Focused specificity of intestinal T<sub>h</sub>17 cells towards commensal bacterial antigens

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T-helper-17 (T<sub>h</sub>17) cells have critical roles in mucosal defence and in autoimmune disease pathogenesis<sup>1–4</sup>. They are most abundant in the small intestine lamina propria, where their presence requires colonization of mice with microbiota<sup><sup>5–7</sup></sup>. Segmented filamentous bacteria (SFB) are sufficient to induce T<sub>h</sub>17 cells and to promote T<sub>h</sub>17-dependent autoimmune disease in animal models<sup>8–14</sup>. However, the specificity of T<sub>h</sub>17 cells, the mechanism of their induction by distinct bacteria, and the means by which they foster tissue-specific inflammation remain unknown. Here we show that the T-cell antigen receptor (TCR) repertoire of intestinal T<sub>h</sub>17 cells in SFB-colonized mice has minimal overlap with that of other intestinal CD<sup>4</sup><sup>+</sup> T cells and that most T<sub>h</sub>17 cells, but not other T cells, recognize antigens encoded by SFB. T cells with antigen receptors specific for SFB-encoded peptides differentiated into RORγt-expressing T<sub>h</sub>17 cells, even if SFB-colonized mice also harboured a strong T<sub>h</sub>1 cell inducer, <i>Listeria monocytogenes</i>, in their intestine. The match of T-cell effector function with antigen specificity is thus determined by the type of bacteria that produce the antigen. These findings have significant implications for understanding how commensal microbiota contribute to organ-specific autoimmunity and for developing novel mucosal vaccines.

How SFB induces T<sub>h</sub>17 cells and how these cells contribute to self-reactive pathological responses remain key unanswered questions. A recent study, using mice with monoclonal TCRs, suggested that induction of T<sub>h</sub>17 cells by SFB or other microbiota is independent of cognate antigen recognition<sup>15</sup>. To further evaluate mucosal effector T-cell induction in a physiological setting, we undertook an examination of the repertoire and specificity of naturally arising T<sub>h</sub>17 cells. To facilitate analysing live T<sub>h</sub>17 cells, we used Il23r<sup>GFP</sup><sup>+</sup> reporter mice<sup>16</sup>, as among CD<sup>4</sup><sup>+</sup> T cells, only this subset expresses IL-23R. We first asked if small intestine lamina propria (SILP) T<sub>h</sub>17 cells are in general responsive to gut luminal commensal antigens. GFP<sup>+</sup> (T<sub>h</sub>17) and GFP<sup>−</sup> (non-T<sub>h</sub>17) CD<sup>4</sup><sup>+</sup> T cells, purified from Il23r<sup>GFP</sup><sup>+</sup><sup>+</sup><sup>C57BL/6</sup><sup>6</sup> (B6) mice that had been colonized with SFB, were incubated with splenic antigen-presenting cells (APCs) and autoclaved small intestinal luminal content of mice from the Jackson laboratory (Jackson) and Taconic Farms (Taconic). We used the measure of forward scatter (FSC) as a surrogate readout for T-cell activation. Intriguingly, only T<sub>h</sub>17 cells mounted a detectable response to Taconic antigens (Extended Data Fig. 1a). SFB is one of the bacteria unique to Taconic flora<sup>9</sup>. Thus we repeated the assay with faecal extract from SFB-colonized mice (Extended Data Fig. 1b, c). We then asked if T<sub>h</sub>17 cells from SFB-colonized mice had a relative weak response towards Jackson antigens, but had a robust response towards Taconic antigens. Significantly, SFB mono-associated mouse faecal antigens stimulated over 60% of the T<sub>h</sub>17 cells (Fig. 1c). In contrast, there was no response of T<sub>h</sub>17 cells to faecal material from germ-free mice (data not shown). Thus, the majority of T<sub>h</sub>17 cells in the SILP of SFB-colonized mice react with SFB-derived antigens, whereas a small proportion respond to non-SFB antigen, indicating that most T<sub>h</sub>17 cells are specific for bacteria in the intestinal lumen.

