**INTRODUCTION**

We cohabitate with trillions of bacteria that reside within our intestines and provide important beneficial metabolic and immunologic functions (1). However, inappropriate immune responses against these bacteria have been implicated in the pathogenesis of inflammatory bowel disease (IBD) (2–4), because germ-free mice are highly resistant to many murine colitis models. Moreover, monoclonization studies of germ-free animals revealed that IBD development may be driven by specific commensal bacterial species (5).

Tolerance to commensal bacteria is thought to depend on CD4+ regulatory T (Treg) cells (6–8), which we have recently shown can arise via peripheral Treg (pTreg) cell development from naïve T (T naive) cells in response to commensal antigens during homeostasis (9). However, intestinal inflammation can result in the exposure of new antigens to the immune system. In murine models, effector T (T eff) cell generation to a commensal bacterial antigen was seen during intestinal inflammation, but not homeostasis (10). Thus, it remains unknown whether effector responses during intestinal inflammation are driven by newly exposed antigens versus the commensal antigens that normally drive Treg cell development during homeostasis.

IBD is associated with marked changes in the gut microbiota, dysbiosis (11). Dysbiosis has been hypothesized to contribute to disease pathogenesis because it is well established that different commensal bacterial species have distinct effects on the intestinal T cell population. For example, segmented filamentous bacteria (SFB) strongly induces T helper 17 (Th17) cells in the small intestine (12), which has now been confirmed by T cell receptor (TCR) transgenic (Tg) studies (13). By contrast, introduction of Clostridium clusters XIVA and IV (7, 14) or altered Schaedler flora (15) markedly increased the frequency of colonic Foxp3+ Treg cells in germ-free mice. Thus, changes in the microbiome may also affect the intestinal T eff versus Treg cell population.

Here, we used TCR repertoire analysis coupled with in vivo studies of commensal-specific TCRs to address the influence of colitis-mediated dysbiosis on bacteria-specific Treg cell development. We also examined the interaction of T cells with luminal versus mucosal-associated (MA) antigens during homeostasis and colonic inflammation. Last, we assessed the impact of commensal-specific T cells during lymphopenic conditions. In summary, our data suggest that the MA pathobionts Helicobacter spp. elicit context-dependent T cell responses during homeostasis and colitis.

**RESULTS**

**Overlap of T eff and Treg TCR antigen specificity during colitis**

To address the effect of colitis on T cell responses to commensal bacteria, we examined induced models because we wanted to compare mice that started with the same microbiota. We settled on a murine model of inflammatory colitis (Fig. 1A) (16) that incorporates 1% dextran sodium sulfate (DSS)–induced mucosal injury along with anti–interleukin-10 receptor antibody (aIL10R) (15, 17), which blocks an important immunoregulatory pathway implicated in human IBD in genome-wide association studies (18). DSS + aIL10R treatment induced colitis as evidenced by weight loss, increased colon weight/length ratio, and a marked enhancement of T eff (CD44+CD62L+) and Treg cells (fig. S1). Because the combination showed a greater effect on the microbiota than DSS or aIL10R alone (fig. S1E), we used DSS + aIL10R for subsequent experiments.

Consistent with previous reports, T naive cells from CT2 and CT6 TCR Tg lines, which express TCRs isolated from colonic Treg cells and recognize commensal antigens (8), showed substantial induction...
of Foxp3, the canonical T<sub>reg</sub> cell transcription factor, by 1 week after transfer into control immunoglobulin G (IgG)–treated mice (Fig. 1, A and B) (9). However, in mice undergoing DSS + αIL10R–mediated colitis, they skewed toward T<sub>eff</sub> cell generation (Fig. 1B and fig. S2). T<sub>eff</sub> cell differentiation was primarily to the T<sub>H</sub>17 cell phenotype based on IL-17A–green fluorescent protein (IL-17A GFP) and interferon-γ–yellow fluorescent protein (IFN<sub>γ</sub>YFP) reporters (Fig. 1C), consistent with observations in human Crohn’s disease (19). In addition, CT2 and CT6 cells underwent extensive proliferation, as assessed by Cell Trace Violet (CTV) dilution and expanded as a fraction of total CD4<sup>+</sup> cells (Fig. 1, D and E). Thus, the normal tolerogenic T<sub>reg</sub> cell developmental response to commensal bacterial antigens can be redirected to a T<sub>H</sub>17 cell effector response during experimental colitis.

