MICROBIOTA

Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation

Koji Atarashi,[1,2] Wataru Suda,[1,3,4] Chengwei Luo,[5,6] Takaaki Kawaguchi,[1,2] Iori Motoo,[2] Seiko Narushima,[2] Yuya Kiguchi,[3] Keiko Yasuma,[1] Eiichiro Watanabe,[2] Takeshi Tanoue,[1,2] Christoph A. Thaiss,[7] Mayuko Sato,[8] Kiminori Toyooka,[8] Heba S. Said,[4,9] Hirokazu Yamagami,[10] Scott A. Rice,[11] Dirk Gevers,[5] Ryan C. Johnson,[12] Julia A. Segre,[12] Kong Chen,[13] Jay K. Kolls,[13] Eran Elinav,[7] Hidetoshi Morita,[14] Ramnik J. Xavier,[5,6] Masahira Hattori,[3,4]* Kenya Honda[1,2]*

Intestinal colonization by bacteria of oral origin has been correlated with several negative health outcomes, including inflammatory bowel disease. However, a causal role of oral bacteria ectopically colonizing the intestine remains unclear. Using gnotobiotic techniques, we show that strains of *Klebsiella* spp. isolated from the salivary microbiota are strong inducers of T helper 1 (T_H1) cells when they colonize in the gut. These *Klebsiella* strains are resistant to multiple antibiotics, tend to colonize when the intestinal microbiota is dysbiotic, and elicit a severe gut inflammation in the context of a genetically susceptible host. Our findings suggest that the oral cavity may serve as a reservoir for potential intestinal pathobionts that can exacerbate intestinal disease.

The average person generates and ingests ~1.5 liters of saliva per day, containing an enormous number of oral-resident bacteria (*1*, *2*). Ingested oral bacteria poorly colonize the healthy intestine (*3*); however, increased levels of microbes of oral origin have been reported in the gut microbiota of patients with several diseases, including inflammatory bowel disease (IBD) (*4*), HIV infection (*5*, *6*), liver cirrhosis (*7*, *8*), and colon cancer (*9*). For instance, the presence of oral bacteria such as Veillonellaceae and Fusobacteriaceae in the intestinal mucosal microbiota strongly correlates with disease status in Crohn's disease (CD) (*4*). Mining of our in-house data sets of 16*S* ribosomal RNA (rRNA) gene sequences revealed that several bacterial taxa—including species belonging to *Rothia*, *Streptococcus*, *Neisseria*, *Prevotella*, and *Gemella* (table S1A), all of which are aerotolerant and typically members of the oral microbiota—were significantly more abundant in the fecal microbiota of patients with ulcerative colitis (UC), primary sclerosing cholangitis (PSC), gastroesophageal reflux disease (GERD) being treated by long-term proton pump inhibitor therapy, and alcoholism, compared with that of healthy controls (Fig. 1A and table S1B). Thus, we hypothesized that a subset of oral microbiota may ectopically colonize and persist in the intestine under certain circumstances to aberrantly activate the intestinal immune system, resulting in chronic inflammatory diseases.

To search the human oral microbiota for bacterial strains showing strong immune-stimulatory activities upon intestinal colonization, we transplanted saliva samples from two patients with CD into C57BL/6 (B6) germ-free (GF) mice by gavage. Each group of mice was housed in separate gnotobiotic isolators for 6 weeks, at which time small intestinal and colonic lamina propria (LP) immune cells were examined. In mice receiving a saliva sample from CD patient #1 (GF+CD#1 mice), there were no significant changes in the intestinal T cells (Fig. 1B). In contrast, in the group that received a saliva sample from CD patient #2 (GF+CD#2 mice), we noticed a marked accumulation of interferon-γ^+ (IFN-γ^+) $CD4^+$ T cells [T helper 1 (T_H1) cells] in the intestinal LP (Fig. 1B). Using 16*S* rRNA gene sequencing, we compared the community composition of the saliva microbiota before administration into GF mice and the fecal microbiota of the colonized animals (Fig. 1C). Although the saliva samples of both patients contained similar microbial communities, the fecal microbiota compositions differed markedly between GF+CD#2 mice and GF+CD#1 mice (Fig. 1C). Importantly, most of the bacterial species observed in the fecal microbiota of the mice had been minor components of the salivary microbiota (Fig. 1C). These results indicate that bacterial species that constitute a small fraction of the oral microbiota can expand and colonize the gut, and a subset of these oral species can induce the accumulation of intestinal T_H1 cells.

To isolate T_H1 cell–inducing bacteria, we anaerobically cultured cecal contents from GF+CD#2 mice using several culture media and picked 224 colonies with different colony appearances. Sequencing of the 16*S* rRNA genes revealed that these colonies contained eight strains from diverse genera—including *Gemella*, *Bifidobacterium*, *Streptococcus*, *Escherichia*, *Fusobacterium*, *Veillonella*, *Anaerococcus*, and *Klebsiella*—and broadly represented the major members of the gut microbiota colonizing GF+CD#2 mice (Fig. 1C). To examine whether these isolated strains had T_H1 cell–inducing capability, we cultured all eight of them and introduced them as a mixture (8-mix) into GF mice. We observed efficient induction of T_H1 cells in the colonic LP of these mice, with a magnitude comparable to that observed in GF+CD#2 mice (compare Fig. 1, B and D). Because *Fusobacterium* and *Veillonella* have been implicated in IBD pathogenesis (*4*), we colonized mice with strains of these two genera (strain IDs Fu-21f and Ve-2E1, respectively); however, this resulted in only marginal elevation of T_H1 cell frequency (Fig. 1D). We tested *Klebsiella pneumoniae* 2H7 (Kp-2H7) because it was the most prominent component of the GF+CD#2 microbiota (Fig. 1C). Oral administration of Kp-2H7 alone significantly induced T_H1 cells, whereas a mixture of the remaining seven strains (7-mix) failed to do so (Fig. 1D), indicating that the Kp-2H7 strain was the major contributor to the accumulation of T_H1 cells observed in GF+CD#2 mice. The effect of Kp-2H7 was relatively specific for T_H1 cells (fig. S1A), which were negative for interleukin-17 (IL-17), RORγt, and Foxp3 but positive for T-bet and CD44 (fig. S1B). Kp-2H7 mainly colonized the colon and cecum (fig. S1C), reflecting greater T_H1 cell induction in the colon than in the small intestine (fig. S1D). There was no increase in the percentage of T_H1 cells in the oral tissues (palate and tongue) of B6 GF+Kp-2H7 mice (fig. S1E). The increase in T_H1 cells was observed in IQI/Jic mice and B6 mice, but not in BALB/c mice (fig. S1F), implying interplay between host genotype and Kp-2H7 for colonic T_H1 cell induction.