We wished to compare the TCR repertoires of T<sub>h</sub>17 cells and those of non-T<sub>h</sub>17 cells. Using antibodies against a panel of TCR V<sub>H</sub>Js, we observed a higher proportion of V<sub>B14</sub><sup>+</sup> T cells in T<sub>h</sub>17 cells than in non-T<sub>h</sub>17 cells from the SILP (Extended Data Fig. 2a, b). This bias was not observed in T<sub>h</sub>17 cells from Jax (Jackson) mice (Extended Data Fig. 2c). Thus SFB acting alone was sufficient to promote the development of T<sub>h</sub>17 cells in the SILP, whereas Jax mice respond to other microbiota, including SFB, in the same TCR V<sub>H</sub>-dependency. SFB-specific TH17 cells in mouse mice (Extended Data Fig. 1b), and any bystander effect in this assay was negligible (Extended Data Fig. 1c). Next, we used an IL-17A ELISPOT assay to quantify the percentage of T<sub>h</sub>17 cells from SFB-colonized mice responding to commensal antigens. GFP<sup>+</sup> cells had a relatively weak response towards Jackson antigens, but had a robust response towards Taconic antigens. Significantly, SFB mono-associated mouse faecal antigens stimulated over 60% of the T<sub>h</sub>17 cells (Fig. 1c).

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Figure 1 | Intestinal T<sub>h</sub>17 cells are specific for SFB- and other microbiota-derived antigens. a, Selective activation of intestinal GFP<sup>+</sup> CD<sup>4</sup><sup>+</sup> T cells from Il23r<sup>GFP</sup><sup>++</sup> mice by faecal extract from SFB-monoassociated mice. Forward scatter (FSC) was evaluated after 2 days. b, Activation of SILP CD<sup>4</sup><sup>+</sup> T cells from B6 Taconic mice and B6 Jackson mice with faecal extract from SFB-monoassociated mice. Jax, Jackson mice; Tac, Taconic mice. c, IL-17A ELISPOT assay of intestinal GFP<sup>+</sup> CD<sup>4</sup><sup>+</sup> T cells from SFB-colonized Il23r<sup>GFP</sup><sup>++</sup> mice treated with indicated stimuli. Left, representative ELISPOT images. Right, compilation of results from multiple animals. Each symbol represents cells from a separate animal.
recapitulated when the CD4+ T cells were stained with antibodies specific for RORγt and IL-17A, two other characteristic markers of T17 cells (Extended Data Fig. 2c). However, intracellular staining for IFNγ and FOXP3 indicated no Vβ14+ cell bias among T11 and T regulatory cells (Extended Data Fig. 2c). To determine if the Vβ14 enrichment of T17 cells is influenced by microbiota, we compared SFB-free B6 Jackson mice with SFB-colonized B6 Taconic mice. The Jackson mice had few RORγt+ T17 cells, and there was no enrichment of Vβ14+ cells among them. In contrast, Jackson mice cohoused with Taconic mice had increased numbers of lamina propria T17 cells, which were among them. In contrast, Jackson mice cohoused with Taconic mice had increased numbers of lamina propria T17 cells, which were enriched for Vβ14+ TCRs (Extended Data Fig. 2d), indicating that the T17 repertoire is shaped by specific microbiota.

We chose to focus on Vβ14+ cells to further elucidate the gut CD4+ T-cell repertoire. First, we used pyrosequencing to examine the repertoire of Vβ14+ SILP T17 and non-T17 cells from SFB-colonized mice. The complementarity-determining region 3 (CDR3) of Vβ14 was characterized for each cell population from eight Il23rGFP/+ mice. Each sample contained a minimum of several hundred unique CDR3 sequences (Extended Data Fig. 3a). Interestingly, the ten most frequently used unique CDR3 sequences accounted for 60% of the T17 and only 40% of the non-T17 repertoire (Extended Data Fig. 3b). Furthermore, the dominant CDR3 sequences in individual mice exhibited a clear bias towards either T17 or non-T17 cells (Supplementary Table 1). Many of these CDR3 sequences were shared between mice and were enriched either in T17 or in non-T17 cells in individual mice (Extended Data Fig. 3c).