To assess whether the responses of CT2 and CT6 TCR Tg cells were representative of the T cell population, we analyzed the effect of colitis on the TCR repertoire (fig. S3, A and B, and table S1). Because the great diversity in polyclonal T cells precludes experimental analysis at the individual TCR level, we used mice in which TCR diversity is limited by a Tg fixed TCR<sub>β</sub> chain (20). Although TCR diversity is diminished, this approach allows high-throughput analysis of the TCR repertoire at the individual TCR level via sequencing of the variable TCR<sub>α</sub> chains. With colitis, both T<sub>reg</sub> and T<sub>eff</sub> repertoires showed increased clonal expansion compared with controls (fig. S3C), consistent with a T cell response. We saw a marked increase in similarity between the T<sub>reg</sub> and T<sub>eff</sub> TCR repertoires within the cLP of individual mice during colitis compared with controls (Fig. 2A and fig. S3D).

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**Fig. 1. Colonic T<sub>reg</sub> TCRs (CT2 and CT6) drive T<sub>eff</sub> cell development in inflammation.** (A) Experimental model. Four- to 5-week-old CD45.1 specific pathogen–free (SPF) mice were administered αIL10R (1 mg per mouse) on day 0 and kept on 1% DSS water for 7 days to initiate colitis. Control mice were given isotype IgG (1 mg per mouse) on day 0. Four days after initiation of colitis, congenically marked naïve CT2/CT6 Tg cells (10<sup>5</sup>) were intravenously transferred and analyzed 7 days later. (B to D) Effector cell induction and expansion with colitis. Transferred TCR Tg cells from the colon lamina propria (cLP) were analyzed by flow cytometry for (B) the development of T<sub>eff</sub> (CD44<sup>hi</sup>CD62L<sup>lo</sup>Foxp3<sup>−</sup>) and T<sub>reg</sub> (Foxp3<sup>+</sup>) markers (P = 0.000003, P = 0.0007, P = 0.0024, P = 0.0157, Student’s t test; n = 10), (C) up-regulation of cytokines using IL-17A<sub>GFP</sub> and IFN<sub>γ</sub>YFP (P = 0.000001, P = 0.0007, P = 0.0029, Student’s t test; n = 10 for CT2 and 7 for CT6), (D) proliferation indicated by CTV dilution (P = 0.000002, P = 0.0002, Student’s t test; n = 10 for CT2 and 7 and 8 for CT6), and (E) expansion indicated by the in vivo frequency among the host CD4<sup>+</sup> T cells (P = 0.000005, P = 0.0275, Student’s t test; n = 10 for CT2 and 10 and 11 for CT6). Bars indicate mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. ns, not significant.
from the cLP were analyzed by flow cytometry for Teff and Treg cells, and the in vivo IL10R hosts as indicated in Fig. 1A. Seven days after transfer, cells IgG or DSS + were injected into three different mice subcutaneously. Each mouse was injected with 10^7 cells. (A) Comparison of TCR αβ repertoires from T naïve (CD44 low CD62 hi), T reg (Foxp3 +), and T eff (CD44 hi CD62L lo) cells in the cLP of TCIIL1R Foxp3-GFP-Thy1.1 TCRαβ mice 2 weeks after initiation of IgG or DSS + αIL10R hosts showed increased T eff but not T reg cell generation (fig. S5), consistent with a previous report in the DSS model (10). Although it remains possible that the CBir1 result in the DSS + αIL10R model is not generalizable to other model systems, in conjunction with the TCR repertoire analysis, these TCR Tg studies suggest that colitis skew T cell development such that proinflammatory T eff cells often use the same TCRs as anti-inflammatory T reg cells.

**Helicobacter spp. are important drivers of pT reg cell development during homeostasis**

The observation that certain commensal antigens can activate both T reg cells during homeostasis and T eff cells during colitis suggested that these bacterial antigens are continually presented to the adaptive immune system. This pattern is different from the CBir1 commensal antigen, which is primarily presented to T cells during colitis (fig. S5) (10). To address this, we assessed the in vitro reactivity of these TCRs to fecal antigen preparations presented on CD11c+ dendritic cells (DCs) (8). Although TCR activation by fecal antigen from control mice was high with T7-1 and detectable with CT2 and CT6, the degree of enhancement in TCR activation by fecal antigens from colitic mice was limited (Fig. 3A and fig. S6). We therefore asked whether these TCRs show more reactivity to MA antigens, which might be predicted to have greater access to the immune system via DC uptake (23), goblet-associated passages (24), or outer membrane vesicles (25). Both CT2 and CT6 showed marked enhancement of TCR activation by MA compared with luminal antigen (Fig. 3A).

To confirm that the reactivity in the MA antigen preparation was dependent on the microbiota, we treated SPF mice with a broad-spectrum antibiotic combination of vancomycin, ampicillin, metronidazole, and neomycin and found that MA antigen no longer stimulated our TCR Tg cells expressing T7-1 in vitro (24). Both CT2 and CT6 showed marked enhancement of TCR activation with MA compared with luminal antigen (Fig. 3A). We then tested additional T reg TCRs—CT1, CT7, and CT9—which we often use the same TCRs as anti-inflammatory T reg cells.