Klebsiella spp. often acquire resistance to multiple antibiotics and can be a cause of health care–associated infection (*10*–*12*). Our isolate Kp-2H7 was resistant to multiple antibiotics, including ampicillin (Amp), tylosin (Tyl), spectinomycin

[1]Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. [2]RIKEN Center for Integrative Medical Sciences, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan. [3]Graduate School of Frontier Sciences, The University of Tokyo, 5-1-5 Kashiwanoha, Kashiwa, Chiba 277-8561, Japan. [4]Cooperative Major in Advanced Health Science, Graduate School of Advanced Science and Engineering, Waseda University, Tokyo, Japan. [5]Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA. [6]Center for Computational and Integrative Biology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, USA. [7]Department of Immunology, Weizmann Institute of Science, Rehovot 76100, Israel. [8]RIKEN Center for Sustainable Resource Science, 1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan. [9]Department of Microbiology and Immunology, Faculty of Pharmacy, Mansoura University, Mansoura 35516, Egypt. [10]Department of Gastroenterology, Osaka City University Graduate School of Medicine, Osaka, Japan. [11]The Singapore Centre for Environmental Life Sciences Engineering, The School of Biological Sciences, Nanyang Technological University, Singapore. [12]Microbial Genomics Section, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892, USA. [13]Richard King Mellon Foundation Institute for Pediatric Research, Department of Pediatrics, University of Pittsburgh School of Medicine, Pittsburgh, PA 15224, USA. [14]Graduate School of Environmental and Life Science, Okayama University, Okayama, Japan
*Corresponding author. Email: m-hattori@aoni.waseda.jp (M.H.); kenya@keio.jp (K.H.)

