitis Foundation of America (Y.W.), the Peter and Carol Goldman Family Research Fund, and the Harry and Leona Helmsley Trust Foundation (SHARE). We are also indebted to S. Finegold for his suggestions on successful culture of B. wadsworthia and Alistipes, J. Tiedje and M. Vital for dsrA primer sequences, L. Hagey for mass spectrometry, E. Huang, B. Theriault and J. Stencel for assistance with experiments, and R. Bouzit for T-cell purification.

Author Contributions S.D. and E.B.C. were involved in all aspects of this study, especially in the development of the hypothesis, experimental plan and data analysis. Y.W., M.M.W., Y.L., H.F.-P. and A.J. helped perform the experiments. D.A.A. and B.J. provided critical feedback and expertise and assisted in the analysis of data.

Author Information Data have been deposited in GenBank under accession numbers JQ890637–JQ894320. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to E.B.C. (echang@medicine.bsd.uchicago.edu).
**METHODS**

**Mice.** All mice were bred in-house and housed in our SPF and GF animal facilities. GF mice were maintained on 18% (by weight) 37% (kcal) MF, lard or PUFA test diets formulated by Harlan-Teklad and irradiated and tested for sterility both before and after use. LF purified diet was based on AIN-93M. All bacterial gavages were performed using 10^8 c.f.u., and taurocholate and glycocholate were added to drinking water. On day 12, mice were changed back to plain tap water, and on day 15 mice were terminated. Weight loss and stool consistency was checked daily.

**Clone library sequencing.** Raw sequence data was processed using the Ribosomal Database Project’s Pipeline Tool (http://rdp.cme.msu.edu/), which includes base calling, quality trimming and alignment as part of the workflow. Additionally, chimeraic sequences were screened and removed using Bellerophon. The RDP Classifier was used to assign the 16S rRNA sequences to a hierarchical taxonomy. The program mothur was used to group sequences into operational taxonomical units (OTUs) using the furthest neighbour algorithm and a 97% sequence similarity criterion. For PCoA, all 16S rRNA gene sequences were imported into R (http://www.r-project.org/) and classified with the QIIME toolkit. Using the QIIME wrappers, OTUs were picked at 97% sequence identity using cdhit and a representative sequence was classified with the QIIME toolkit. Throughput Genome Analysis Core (HGAC; part of the Institute for Genomics and Systems Biology) at Argonne National Laboratory, Sequences were then trimmed and classified with the QIIME toolkit. Using the QIIME wrappers, OTUs were picked at 97% sequence identity using cdhit and a representative sequence was then chosen for each OTU by selecting the most abundant sequence in that OTU. These representative sequences were aligned using PyNAST and taxonomy was assigned to them using the RDP Classifier. The PyNAST-aligned sequences were also used to build a phylogenetic tree with FastTree and unweighted UniFrac distances then computed between all samples for additional ecological analyses, including PCoA.

**DSS colitis.** SPF C57BL/6 mice were fed LF, PUFA or MF for 1 week (n = 5 per group). On day 7, 1.5% DSS was added to drinking water. On day 12, mice were changed back to plain tap water, and on day 15 mice were terminated. Weight loss and stool consistency was checked daily.

**Bile composition analysis.** Bile acid conjugates in pooled gall bladder aspirates were analysed by HPLC as described previously. Conjugated bile acids were quantified in the column effluent by monitoring the absorbance at 205 nm (for the amide bond). Peaks were identified using the relative retention time of known standards. Next, electrospray mass spectrometry was performed on the pooled gall bladder aspirates using a Perkin-Elmer Sciex API-III instrument modified with a nanoelectrospray source. The instrument was operated in the negative mode with Q1 IS voltage set to 600 V. The IN and ORI voltages were set to 110 V and 90 V respectively. Chemical identity of peaks was confirmed by the fragmentation pattern of selected ions (Q3 mode) using argon collision gas. The presence of conjugated bile salts was confirmed by selection for m/z 74 (glycine), m/z 97 (sulphate), m/z 124 (taurine).

**Sulphite-reducing bacteria quantification.** Sulphite-reducing bacteria were quantified using specific primers developed by M. Vital and J. Tiedje (MSU) for the dsrA gene. Forward, 5'-CCTTGAGCCTGCGCTCCA-3'; reverse, 5'-CTGCAGACCTAATGAGG-3'.

Data are available to the public via the MG-RAST system (http://metagenomics.anl.gov/), including instant availability of the sequence data, bioinformatic analyses and tools, plus the support for export to QiIME.

**T-cell and dendritic-cell purification.** For CD4+ T-cell isolation, spleens and MLNs were mechanically disrupted through a 70-μm cell strainer. CD4+ T cells were isolated first by CD25 negative selection using anti-CD25 APC with anti-APC microbeads on automacs. This was followed by positive immunoselection using CD4+ (L3T4) microbeads (Miltenyi Biotec).

For dendritic-cell isolation, MLN and spleen were digested with 400 units ml^-1 collagenase type IV (Sigma-Aldrich). Cells were filtered, resuspended in 22.5% Optiprep (Sigma-Aldrich), overlaid with Hank’s buffered saline (HBS) and centrifuged at 670g for 30 min. Dendritic cells were then enriched from the interface by positive immunomagnetic selection using anti-CD11c-coated beads according to the manufacturer’s recommendations (Miltenyi Biotec). Purification yielded up to 90% CD11c+ cells.

**In vitro T-cell differentiation assay.** Dendritic cells from MLNs and spleen plus retinoic acid/TGF-β were incubated for 24 h with 25 μg ml^-1 lysate from either *B. wadsworthia*, *Alistipes* or *L. murinus*. Supernatants were analysed for IL-12 and IL-10 by ELISA and diluted 25%, then applied to purified CD4+ T cells stimulated with plate-bound anti-CD3 (1 μg ml^-1) and anti-CD28 (2 μg ml^-1) for 3 days. The supernatants from these cultures were then analysed for IFN-γ and IL-17.

**Bile acid conjugates in pooled gall bladder aspirates.** The following conjugated antibodies were purchased from E Bioscience (San Diego): CD4 (GK1.5), CD11c (N418), IFN-γ (XMG1.2), IL-17 (eBio17B7) and isotype controls. Cells were permeabilized with the CytoFix/CytoPerm kit (BD Biosciences) for intra-cytoplasmic detection of IFN-γ and IL-17 cytokines. Flow cytometry analysis was performed with a FACSan (BD Biosciences).

26. Rossi, S. S., Converse, J. L. & Hofmann, A. F. High pressure liquid chromatographic analysis of conjugated bile acids in human bile: simultaneous resolution of sulfated and unsulfated lithocholyl amidates and the common conjugates bile acids. *J. Lipid Res.* 28, 589–595 (1987).