Examination of the top 25 T eff TCRs per mouse showed that many of these TCRs are also found in the T reg cell subset during colitis (Fig. 2B), consistent with our TCR Tg studies (Fig. 1). In control mice, the relatively low degree of overlap between the T eff and T reg cell subset within individual mice (Fig. 2, A and B, and fig. S3D) is consistent with our previous studies (8, 21). As before, we also observed mouse-to-mouse variability of T eff and T reg TCR repertoires between mice (Fig. 2A and fig. S3, D and E). To confirm the TCR repertoire analysis, we cloned T7-1 (Fig. 2C), the most abundant TCR found in the T eff subset of colitic mice by mean percentage (fig. S3E). As predicted by the TCR repertoire study, analysis of adoptively transferred peripheral TCIIL1R TCR Tg cells expressing T7-1 showed that this TCR facilitated Foxp3 induction during homeostasis but skewed toward Teff cell generation with colitis (Fig. 2D and fig. S4). Thus, these data demonstrate an increased overlap between the T eff and T reg TCR repertoires during colitis that does not occur at homeostasis.

Our TCR Tg data suggest that the overlap in T reg and T eff TCR usage during colitis is due to the enhanced T eff generation by clones that might normally differentiate into T reg cells (Fig. 1). Alternatively, the overlap may arise from increased T reg cell generation by T eff TCRs during colitis. However, transfer of CBir1 Tg cells (22) into DSS + αIL10R hosts showed increased T eff but not T reg cell generation (fig. S5), consistent with a previous report in the DSS model (10). Although it remains possible that the CBir1 result in the DSS + αIL10R model is not generalizable to other model systems, in conjunction with the TCR repertoire analysis, these TCR Tg studies suggest that colitis skews T cell development such that proinflammatory T eff cells often use the same TCRs as anti-inflammatory T reg cells.

**Helicobacter spp. are important drivers of pT reg cell development during homeostasis**

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To confirm that the reactivity in the MA antigen preparation was dependent on the microbiota, we treated SPF mice with a broad-spectrum antibiotic combination of vancomycin, ampicillin, metronidazole, and neomycin and found that MA antigen no longer stimulated our four MA antigen–reactive T reg TCR panel (CT2, CT6, CT9, and T7-1) (fig. S7A). This antibiotic cocktail includes vancomycin, which has been reported to decrease T reg cell numbers and Clostridium spp.
Hybridoma cells expressing different TCRs were cultured with CD11c+ DCs, and the indicated antigen was obtained.

Fig. 3. Colonic Treg TCRs react to MA Helicobacter species. (A) Treg TCRs preferentially react to MA antigens (Ags). Hybridoma cells expressing different TCRs were cultured with CD11c+ DCs, and the indicated antigen was obtained 2 weeks after the initiation of colitis. NFAT (nuclear factor of activated T cells)–GFP up-regulation was assessed by flow cytometry 1.5 days later. (B) Selective elimination of MA antigen using individual antibiotics. TCRs from (A) that react to MA antigen were stimulated with colonic MA antigens isolated from antibiotic-treated or untreated mice as per (A). (C) Changes in H. typhlonius and H. apodemus OTUs correlate with in vitro reactivity to MA antigen in (B). Data are the percentage of 16S OTUs from the MA preparations of individual antibiotic-treated or untreated mice. (D) In vitro recognition of H. typhlonius or H. apodemus. Cultured isolates were tested for TCR reactivity in vitro as per (A). Two to three independent experiments. Bars indicate mean.

We then asked whether these Helicobacter spp. could induce TCR-specific Treg cell responses in vivo. As expected, transfer of CT2/CT6/CT9/T7-1 TCR+ cells into ampicillin-treated mice to eliminate pre-existing Helicobacter spp. (Fig. 3C) resulted in little proliferation or differentiation (Fig. 4A). However, inoculation of ampicillin-treated mice with a single Helicobacter spp. elicited robust expansion and Foxp3 up-regulation in appropriate TCR+ Tg cells (Fig. 4A). Similarly, Helicobacter-free mice obtained from Charles River Laboratories showed little ability to induce TCR-dependent proliferation or Treg cell generation, unless the appropriate Helicobacter spp. identified in vitro (Fig. 3D) was subsequently inoculated in vivo (Fig. 4B and fig. S9). Because CT2 and CT6 Tg cells were cotransferred into the existing Helicobacter spp., (Fig. 3C) and fig. S7B).