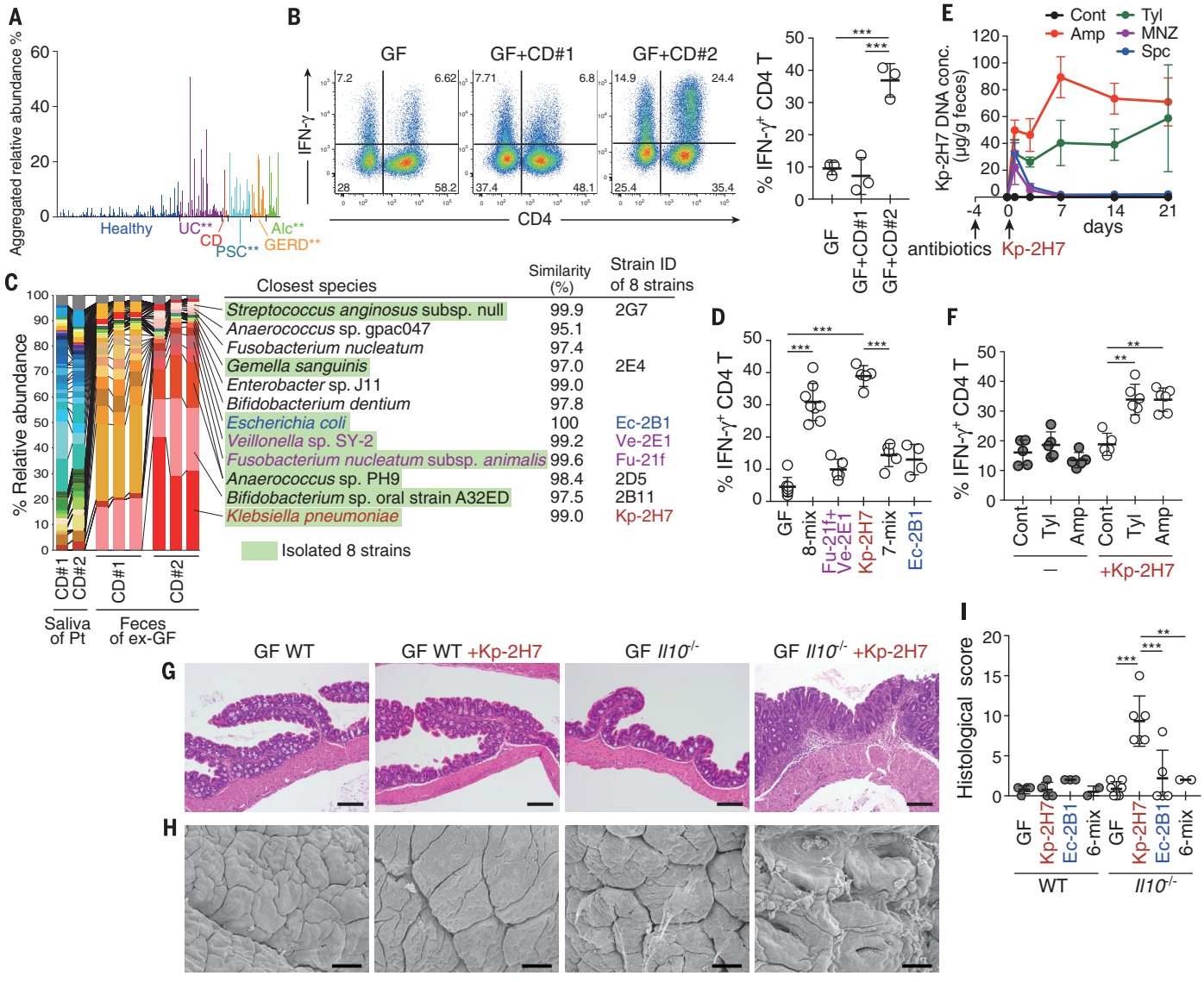

Fig. 1. Isolation of a T_H1 cell–inducing, multiple antibiotic–resistant, and proinflammatory *Klebsiella pneumoniae* strain from the microbiota of human saliva. (**A**) Aggregated relative abundance of operational taxonomic units (OTUs) typical of human oral microbiota in the fecal microbiota of healthy individuals (n = 150), patients with ulcerative colitis (UC; n = 51), Crohn's disease (CD; n = 7), primary sclerosing cholangitis (PSC; n = 27), gastroesophageal reflux disease (GERD; n = 18), and alcoholism (Alc; n = 16). $^{**}P < 0.001$; Wilcoxon rank-sum test (fig. S1B). (**B**) Representative FACS (fluorescence-activated cell sorting) plots (left) and frequencies of IFN-γ^+ cells (right) among colonic LP CD4$^+$TCRβ^+ T cells from ex–germ-free (exGF) B6 mice inoculated with saliva samples from patients with CD. Each point (right) represents an individual mouse. (**C**) Pyrosequencing of 16S rRNA genes from the saliva microbiota of patients (Pt) and from the resulting fecal microbiota of exGF mice (n = 3 per group). Quality filter–passed sequences were classified into OTUs on the basis of sequence similarity (96% identity), and the relative abundance of OTUs and closest known species for each OTU are shown. OTUs corresponding to the eight isolated strains are marked in green. (**D**) The percentage of T_H1 cells in the colonic LP of exGF B6 mice colonized with 8-mix, Fu-21f+Ve-2E1, Kp-2H7, 7-mix, or Ec-2B1. (**E** and **F**) SPF B6 mice were untreated (Cont) or continuously treated with antibiotics in drinking water, starting 4 days before oral administration of 2×10^8 colony-forming units (CFU) of Kp-2H7. The relative abundance of Kp-2H7 DNA over time in fecal samples was determined by qPCR (E). The percentage of T_H1 cells among colonic LP CD4$^+$ cells was analyzed by flow cytometry on day 21 after Kp-2H7 administration (F). Amp, ampicillin; Tyl, tylosin; Spc, spectinomycin; MNZ, metronidazole. (**G** to **I**) Representative hematoxylin and eosin staining (G), representative scanning electron micrograph (SEM) (H), and histological colitis scores (I) of the proximal colon of Kp-2H7–, Ec-2B1–, or 6-mix–colonized WT or *Il10*$^{-/-}$ mice. Scale bars, 200 μm (G) and 30 μm (H). Each point in (B), (D), and (F) represents an individual mouse (thick bars, means); points in (E) represent means of a group of six mice. Error bars, SD. $^{**}P < 0.01$; $^{***}P < 0.001$; one-way analysis of variance (ANOVA) with post hoc Tukey's test. Data represent at least two independent experiments with similar results.

(Spc), and metronidazole (MNZ) (fig. S2). Specific-pathogen-free (SPF) mice were untreated or continuously treated with Amp, Tyl, Spc, or MNZ in drinking water starting 4 days before oral gavage with Kp-2H7. Antibiotic-naïve mice were resistant to colonization by Kp-2H7, but Amp or Tyl treatment allowed Kp-2H7 to persist in the intestine (Fig. 1E). Persistent colonization with Kp-2H7 was accompanied by a significant increase in the frequency of colonic T_H1 cells (Fig. 1F). In contrast to Amp and Tyl treatments, MNZ and Spc treatments did not allow Kp-2H7 colonization, although the Kp-2H7 strain is resistant to both of these antibiotics (Fig. 1E). These results suggest that antibiotic exposure potentiates orally