The finding that intestinal T17 cells have a distinct repertoire prompted us to further determine their antigen specificity. Thus, we sorted single T cells from four mice and sequenced their Vβ14 and paired Vα chains (Extended Data Fig. 4a). Notably, each mouse carried some Vβ14 sequences that were present in several sorted cells, and these sequences strongly biased towards T17 or non-T17 cells (Extended Data Fig. 4b), corroborating our findings from the high-throughput sequencing analysis. To define the antigen specificity of the TCRs from intestinal T17 and non-T17 cell clones, we expressed a cohort of nineteen predominate clonotypic TCRs (ten T17 clones, eight non-T17 clones, and one neutral clone) in a NFAT–GFP+ hybridoma that can report on TCR signalling5. Upon co-culture of the hybridomas with splenic APCs and heat-inactivated mouse intestinal luminal content, several T17-T17 hybridomas, but not non-T17-T17 hybridomas, responded to Taconic antigens, and not to Jackson antigens (Extended Data Fig. 4c). Furthermore, when SFB-mono antigens were used, we detected responses from 7/10 T17-T17 and the neutral TCR hybridoma, but none of the non-T17 cell hybridomas (Fig. 2a). These responses were abrogated if the APCs were from MHCII-deficient mice (Extended Data Fig. 4d).

We next sought to identify epitopes recognized by T17 cell TCRs using a whole-genome shotgun cloning and expression screen, an unbiased approach previously used to identify T-cell antigens from other bacteria14 (Extended Data Fig. 5a). One bacterial clone, designated 3F12-E8, stimulated 7B8 and four other T17-T17 hybridomas (Extended Data Fig. 5b–d). Based on the recent annotation of the SFB genome19,20, we assigned the 672-base pair 3F12-E8 insert to an SFB gene (SFBNYU_003340). We confirmed the specificity by cloning the full-length gene and demonstrating that its product stimulated the aforementioned five TCRs, but not any other TCRs (Fig. 2b, left). We further mapped a minimal epitope that stimulated all five TCRs and a shorter 8-amino-acid epitope that stimulated only the 7B8 and 2A6 hybridomas (Extended Data Fig. 5e).

Another expression screen was performed using the 1A7 hybridoma, which along with three other TCRs formed a distinct cluster with an identical Vα and highly similar Vβ14 CDR3 sequences (Extended Data Fig. 6a). A stimulatory clone, designated 2D10-A10 (Extended Data Fig. 6b, c), contained the amino-terminal sequence of another SFB gene (SFBNYU_004990). We mapped the epitope for the 1A7 hybridoma to 9 amino acids (Extended Data Fig. 6d). Both the full-length gene product and a 9-amino-acid peptide stimulated all four TCRs, indicating that...
these TCRs indeed recognize the same epitope (Fig. 2b, right). However, the single TCR derived from non-T\(_{h17}\) cells (3F4) displayed a much weaker dose–response to peptide antigen than the other TCRs (Extended Data Fig. 6e).

Thus, eight out of eleven Vß14\(^+\) T\(_{h17}\)-TCR hybridomas recognized two distinct antigens encoded by SFB (Fig. 2c). Both proteins are unique to SFB, expressed at a medium to high level, and predicted to be secreted or at the cell surface (Fig. 2d). Importantly, primary Vß14\(^+\) T\(_{h17}\) cells responded to the two immunodominant SFB epitopes (Extended Data Fig. 7a). Although Vß14\(^+\) cells consistently responded slightly better, Vß14\(^+\) T\(_{h17}\) cells were also stimulated by SFB (Extended Data Fig. 7b), indicating that these cells respond to other SFB epitopes. An in silico search was conducted for potential epitopes within the SFB proteome (Extended Data Fig. 7c, d), which yielded several more stimulatory peptides (Extended Data Fig. 7e). Among these, peptide N5, also derived from SFBNYU_003340, was a strong stimulator of intestinal T\(_{h17}\) cells, activating both Vß14\(^+\) cells and Vß14\(^-\) cells (Extended Data Fig. 7f). Thus, in the small intestine, SFB is the dominant antigen source for polyclonal T\(_{h17}\) cells, but for few, if any, non-T\(_{h17}\) cells.