To determine whether Helicobacter spp. were being directly recognized by these TCRs, we tested cultured isolates that matched the 16S rRNA sequence (Fig. 3D). There were specific patterns of TCR reactivity, with T7-1 and CT9 recognizing both species, whereas CT2 reacted only to H. typhlonius, and CT6 to H. apodemus. Recognition of Helicobacter spp. in vitro appeared to be TCR-specific and not via a superantigen or other antigen-independent mechanism. First, each Helicobacter isolate stimulated only some, but not all, TCRs. Second, addition of antimicrobial histocompatibility complex (MHC) II blocked TCR activation (fig. S7C). Third, testing of these TCRs against multiple bacterial isolates in vitro from different genera, including Clostridium, Bifidobacterium, and Lactobacillus, failed to activate our TCR panel to the same degree as Helicobacter (fig. S8). However, these TCRs could still show cross-reactivity to different bacterial species due to shared epitopes. T7-1 likely recognizes additional bacterial antigens, because it reacted to luminal antigens with high efficiency (Fig. 3A). CT6 may also recognize other bacterial species, because vancomycin reduced CT6 response to MA antigen without a corresponding decrease in H. apodemus frequency by 16S (Fig. 3, B and C). We have also shown previously that CT6 reacted to an uncharacterized Clostridium spp. (8) and Fig. 4A). Although the extent of bacterial cross-reactivity remains to be defined, these data demonstrate that four of our six colonic Treg TCRs recognize Helicobacter spp. in vitro.

We then tested the individual antibiotics to attempt to identify the bacterial species recognized by these TCRs by correlating in vitro TCR reactivity to MA antigen with changes in the bacteria composition.

Unexpectedly, these data suggested that Helicobacter spp., and not the predicted Clostridium spp. (7), were being recognized by the TCR panel. First, of the individual antibiotics, ampicillin and neomycin, but not vancomycin or metronidazole, markedly decreased the ability of MA antigen to stimulate the TCR panel compared with untreated controls (Fig. 3B). Second, 16S ribosomal RNA (rRNA) gene sequences from the MA antigen preparations revealed that the two most frequent operational taxonomic units (OTUs) lost with ampicillin or neomycin were Helicobacter typhlonius and Helicobacter apodemus (Fig. 3C and fig. S7B).
same host in this experiment, it appears that these TCR clones show a high degree of specificity for individual Helicobacter strains (Fig. 4B). Thus, these data demonstrate that Helicobacter spp. are a major driver of bacterial-specific T reg cell responses during homeostasis.

**Marked changes in bacterial composition with colitis**

The observation that colitis enhanced the in vitro stimulatory ability of MA antigen (Fig. 3A), and increased the proliferation of adoptively transferred CT2 and CT6 TCR Tg cells (Fig. 1B), suggested that there might be alterations in the colonic microbiota. We therefore analyzed the changes in the MA and luminal 16S rRNA profile with colitis. Consistent with previous reports, the bacterial composition in the lumen versus mucosa is quite distinct (Fig. 5, A to C) (26, 27). In the MA preparation, we noted a high frequency of Helicobacter spp. in both control and colitic mice (Fig. 5B), consistent with the activation and differentiation of CT2 and CT6 Tg cells in vivo under both conditions (Fig. 1). The frequency of H. typhlonius was further increased in colitic mice (Fig. 5, D and E), which may explain the increased in vitro MA antigen reactivity of CT2 and CT7 T reg TCRs with colitis (Fig. 3A). In the lumen, besides the increase in H. typhlonius (Fig. 5, D and E), we noted that colitis induced a marked expansion of Bacteroides spp., particularly Bacteroides vulgatus, which represented more than 20% of 16S reads in some mice (Fig. 5, D and F). Because there was no obvious decrease in bacterial density (fig. S10), these data suggest that DSS + αIL10R colitis is associated with a major bloom of Bacteroides spp. in the lumen.

**Lack of T cell responses to the bloom of luminal Bacteroides species during colitis**

Because Bacteroides species have been suggested to be important triggers of intestinal inflammation in other murine models (28, 29), we asked whether the dramatic expansion of Bacteroides species in DSS + αIL10R–treated mice induced strong antimicrobial T eff cell responses. We assessed the in vivo responses of several TCRs that recognize Bacteroides strains isolated from DSS + αIL10R mice in vitro, including the B. vulgatus–reactive TCR DP1 (Fig. 6A and table S2). These TCRs recognized Bacteroides strains through MHC II and did not respond in vitro to the Helicobacter strains tested (fig. S11, A and B). To our surprise, adoptively transferred T cells expressing these Bacteroides–reactive TCRs did not show increased proliferation, expansion, or T eff cell development in DSS + αIL10R–treated mice compared with controls (Fig. 6, B and C). Consistent with the poor expansion, very few transferred cells were recovered from the colon. The percentage of the transferred TCR-expressing cells among total CD4+ T cells was reduced during DSS + αIL10R colitis (Fig. 6C), implying that these antigens are relatively less important during colitis despite their bloom in the lumen. We confirmed in vitro that the luminal antigens for DP1 and NT2 increased with colitis (Fig. 6D), as predicted based on 16S rRNA sequencing (Fig. 5F). By contrast, MA antigen showed lower stimulatory ability than luminal antigen for DP1 and NT2 even during colitis (Fig. 6D). We also checked that the Helicobacter spp.–reactive TCRs (Fig. 3D) did not respond to these Bacteroides strains (fig. S11C). Although it remains possible that these Bacteroides–reactive TCR clones do not compete well in the gut or are not representative of polyclonal T cell responses to Bacteroides, these data suggest that the marked expansion of luminal Bacteroides spp. in this model of colitis does not elicit a corresponding increase in antigen-specific T cell responses.