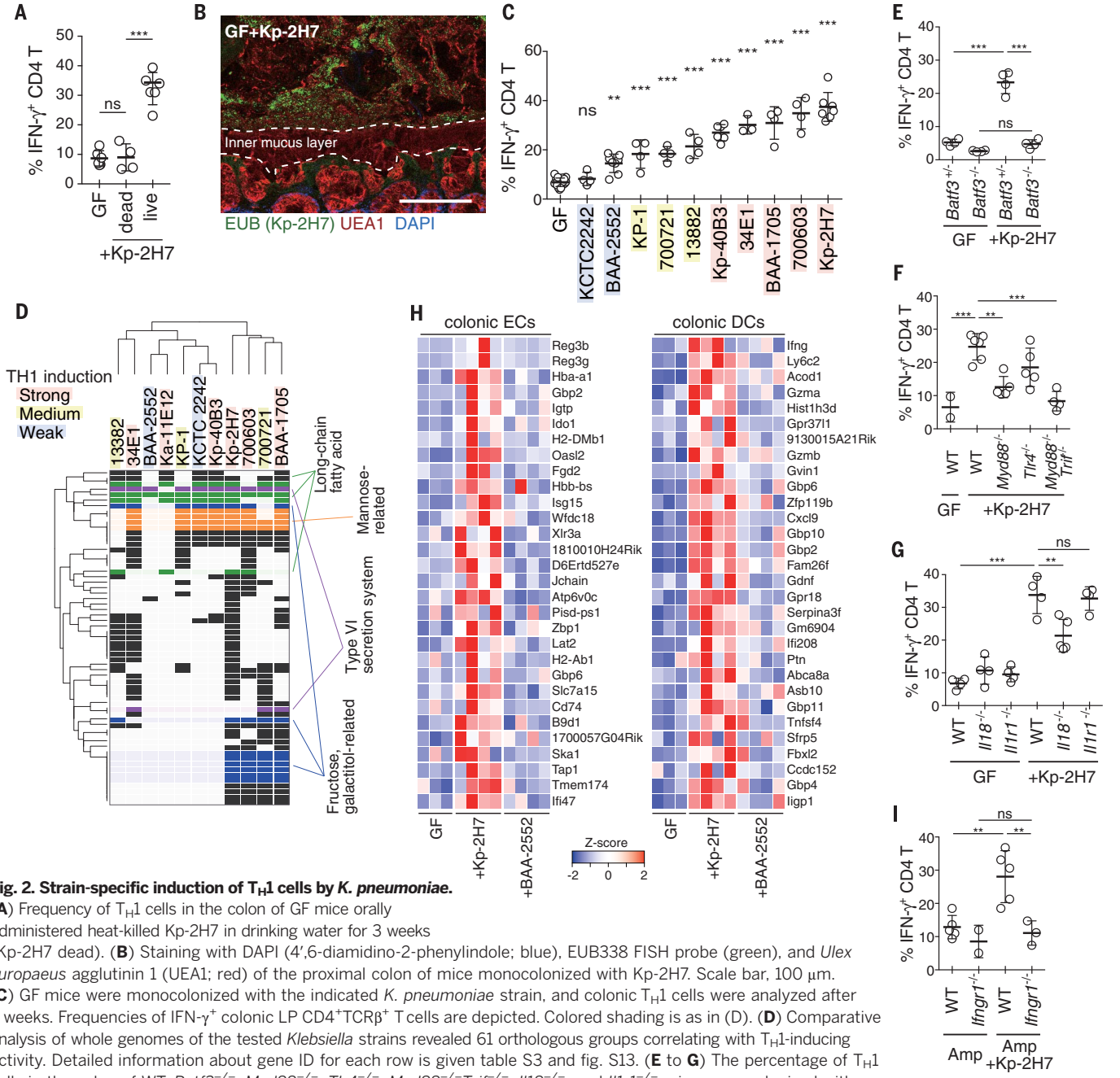

Fig. 2. Strain-specific induction of T_H1 cells by *K. pneumoniae*. (**A**) Frequency of T_H1 cells in the colon of GF mice orally administered heat-killed Kp-2H7 in drinking water for 3 weeks (Kp-2H7 dead). (**B**) Staining with DAPI (4′,6-diamidino-2-phenylindole; blue), EUB338 FISH probe (green), and *Ulex europaeus* agglutinin 1 (UEA1; red) of the proximal colon of mice monocolonized with Kp-2H7. Scale bar, 100 μm. (**C**) GF mice were monocolonized with the indicated *K. pneumoniae* strain, and colonic T_H1 cells were analyzed after 3 weeks. Frequencies of IFN-γ^+ colonic LP CD4$^+$TCRβ$^+$ T cells are depicted. Colored shading is as in (D). (**D**) Comparative analysis of whole genomes of the tested *Klebsiella* strains revealed 61 orthologous groups correlating with T_H1-inducing activity. Detailed information about gene ID for each row is given table S3 and fig. S13. (**E** to **G**) The percentage of T_H1 cells in the colon of WT, *Batf3*$^{-/-}$, *Myd88*$^{-/-}$, *Tlr4*$^{-/-}$, *Myd88*$^{-/-}$*Trif*$^{-/-}$, *Il18*$^{-/-}$, and *Il1r1*$^{-/-}$ mice monocolonized with Kp-2H7. (**H**) Differential gene expression in the colonic ECs and DCs from WT mice monocolonized with Kp-2H7 or BAA-2552 for 1 week. Heatmap colors represent the *z*-score–normalized FPKM (fragments per kilobase of exon per million fragments mapped) values for each gene. (**I**) SPF WT and *Ifngr1*$^{-/-}$ mice were treated with Amp and gavaged with Kp-2H7 as in Fig. 1E. The percentage of T_H1 cells among colonic LP CD4$^+$ cells is depicted. Symbols represent individual mice. Thick bars, means; error bars, SD. $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant ($P > 0.05$); one-way ANOVA with post hoc Tukey's test. Data represent at least two independent experiments with similar results.

derived Kp-2H7 colonization by disrupting the colonization resistance provided by specific members of the gut microbiota that are Amp- and Tyl-sensitive but MNZ- and Spc-resistant.

Despite induction of T_H1 cells, Kp-2H7 colonization did not induce any inflammatory changes in the intestine of wild-type (WT) hosts—either GF mice or Amp-treated SPF mice (Fig. 1, G to I, and fig. S3, A and B). Because microbial and host genetic factors both contribute to the pathogenesis of IBD (*13*), we tested the influence of Kp-2H7 colonization in colitis-prone *Il10*$^{-/-}$ mice. GF WT and GF *Il10*$^{-/-}$ mice were orally administered Kp-2H7, *Escherichia coli* 2B1 (Ec-2B1), or a mixture of the six other strains (6-mix). *K. pneumoniae* and *E. coli* are both in the family Enterobacteriaceae, which has been implicated in IBD pathogenesis (*4*, *14*). One week after colonization, more potent induction of colonic T_H1 cells was observed in *Il10*$^{-/-}$+Kp-2H7 mice than in the other groups of mice (fig. S3C). In addition, there was a greater induction of colonic LP IL-17$^+$IFN-γ^+ CD4$^+$ T cells (fig. S3C) and epithelial tumor necrosis factor–α mRNA expression (fig. S3D) in the *Il10*$^{-/-}$+Kp-2H7 mice. Histological analysis revealed that Kp-2H7 induced more severe inflammation than Ec-2B1 or the 6-mix in the proximal colon of *Il10*$^{-/-}$ mice (Fig. 1, G to I, and fig. S3E). Colitis exacerbation was similarly observed in Amp-treated SPF *Il10*$^{-/-}$ mice colonized with Kp-2H7 (fig. S3, A and