![Fig. 4. Helicobacter species induce pTreg cell differentiation during homeostasis.](http://immunology.sciencemag.org/)

**Fig. 4. Helicobacter species induce pTreg cell differentiation during homeostasis.**

(A) In vivo validation of TCR reactivity to Helicobacter species. Three-week-old SPF mice were treated with ampicillin (amp) for 2 weeks via drinking water. Two days after the last treatment, H. typhlonius or H. apodemus were gavaged for a total of three times every other day. With the last gavage, congenically marked naïve CT2/CT6 Tg cells or retrovirally expressed CT9/T7-1 cells were transferred. Seven days after transfer, cLP cells were analyzed by flow cytometry for (top) the development of Treg (Foxp3+) cells (right) (**P = 0.07**), (**P < 0.0001**) (Student’s t test; n = 4 and 5 for CT2 and 3 and 5 for CT6), (middle) frequency of transferred TCR-expressing cells among the host CD4+ T cells (**P = 0.0442**, P = 0.021, P = 0.0046, P = 0.0705, Student’s t test; n = 4 and 5 for CT2 and CT6, 6 and 7 for CT9, 5 for T7-1, and 3 and 5 for CT6), and (bottom) CT7 cytokine secretion (P = 0.00000002, P = 0.00000007, Student’s t test; n = 4 and 5 for CT2 and 3 and 5 for CT6). (B) T cell response to Helicobacter in vivo is species-specific. Three-week-old SPF mice obtained from Charles River Laboratories were gavaged with H. typhlonius or H. apodemus (for a total of three times every other day). With the last gavage, congenically marked naïve CT2 and CT6 Tg cells (10^6 each) were cotransferred. One week after transfer, cells from the distal mesenteric lymph node (dMLN) were analyzed by flow cytometry for the frequency of transferred TCR-expressing cells among the host CD4+ T cells (left), CT7 cytokine secretion (middle), or development of Treg (Foxp3+) cells (right) (**P < 0.0001**). Bars indicate mean ± SEM. **P < 0.05, ***P < 0.01, ****P < 0.0001.
**Pathogenic potential of Helicobacter-reactive TCRs**

The differentiation of colonic T<sub>reg</sub> TCRs into T<sub>eff</sub> cells during colitis (Fig. 1) suggests that the altered T cell response to bacterial antigens may contribute to colonic inflammation. To test the pathogenic potential of these Helicobacter spp.–reactive TCRs, we adoptively transferred naïve CT6 Tg cells into Rag1<sup>−/−</sup> hosts with or without *H. apodemus* inoculation. In contrast with wild-type hosts (Fig. 4), *H. apodemus* drove antigen-specific T<sub>eff</sub> cell generation of CT6 Tg cells (Fig. 7A...
Fig. 6. Expansion of Bacteroides species during colitis does not enhance TCR-specific T cell responses. (A) Antigenic reactivity of Bacteroides-reactive TCRs was used. In vitro stimulation by the Bacteroides isolates are shown as per Fig. 3A. Two to three independent experiments. (B and C) In vivo expansion and effector cell development of Bacteroides-reactive T cells. Congenically marked naïve DP1 Tg cells (10⁵) were transferred into CD45.1 hosts as indicated in Fig. 1A and analyzed 7 days after transfer by flow cytometry for (B) CTV dilution in the dMLN (n = 7), (C) (left) frequency among the host CD4+ T cells (n = 11 for CT2 and NT2; Student’s t test; **P < 0.01) (Fig. S6A), and (right) development of T eff cells (CD44hi CD62Llo Foxp3−) (n = 7 for DP1 and NT2 and 9 for CT7); Student’s t test; n = 7 for DP1 and NT2 and 9 for CT7) in Rag1−/− (black) or Rag1+ (blue) mice before transfer of 2 × 10⁵ cells. (D) In vitro reactivity to in vivo antigen preparations is consistent with Bacteroides expansion by 16S rRNA analysis. Colonic lumen contents or MA preparations from IgG or DSS + αIL10R mice were tested as per Fig. 3A. Two independent experiments. Bars indicate mean ± SEM. *P < 0.05, **P < 0.01.