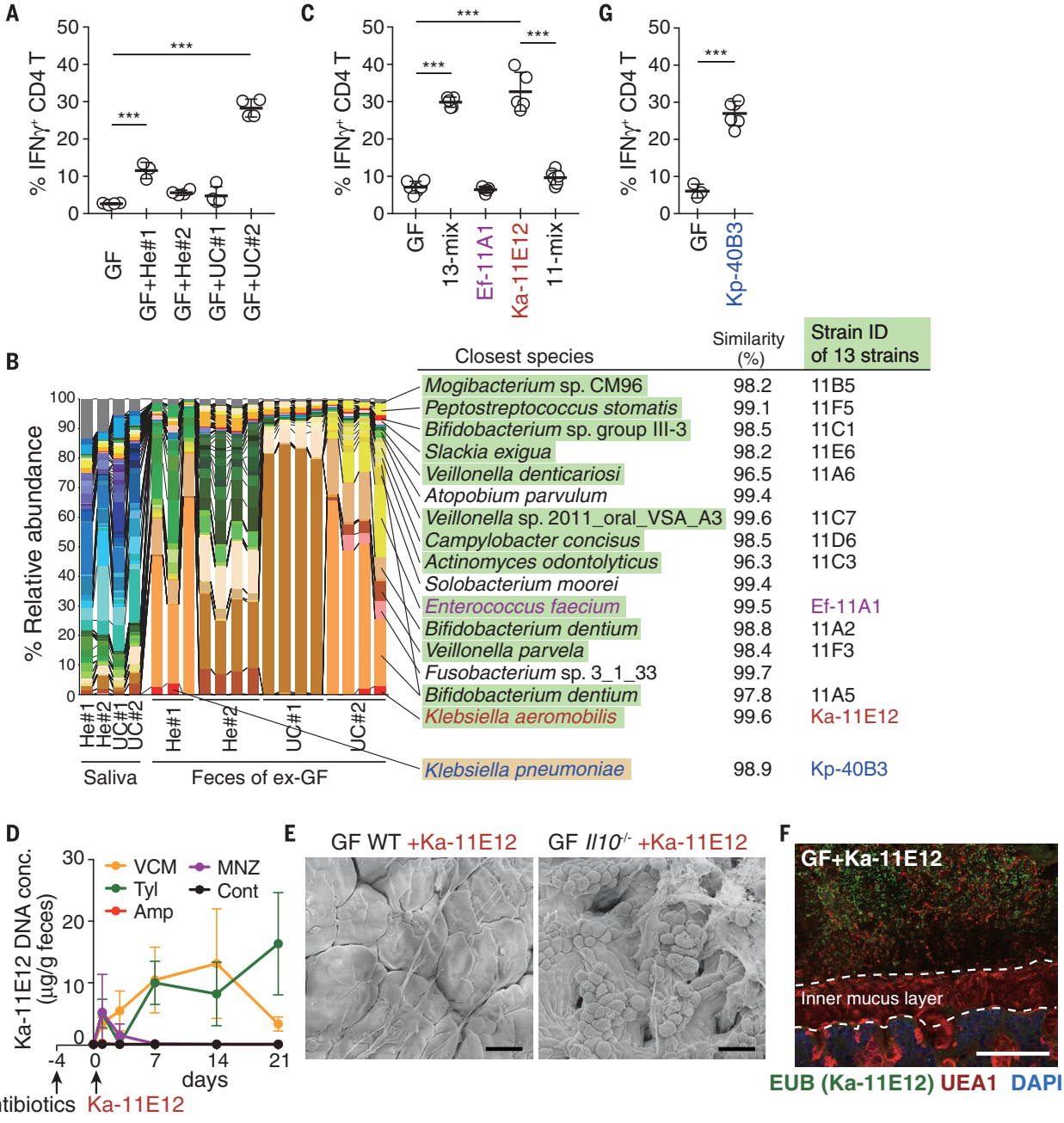

Fig. 3. *Klebsiella aeromobilis* is another T_H1 cell–inducing oral cavity-derived bacterial species. (**A**) Frequencies of IFNγ$^+$ within colonic LP CD4$^+$TCRβ$^+$ T cells from exGF mice inoculated with saliva samples from healthy donors and UC patients. (**B**) Pyrosequencing of 16S rRNA genes of the saliva microbiota of healthy controls and patients with UC and of the resulting fecal microbiota of exGF mice (n = 3 to 4 mice per group). The relative abundance of OTUs and closest known species for each OTU are shown. OTUs corresponding to the isolated 13 strains and Kp-40B3 are marked in green and yellow, respectively. (**C**) The percentage of T_H1 cells in the colonic LP of B6 mice colonized with 13-mix, Ef-11A1, Ka-11E12, or 11-mix. (**D**) SPF B6 mice were untreated or continuously treated with antibiotics in drinking water starting 4 days before oral administration of 2 × 10^8 CFU of Ka-11E12. Ka-11E12 abundance in fecal samples was determined by qPCR. (**E**) Representative SEM images of the colon of Ka-11E12–monocolonized WT or *Il10*$^{-/-}$ mice. (**F**) Staining with DAPI (blue), EUB338 FISH probe (green), and UEA1 (red) of the colon of mice monocolonized with Ka-11E12. Scale bars, 30 μm (E) and 100 μm (F). (**G**) The percentage of T_H1 cells in the colonic LP of B6 mice colonized with Kp-40B3. Each point in (A), (C), and (G) represents an individual mouse (thick bars, means); points in (D) represent means of a group of five or six mice. Error bars, SD. ***P < 0.001; one-way ANOVA with post hoc Tukey's test [(A) and (C)] and two-tailed unpaired Student's t test (G).

B). These results suggest that Kp-2H7 acts as a gut pathobiont in the context of a genetically susceptible host. When Kp-2H7 was intratracheally injected into the lung, a T_H17 response predominated over a T_H1 response in lung T cells, consistent with previous reports (*15*, *16*), and this response was accompanied by severe lung pathology, even in genetically normal mice (fig. S4). Therefore, the effect of Kp-2H7 on T_H1 induction appears to be a gut-specific response.

To investigate the mechanism of Kp-2H7–mediated T_H1 cell induction, we orally administered heat-killed Kp-2H7 to GF WT B6 mice in drinking water for 3 weeks. Heat-killed bacteria had no effect on the frequency of T_H1 cells (Fig. 2A). Fluorescence in situ hybridization (FISH) of the colon of mice colonized with Kp-2H7 revealed the presence of Kp-2H7 on the mucus layer, with no