and fig. S12) in Rag1−/− hosts likely due to expansion in a lymphopenic environment. In line with a T eff cell response, we observed colonic inflammation in CT6-transferred hosts only in the presence of H. apodemus, as evidenced by increased colon weight/length ratio and crypt dropout in histology (Fig. 7, B and C). Similarly, T eff cell response of CT2 Tg cells to H. typhlonius led to colonic inflammation in Rag1−/− hosts (Fig. S13). We did not note major weight loss induced by these TCR Tg cells and/or Helicobacter spp. during the time frame of this experiment (fig. S13, D and E), in contrast with polyclonal T cell transfer models (30). This could be due to limited systemic effects from a single TCR clone or differences in gut microbiota composition. Although Helicobacter spp. without T cell transfers have been reported to induce colitis in Rag-deficient mice (31–34), we did not observe inflammation caused by these two strains during the period of the experiment (Fig. 7 and fig. S13). Thus, these data demonstrate the potential pathological consequences of antigen-specific T eff cell expansion against gut bacteria.

Last, MA enterohepatic Helicobacter spp. in humans have been suggested to be associated with IBD (35). We have also detected the presence of Helicobacter spp. in patients with Crohn’s disease or ulcerative colitis by 16S rRNA sequencing of fecal or colonoscopy samples (fig. S14). However, it remains to be established whether these Helicobacter spp. play a similar immunomodulatory role in humans, as seen in our mouse studies.

DISCUSSION

On the basis of this study of T cell responses to commensal antigens during experimental colitis, we make the following observations: First, T eff cell responses during colitis are often to commensal antigens normally presented to the immune system during homeostasis, and not to antigens normally excluded by the mucosal barrier. Second, commensal bacterial species vary widely in their ability to induce T cell responses due, in part, to their anatomic location relative to the mucosal surfaces. Third, constitutively presented antigens appear to primarily elicit T reg cell responses during homeostasis, implying that tolerance to these antigens is important to preventing colitis. Last, Helicobacter spp. are important inducers of T cell responses during homeostasis and colitis. Thus, these data suggest a model whereby T reg cell–mediated tolerance, and not T eff cell–mediated immunity, is required against commensal bacteria such as Helicobacter spp. that routinely interact with the host immune system.
interact with T cells during homeostasis. These data do not exclude the possibility that dysbiosis of _Bacteroides_ spp. may exert non–antigen-dependent effects on T cell immunity through changes in short-chain fatty acids (38–40), polysaccharides (41), and aryl hydrocarbon receptor ligands (42). Furthermore, because our studies were done in SPF mice, variability in gut flora composition and strain-level differences could affect the functional outcome of specific bacteria. Nonetheless, these data suggest that dysbiosis during intestinal inflammation may not provide an important antigen-specific trigger of effector responses that contribute to colitis.

The relative inefficiency of _Bacteroides_ spp. to induce T cell activation is correlated with their bacterial distribution because they are preferentially found in the lumen. By contrast, MA _Helicobacter_ spp. were potent stimulators of T cell response. This is consistent with a recent study of SFB showing that tight attachment to the intestinal epithelium plays an important role in eliciting Th17 cells (43). Thus, understanding the adaptive immune response to commensal bacteria may be facilitated by analysis of MA or epithelial-associated, and not luminal, bacteria.

The recognition that _Helicobacter_ spp. are important inducers of antigen-specific Treg cells during homeostasis is consistent with a previous study (44) and suggests that our current notion of Treg-inducing bacteria as being “good bacteria” that are protective against colitis may be incomplete (7, 45). _Helicobacter_ spp. are widely prevalent in mice colonies around the world (46) and have often been thought of as pathobionts based on their ability to induce or enhance colitis in lymphopenic mice (32–34) or mice deficient in critical immunoregulators such as IL-10 (31, 47, 48). To reconcile these different observations regarding _Helicobacter_ spp., our data suggest that T cell development is context-dependent, which is tolerogenic during homeostasis and inflammatory during lymphopenia or experimental colitis.

Our initial goal was to study the T cell response to alterations in the gut microbiota structure, also known as dysbiosis, that occurs with colitis and IBD and has been proposed to be involved in disease pathogenesis (4, 36, 37). However, rather than observing a dominant Teff cell response to _Bacteroides_ spp. that bloom during colitis, the major stimulators were _Helicobacter_ spp. that routinely
to the immune system primarily during inflammation (10). In this case, the CB1R antigen is normally not seen by the immune system and might be considered a pathogen when seen in the context of inflammation. However, in the DSS + αIL10R model of colitis, this type of immune response to commensal antigens appears to be less relevant. Thus, our studies bring up an interesting question, that is, to what extent is the human intestinal inflammatory T cell response directed toward commensal antigens that the host is commonly in contact with, that is, akin to “self” antigens, or commensal antigens that are only presented during injury, that is, “foreign” antigens.

**MATERIALS AND METHODS**

**Study design**

Animal experiments were performed in an SPF facility in accordance with the guidelines of the Institutional Animal Care and Use Committee at the Washington University. Host mice were housed together and interbred to maintain microbial integrity. Gender-matched littermate host mice were used for all comparisons. Cages were randomly assigned into different treatment groups. Both male and female mice were used. All in vivo and in vitro experiments were performed independently at least two times unless otherwise stated.

**Mice**

CT2, CT6 (8, 9), and DP1 Tg mice were generated as described (50) and bred to Rag1−/− [the Jackson Laboratory (JAX #002216), Foxp3IKRES-GFP (JAX #006772)] or Foxp3IKRES-Thy1.1 (51), IL-17AIRES-GFP (JAX #18472), and IFNγIKRES-YFP (GREAT) (52), TCI TCRβ Foxp3IKRES-Thy1.1 TCRA−/− (53) and TGI TCRβ (54) Foxp3IKRES-Thy1.1 Rag1−/− mice were previously described. IFNγIKRES-YFP mice were a gift from R. Locksley (University of California, San Francisco); Foxp3IKRES-Thy1.1 mice were a gift from A. Rudensky (Memorial Sloan Kettering Cancer Center). Experiments in Fig. 4B used Ly5.1 mice from Charles River Laboratories as hosts; experiments in fig. S6 used Ly5.2 mice inbred in our colony as hosts; all other in vivo experiments used Foxp3IKRES-GFP CD45.1 (JAX #006772) mice. All mice were on a C57BL/6 genetic background.

**Reagents, antibodies, and flow cytometry**

αIL10R blocking antibody (BE0050), isotype IgG1 (BE0088), and anti–MHC II antibody (BE0108) were purchased from Bio X Cell. Fluorescently conjugated antibodies were purchased from BioLegend, eBioscience, and Becton Dickinson. Cells were analyzed using a FACSaria IIu (Becton Dickinson), and data were processed with FlowJo (Treestar).

**Cell isolation from the cLP**

Cells were isolated as described (9). Colonic segments were treated with RPMI containing 3% fetal bovine serum and 20 mM Hepes (HyClone) with dithiothreitol (DTT) (Sigma) and EDTA (Thermo Fisher) for 20 min at 37°C with constant stirring. Tissue was further digested with Liberase TL (28.3 µg/ml) (Roche) and deoxyribonuclease I (200 µg/ml) (Roche), with continuous stirring at 37°C for 30 min. Digested tissue was forced through a Cell ucer tissue sieve (Bellco Glass) and passed through a 40-μm cell strainer.

**T cell hybridoma stimulation assay**

T cell hybridoma cells expressing GFP under NFAT promoter (55) were retrovirally transduced with TCRα chains of interest, as previously described (8). Hybridoma cells (1.5 × 10^5) were cultured with αIL10R-blocking antibody, vβ6+Vα2+ cells were analyzed for GFP expression after 1.5 days by flow cytometry.

**Antigen preparations**

Total colonic lumen contents were collected from longitudinally opened colon and cecum, diluted with phosphate-buffered saline (PBS) (HyClone), vortexed, filtered through a 40-μm strainer, and autoclaved. For MA preparation, lumen contents were removed with forceps, and the remaining tissue was rinsed with PBS. MA particles were released from colonic tissues using PBS with DTT (1 μM/ml) and EDTA (5 μM/ml) for 20 min at 37°C with constant stirring, followed by PBS with EDTA (2 μM/ml) for three times. From each of these steps, MA particles were filtered through a 40-μm strainer. Larger particles such as cells were removed by centrifugation at 1500 rpm for 10 min. The remaining particles in suspension were pelleted at 2500 rpm for 15 min, resuspended in PBS, and then autoclaved. For bacterial isolates, in vitro cultures were pelleted at 2500 rpm for 15 min, washed twice with PBS, resuspended in PBS, and autoclaved for 45 min.

**Adoptive transfer experiments**

For experiments with CT2, CT6, or DP1 Tg cells, T naïve cells were fluorescence-activated cell sorting (FACS)–purified (CD4+ Foxp3+ CD25+ CD44hi CD62Lhi) from peripheral lymph nodes and spleen of CD45.2 Foxp3IKRES-GFP Rag1−/− or Foxp3IKRES-Thy1.1 IL-17AIRES-GFP IFNγIKRES-YFP Rag1−/− TCR Tg mice. Naïve TCR Tg cells (105) were injected retro-orbitally into congenic CD45.1 Foxp3IKRES-GFP mice. In some experiments, cells were first labeled with CTV (Thermo Fisher) before injection.