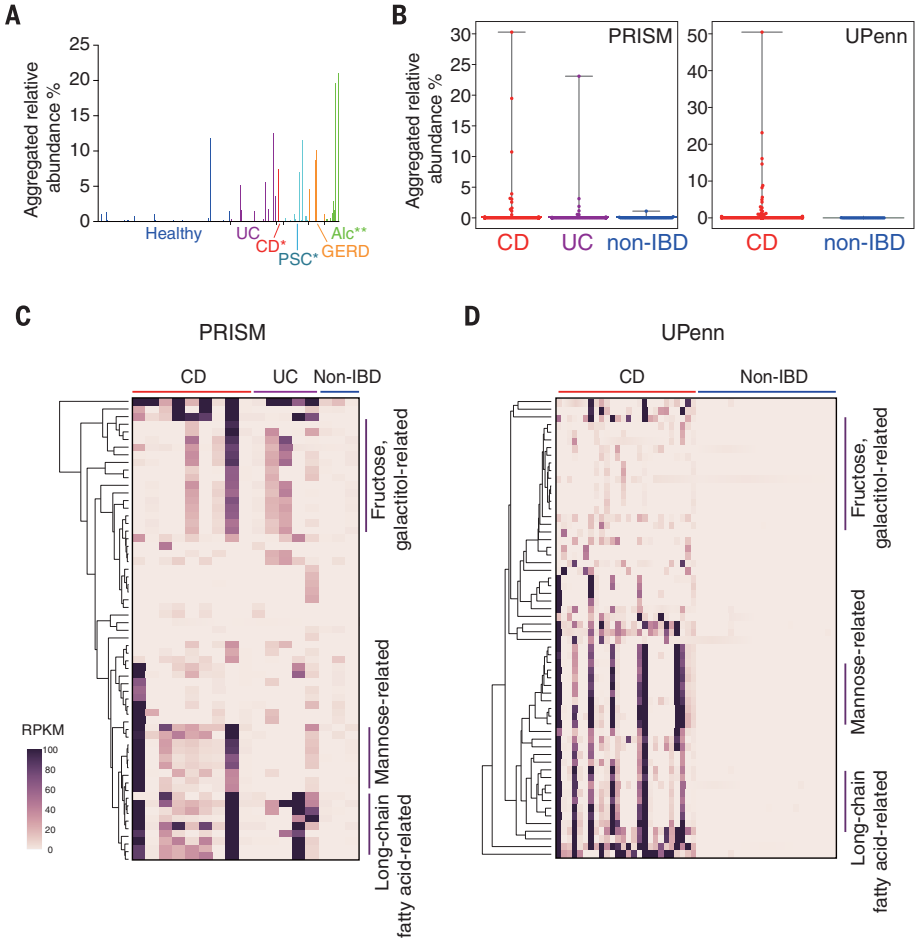

Fig. 4. Association of *Klebsiella* species and T_H1-related genes with IBD. (**A**) Aggregated relative abundance of OTUs assigned to the genus *Klebsiella* in the samples from healthy donors and patients with the indicated diseases. *$P < 0.05$; **$P < 0.01$; Wilcoxon rank-sum test. (**B** to **D**) The reads of individual samples from PRISM and UPenn cohorts were mapped to *Klebsiella* species (B) and to the gene sequences correlated with T_H1 induction [(C) and (D)]. Detailed information about gene ID for each row is given in table S3 and fig. S13. Bars in (B) represent mean values, with error bars indicating the range. Heatmaps in [(C) and (D)] show values of reads per kilobase per million reads (RPKM) for the T_H1-related genes.

evidence of adhesion or invasion of bacteria on or into the epithelial layer (Fig. 2B). Phylogenetically distinct human, mouse, and environmental *K. pneumoniae* strains (fig. S5A and table S2) showed considerable variability in the ability to elicit colonic T_H1 cell induction when monocolonized in B6 WT mice (Fig. 2C). In particular, KCTC2242 and BAA2552 did not affect, or only weakly affected, T_H1 cell frequency. The intensity of induction was independent of bacterial load and was not accompanied by inflammation (fig. S5B). There was no correlation between T_H1 cell induction and multilocus sequence typing, K-typing, or phylogeny (fig. S5A and table S2). Comparative analysis of the whole genomes revealed that 61 orthologous groups of genes were positively correlated with the T_H1 induction ability, including genes predicted to encode hemolysin-coregulated protein (Hcp) and enzymes involved in fructose-, galactitol-, mannose-, and long-chain fatty acid–related uptake and metabolic pathways (Fig. 2D and table S3). These genes have been reported to be enriched in the fecal microbiome of patients with inflammatory diseases and have been suggested to have immunomodulatory effects, and they thereby may contribute to the induction of T_H1 cells (*8*, *14*, *17*–*19*).

Mice deficient in basic leucine zipper transcription factor ATF-like 3 (*Batf3*$^{-/-}$), which lack the intestinal CD11b$^-$CD103$^+$ dendritic cell (DC) subset, failed to mount a colonic T_H1 cell response (Fig. 2E). Assessment of the antigen specificity of colonic T_H1 cells induced by Kp-2H7 revealed that a substantial fraction recognized *Klebsiella* antigens (fig. S6), including OmpX (outer membrane protein X) (*20*). Colonic T_H1 induction by Kp-2H7 monocolonization was significantly suppressed in *Myd88*$^{-/-}$ and *Myd88*$^{-/-}$ *Trif*$^{-/-}$ mice and partially attenuated in *Tlr4*$^{-/-}$ mice and *Il18*$^{-/-}$ mice, but occurred normally in *Il1r1*$^{-/-}$ mice (Fig. 2, F and G). Increased production of IL-18 by colonic epithelial cells (ECs) was detected after monocolonization with Kp-2H7 (fig. S6B). Together, these results suggest contributions from Toll-like receptors (TLRs) and IL-18 signaling to the DC-mediated *Klebsiella* antigen–specific T_H1 cell induction.

RNA-sequencing (RNA-seq) data for colonic ECs and CD11c$^+$ DCs isolated from WT mice monocolonized with Kp-2H7 or BAA2552 for 1 week, and from WT, *Myd88*$^{-/-}$, *Myd88*$^{-/-}$*Trif*$^{-/-}$, and *Tlr4*$^{-/-}$ mice monocolonized with Kp-2H7 for 3 weeks, were compared with data for GF mice (Fig. 2H and figs. S7 and S8). IFN-inducible (IFI) genes—such as those encoding guanylate-binding proteins (GBPs), chemokine (C-X-C motif) ligand 9 (Cxcl9), major histocompatibility complex–related molecules (MHCs; e.g., *H2-DMb1*, *H2-Ab1*, and *Tap1*), and dual oxidase 2 (Duox2)—were significantly up-regulated in colonic ECs and DCs from GF WT+Kp-2H7 mice. Expression differences were confirmed by quantitative polymerase chain reaction (qPCR) analysis (fig. S9A). The up-regulation of IFI genes in colonic ECs of GF+Kp-2H7 mice began within 3 days of colonization (fig. S9B), when T_H1 induction was limited (fig. S9C). Bacteria-free cecal suspensions from GF+Kp-2H7 mice up-regulated IFI genes in a colonic EC line in vitro (fig. S10), suggesting direct effects of bacterial products on the induction of IFI genes. In the later phase of Kp-2H7 colonization, IFI genes were further up-regulated, coinciding with the increase of T_H1 cells (fig. S9, A to C). GBPs are known to function as microbial receptors (*21*). MHCs and Cxcl9 mediate development and recruitment of T_H1 cells. Duox2 mediates production of hydrogen peroxide, which may facilitate further colonization of reactive oxygen–tolerant *Klebsiella* spp. (*22*, *23*). IFN-γ receptor 1–deficient (*IFNγR1*$^{-/-}$) mice mounted a defective T_H1 cell response upon colonization with Kp-2H7 (Fig. 2I). These results suggest that a feedforward loop involving IFN-γ and IFIs was created among ECs, DCs, and T cells for sustained accumulation of T_H1 cells.