For experiments using TCRs CT7, CT9, NT2, or T7-1, T naïve cells (CD4+ Foxp3+ CD25+ CD44hi CD62Lhi Vα2+) were FACS-purified from CD45.2 TCI TCRβ Foxp3IKRES-Thy1.1 Tg mice (Rag1−/− or Rag1−/−) and activated in vitro with soluble anti-CD3 (0.1 µg/ml; 145-2C11, Bio X Cell) and anti-CD28 (1 µg/ml; 37.51, Bio X Cell) in tissue culture plates coated with rabbit antibody to hamster IgG (127-005-099, Jackson ImmunoResearch) in the presence of anti–cytokine antibodies from Bio X Cell: anti–transforming growth factor–β (20 µg/ml; catalog no. BE0057), anti–IFNγ (5 µg/ml; catalog no. BE0054), anti–IL–4 (5 µg/ml; catalog no. BE0045), and anti–IL–12 (5 µg/ml; catalog no. BE0052). One day after activation, cells were retrovirally transduced with individual TCRα chain of interest (8). Total cells (2 × 105) (both transduced and nontransduced) were transferred into congenic CD45.1 Foxp3IKRES-GFP mice.

The cLP and dMLN were harvested at indicated times after transfer and analyzed by flow cytometry. Transferred TCRα cells were identified as CD4+CD45.2+CD44hiVα6+Vα2+ (CT2/CT6/CT7/CT9/DP1/NT2) or CD4+CD45.2+CD45.1−Vβ6+GFP+ (T7-1).

**Antibiotic treatment**

Littermates were divided and treated at 3 weeks of age. Vancomycin (0.5 mg/ml), ampicillin (1 mg/ml), and neomycin (1 mg/ml) were administered ad libitum via drinking water continuously for the time indicated. Metronidazole (1 mg/ml) was administrated by oral gavage every day for 1 week followed by gavage every other day (56).

**Bacterial isolation, culture, and inoculation**

Colonic lumen contents from DSS + αIL10R–treated mice were homogenized, and serial dilutions were plated on brain heart infusion...
(BHI; BD Difco) agar supplemented with 10% (v/v) defibrinated horse blood (Colorado Serum Co.). Plates were grown for 3 days at 37°C under anaerobic conditions (5% H₂, 20% CO₂, and 75% N₂) in a Coy chamber. Twenty colonies were picked, and 16S rRNA gene was sequenced. Colonies matched to *B. vulgatus, Bacteroides acidifaciens*, and *Bacteroides uniformis* were cultured in BHI medium (EMD) supplemented with yeast extract (5 g/liter), l-cysteine–HCl (0.5 g/liter), vitamin K₃ (1 mg/liter), and hematin (1.2 mg/liter) (all from Sigma) for the T cell hybridoma assay. 

**Statistical analysis**

GraphPad Prism v7, R v3.3.0, and Qime v1.9 were used for statistical and graphical analysis. Student’s t test, Mann-Whitney U test, one-way ANOVA, and two-way repeated-measures ANOVA were used for between-subjects analyses. Benjamini-Hochberg false discovery rate correction was used on Mann-Whitney U calculations for OTU comparisons. Morisita-Horn statistical test was used for TCR repertoire comparison. TCR Renyi diversity profiles were generated, as previously indicated (53), using Renyi entropy values with α/order values ranging from 0 (natural logarithm of species richness) to 2 (natural logarithm of the inverse Simpson index). This includes α = 1, which represents the commonly used Shannon entropy.

**SUPPLEMENTARY MATERIALS**

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MATERIALS AND METHODS

Fig. S1. 16S rRNA gene sequencing is in (61). The V4 region of 16S rRNA gene was PCR-amplified using barcoded primer described previously (62) and sequenced using the Illumina MiSeq Platform (2 × 250–bp paired-end reads). OTU picking was performed using UPARSE (usearch v8.0.162) (63), and taxonomy was assigned using the uclust method with the Greengenes 13.8 database (QIIME v1.9) (64).

**Colon histology**

Mouse colons were collected, and luminal contents were removed, cut open longitudinally, and pinned and fixed with 10% (v/v) formalin. Fixed samples were paraffin-embedded, cut into 5-μm sections, and stained with H&E by standard procedures. Crypt height was measured from digital photographs of H&E-stained colon sections taken with a Nikon ECLIPSE 50i microscope.

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**Helicobacter** species are potent drivers of colonic T cell responses in homeostasis and inflammation

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**Context is critical in IBD**

The intestine hosts trillions of commensal microbes; however, exactly how these microbes contribute to a balanced immune response in the intestine is still being explored. Now, Chai et al. report that mucosal-associated **Helicobacter** species can trigger either regulatory T (Treg) or effector T (Teff) cell activation in mouse intestine, depending on context. T cells specific to the bacteria activated Treg cells in homeostatic conditions. In contrast, in a mouse model of colitis, **Helicobacter** species induced Teff cells. These data suggest that a pathobiont such as **Helicobacter** species may induce immune tolerance in homeostatic conditions but switch to contribute to pathogenesis in the presence of inflammation.