To confirm the link between oral-derived bacteria and T_H1 cell induction, we obtained additional saliva samples from two healthy donors (He#1 and He#2) and two patients with active UC (UC#1 and UC#2) and orally administered these samples to GF WT B6 mice. T_H1 cells accumulated in the colonic LP of mice inoculated with a saliva sample from UC patient #2 (GF+UC#2 mice), comparably to the accumulation observed in GF+CD#2 mice (Fig. 3A). We cultured cecal contents from GF+UC#2 mice and isolated 13 strains, which resembled the microbiota composition of GF+UC#2 mice (Fig. 3B). Oral administration of the 13 strains (13-mix) into GF mice fully replicated the phenotype observed in GF+UC#2 mice in terms of colonic T_H1 cell induction (Fig. 3C). Among the 13 strains, *Enterococcus faecium* 11A1 (Ef-11A1) and *Klebsiella aeromobilis* 11E12 (Ka-11E12) drew our attention, because both species have been implicated in IBD pathogenesis and reported to be important multidrug-resistant pathobionts (*24*–*27*). GF mice were gavaged either with Ef-11A1, Ka-11E12, or a mixture of the 11 other strains (11-mix). Ka-11E12 induced T_H1 cells in the colon comparably to the 13-mix, whereas Ef-11A1 and the 11-mix failed to do so (Fig. 3C). Therefore, Ka-11E12 was most likely the major driver for the induction of

T_H1 cells observed in GF+UC#2 mice, even as a minor member of the gut microbiota (Fig. 3B). Ka-11E12 was also resistant to multiple antibiotics (fig. S11, A and B) and persisted in the intestine of vancomycin- or Tyl-treated SPF mice (Fig. 3D). Ka-11E12 colonization resulted in severe inflammation in *Il10*$^{-/-}$ mice (fig. S11, C and D, and Fig. 3E), suggesting that the orally derived *K. aeromobilis* strain may act similarly to Kp-2H7. Likewise, Ka-11E12 did not attach to the EC surface, as shown by FISH (Fig. 3F). However, genome sequence analysis and electron microscopy (EM) revealed that, in contrast to Kp-2H7, Ka-11E12 had a flagellar assembly system (table S4 and fig. S12A) and was highly motile and stimulatory for TLR5 (fig. S12, B and C). Therefore, distinct mechanisms may drive T_H1 cell induction by different *Klebsiella* strains.

Somewhat unexpectedly, the microbiota sample from one healthy donor (He#1) also induced a substantial increase in T_H1 cells (Fig. 3A). In this case, the fecal microbiome contained a *K. pneumoniae* sequence. We isolated a *K. pneumoniae* strain, Kp-40B3 (Fig. 3B). This strain induced marked T_H1 cell accumulation in the colon of monocolonized mice (Fig. 3G). Whole-genome sequence analysis revealed that Kp-40B3 possessed several genes that correlated with Kp-2H7–mediated T_H1 cell induction (Fig. 2D). These results suggest that *Klebsiella* spp. with T_H1 cell induction capability may exist in the oral cavity of not only IBD patients but also healthy humans.

We mined the 16*S* rRNA gene-sequencing data sets used in the analysis in Fig. 1A and found that the relative abundance of members of *Klebsiella* was significantly higher in patients with CD (P = 0.0157), PSC (P = 0.0309), and alcoholism (P < 0.0001) compared with that in healthy controls (Fig. 4A). We extended our analysis to the metagenome databases of intestinal microbiota of IBD patients and non-IBD controls from the Prospective Registry in IBD Study at Massachusetts General Hospital (PRISM) (*14*) and the CD cohort from the University of Pennsylvania (UPenn cohort) (*28*). The aggregated relative abundance of *Klebsiella* species was significantly higher in patients with IBD (P < 0.01) (Fig. 4B). The genes that were correlated with Kp-2H7–mediated T_H1 induction (shown in Fig. 2D) were enriched in the fecal microbiome of IBD patients who carried *Klebsiella* species, but not in non-IBD individuals (Fig. 4, C and D; P = 0.00988, Mann-Whitney U test).

Ectopic colonization of the colon by orally derived *Klebsiella* spp. is associated with aberrant activation of the immune system. The lower gastrointestinal tract is the expected niche for *Klebsiella* spp., where they constitute a minor population that can proliferate after antibiotic treatment and other conditions (*24*). Our data suggest that the oral cavity may serve as another reservoir for *Klebsiella* pathobionts. Indeed, the oral microbiota contains the highest relative abundance of Enterobacteriaceae compared with other mucosal sites (*29*). The inflammatory state in IBD may render the intestine more permissive to aerotolerant oral-derived bacteria than the steady-state intestine, and ongoing colonization by oral bacteria may help perpetuate gut microbiota dysbiosis and chronic inflammation. Overrepresentation of *Klebsiella* spp. in the gut microbiota, coupled with elevated levels of serum antibodies against *Klebsiella* antigens, has been reported in patients with IBD (*30*, *31*). In mouse models of IBD, such as *T-bet*$^{-/-}$*Rag2*$^{-/-}$ mice, *K. pneumoniae* is known to proliferate and play an important role in triggering disease (*32*). Furthermore, persistence of *Klebsiella* spp. is also observed in several other diseases (Fig. 4A) (*33*). Thus, our findings indicate that targeting oral-derived bacteria, particularly *Klebsiella*, could provide a therapeutic strategy to correct IBD and many other disease conditions.

Our results highlight the disease potential of antibiotic-resistant, proinflammatory *Klebsiella* in chronic human disease. *K. pneumoniae* and *K. aeromobilis* commonly cause hospital infections (*10*–*12*, *24*, *25*). Our isolates were resistant to several antibiotics, and treatment with antibiotics allowed their colonization in the intestine of SPF mice. The colonization efficiency of Kp-2H7 and Ka-11E12 varied, depending on the spectrum of antibiotics used as treatment. Therefore, identifying members of the normal gut microbiota that can provide colonization resistance against orally derived bacteria could foster future avenues for the development of effective treatments for multidrug-resistant bacteria and chronic inflammation.

REFERENCES AND NOTES

1. I. Nasidze, J. Li, D. Quinque, K. Tang, M. Stoneking, *Genome Res.* **19**, 636–643 (2009).
2. S. P. Humphrey, R. T. Williamson, *J. Prosthet. Dent.* **85**, 162–169 (2001).
3. H. Seedorf *et al.*, *Cell* **159**, 253–266 (2014).
4. D. Gevers *et al.*, *Cell Host Microbe* **15**, 382–392 (2014).
5. C. A. Lozupone *et al.*, *Cell Host Microbe* **14**, 329–339 (2013).
6. I. Vujkovic-Cvijin *et al.*, *Sci. Transl. Med.* **5**, 193ra91 (2013).
7. N. Qin *et al.*, *Nature* **513**, 59–64 (2014).
8. Y. Chen *et al.*, *Sci. Rep.* **6**, 34055 (2016).
9. C. L. Sears, W. S. Garrett, *Cell Host Microbe* **15**, 317–328 (2014).
10. E. S. Snitkin *et al.*, *Sci. Transl. Med.* **4**, 148ra116 (2012).
11. K. E. Holt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **112**, E3574–E3581 (2015).
12. E. G. Pamer, *Science* **352**, 535–538 (2016).
13. H. Chu *et al.*, *Science* **352**, 1116–1120 (2016).
14. X. C. Morgan *et al.*, *Genome Biol.* **13**, R79 (2012).
15. K. Chen *et al.*, *Immunity* **35**, 997–1009 (2011).
16. H. Xiong *et al.*, *Cell* **165**, 679–689 (2016).
17. A. R. Records, *Mol. Plant Microbe Interact.* **24**, 751–757 (2011).
18. S. Fukuda *et al.*, *Nature* **469**, 543–547 (2011).
19. A. N. Thorburn, L. Macia, C. R. Mackay, *Immunity* **40**, 833–842 (2014).
20. K. Chen *et al.*, *J. Immunol.* **192**, 141.12 (2014).
21. B. H. Kim *et al.*, *Nat. Immunol.* **17**, 481–489 (2016).
22. S. E. Winter, C. A. Lopez, A. J. Bäumler, *EMBO Rep.* **14**, 319–327 (2013).
23. Y. Haberman *et al.*, *J. Clin. Invest.* **124**, 3617–3633 (2014).
24. Y. Taur, E. G. Pamer, *Curr. Opin. Infect. Dis.* **26**, 332–337 (2013).
25. A. Davin-Regli, J. M. Pagès, *Front. Microbiol.* **6**, 392 (2015).
26. S. Mondot *et al.*, *Inflamm. Bowel Dis.* **17**, 185–192 (2011).
27. S. M. Diene *et al.*, *Mol. Biol. Evol.* **30**, 369–383 (2013).
28. J. D. Lewis *et al.*, *Cell Host Microbe* **18**, 489–500 (2015).
29. N. R. Shin, T. W. Whon, J. W. Bae, *Trends Biotechnol.* **33**, 496–503 (2015).
30. H. Tiwana *et al.*, *Br. J. Rheumatol.* **37**, 525–531 (1998).
31. T. Rashid, A. Ebringer, C. Wilson, *Int. J. Rheumatol.* **2013**, 610393 (2013).
32. W. S. Garrett *et al.*, *Cell Host Microbe* **8**, 292–300 (2010).
33. A. Ebringer, T. Rashid, H. Tiwana, C. Wilson, *Clin. Rheumatol.* **26**, 289–297 (2007).

ACKNOWLEDGMENTS

K.A. and K.H. acknowledge funding from the Uehara Memorial Foundation, the Takeda Science Foundation, the Mitsubishi Foundation, Core Research for Evolutionary Medical Science and Technology, and Leading Advanced Projects for Medical Innovation, a program of the Japan Agency for Medical Research and Development. C.L. and R.J.X. acknowledge funding from the U.S. NIH (grants DK043351 and DK92405), the Helmsley Charitable Trust, and the Crohn's & Colitis Foundation. We thank P. Wilmes, J. Baginska, M. Wakazaki, O. Ohara, and Y. Arakawa for their technical support and P. Burrows for helpful comments. M.H., H.M., and Y.K. conceived the research and performed initial experiments; K.H. planned experiments, analyzed data, and wrote the manuscript together with K.A., C.L., and R.J.X.; K.A., T.K., S.N., Y.K., I.M., K.Y., E.W., T.T., and C.A.T performed gnotobiotic studies, immunological analyses, and bacterial cultures; C.L., D.G., R.C.J., J.S., R.J.X., E.E., W.S., H.S.S., and M.H. performed bacterial sequence and microbiome analyses; M.S., K.T., and S.N. performed EM analyses; H.Y. provided clinical samples; and K.C., J.K.K., and S.A.R. provided essential materials and contributed to data discussions. J.K.K. is a recipient of Public Health Service grant R37 HL079142. K.H. is a scientific advisory board member of Vedanta Biosciences. All data and code to understand and assess the conclusions of this research are available in the main text, the supplementary materials, and the indicated repositories. Sequences of the genome of *Klebsiella* spp. and the 16*S* rRNA sequence data set are deposited in the DNA Data Bank of Japan under accession numbers PRJDB5883-5886 and PRJDB5967, respectively. The raw and processed RNA-seq data are deposited in the National Center for Biotechnology Information's Gene Expression Omnibus under accession number GSE23056. *Klebsiella* strains are available under a material transfer agreement with Keio University.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/358/6361/359/suppl/DC1
Materials and Methods
Figs. S1 to S12
Tables S1 to S4
References (*34*–*39*)

2 May 2017; accepted 7 September 2017
10.1126/science.aan4526

Science

Ectopic colonization of oral bacteria in the intestine drives T_H1 cell induction and inflammation

Koji Atarashi, Wataru Suda, Chengwei Luo, Takaaki Kawaguchi, Iori Motoo, Seiko Narushima, Yuya Kiguchi, Keiko Yasuma, Eiichiro Watanabe, Takeshi Tanoue, Christoph A. Thaiss, Mayuko Sato, Kiminori Toyooka, Heba S. Said, Hirokazu Yamagami, Scott A. Rice, Dirk Gevers, Ryan C. Johnson, Julia A. Segre, Kong Chen, Jay K. Kolls, Eran Elinav, Hidetoshi Morita, Ramnik J. Xavier, Masahira Hattori and Kenya Honda

Science **358** (6361), 359-365.
DOI: 10.1126/science.aan4526

Gut reasons to brush your teeth

Some gut conditions, such as inflammatory bowel disease (IBD), ulcerative colitis, and Crohn's disease (CD), are associated with imbalances in the gut microbe community. The causes of these intractable diseases have been difficult to discern. Atarashi *et al.* took samples from the mouths of IBD and CD patients and inoculated the extracted bacteria into germ-free mice (see the Perspective by Cao). Some of the inoculated mice showed strong proliferation of T helper 1 cells associated with the establishment of oral *Klebsiella* species in the colon. *Klebsiella* can be resistant to multiple antibiotics and are able to replace normal colon microbes after antibiotic therapy. Now we know that they probably originate from the mouth and could potentially contribute to bowel disease.

Science, this issue p. 359; see also p. 308

Science (print ISSN 0036-8075; online ISSN 1095-9203) is published by the American Association for the Advancement of Science, 1200 New York Avenue NW, Washington, DC 20005. The title *Science* is a registered trademark of AAAS.