We then asked what happens to T cells expressing SFB-specific TCRs. We generated 7B8 TCR transgenic mice (7B8Tg)\(^{21}\), and transferred naive T cells from these mice into C57BL/6 mice\(^{22}\). After one week, we readily detected donor-derived T cells in the ILP of mice that had been exposed to SFB, whereas they were completely absent in SFB-deficient recipients (Extended Data Fig. 8a). Remarkably, almost all donor-derived cells became positive for ROR\(\gamma\)T (Fig. 3a). Similar results were obtained upon transfer of T cells from two other TCR transgenic strains (1A2Tg and 5A11Tg) into SFB-colonized recipient mice (Extended Data Fig. 8b). The donor-derived T cells lacked expression of the transcription factors associated with alternative CD4 T-cell programs (for example, FOXP3, GATA3, and T-bet) (Extended Data Fig. 8c).

To visualize endogenous SFB-antigen-specific T cells, we produced MHCII-tetramers containing peptide A6 from SFBNYU_003340 (3340-A6 tetramer)\(^{23}\). The I-A\(^\beta\)/3340-A6 tetramer specifically stained GFP\(^+\) SILP CD4\(^+\) T cells from SFB-colonized H2b\(^{GFP/+}\) mice (Extended Data Fig. 8d). Furthermore, a sizable population of I-A\(^\beta\)/3340-A6 tetramer-positive cells was present in B6 Taconic, but not in B6 Jackson mice (Extended Data Fig. 8e), and these cells were uniformly ROR\(\gamma\)T-positive, indicating that they were SFB-elicited T\(_{h17}\) cells (Fig. 3b).

We next aimed to determine whether polarization of the antigen-specific T\(_{h17}\) cells in response to SFB colonization is dictated by the nature of the antigenic protein or properties of the microbe. Listeria monocytogenes, an enteric pathogenic bacterium that also colonizes the small intestine, typically elicits a TH1 response\(^{24}\). Mice were orally infected with L. monocytogenes expressing SFBNYU_003340 (Listeria-3340) (Extended Data Fig. 8f) or SFB before intravenous transfer of 7B8Tg T cells. 7B8Tg T cells accumulated in the SILP of both sets of mice, but, importantly, they expressed T-bet rather than ROR\(\gamma\)T when the hosts were colonized with Listeria-3340 (Fig. 3c).

To further investigate a relationship between the fate of SILP T helper cells and the bacterial origins of antigens, we transferred 7B8Tg T cells into mice that were colonized with both SFB and Listeria and simultaneously tracked CD4\(^+\) T-cell responses specific for both bacteria in the SILP using the Ly5.1\(^+\) congenic marker for 7B8Tg cells and listeriolysin O (LLO)-tetramers that stain endogenous Listeria-specific T cells derived from the host (Extended Data Fig. 9a). In the presence of both T\(_{h17}\) and T\(_{h1}\)-inducing bacteria, 7B8Tg T cells expressed ROR\(\gamma\)T, but not T-bet, whereas LLO-tetramer\(^+\) cells expressed T-bet, but not ROR\(\gamma\)T (Fig. 4a and Extended Data Fig. 9b, c). This result is in contrast to the T\(_{h1}\) polarization of TCR transgenic T cells specific for the commensal CBir1 flagellin antigen observed upon infection with the protozoan parasite Toxoplasma gondii\(^{25}\), a T\(_{h1}\)-inducing intestinal pathogen. This suggests that, unlike CBir1-encoding Clostridia, SFB has the ability to direct a dominant signal specialized for induction of T\(_{h17}\) cells.

SFB colonization of the small intestine is potentially beneficial, attenuating pathogenic bacteria-induced colitis\(^{6}\), but it can also trigger or exacerbate systemic autoimmune disease\(^{10,11}\), raising the question as to whether SFB-specific T\(_{h17}\) cells can circulate beyond the small intestine. We examined the colons and spleens of SFB-positive recipients of 7B8Tg naive T cells, and found these cells in both organs. Importantly, more than 80% of these SFB-specific T cells in colon and 40% in spleen expressed ROR\(\gamma\)T (Fig. 4b). Consistent with this result, staining of endogenous T cells from Taconic mice revealed 3340-A6 tetramer-positive cells in the large intestine and most of these cells expressed ROR\(\gamma\)T (Extended Data Fig. 10a, b).

Our results therefore indicate that intestinal antigen-specific CD4\(^+\) T cells differentiate to become either T\(_{h1}\) or T\(_{h17}\) cells, depending on which luminal bacterium delivers the antigen. We propose a deterministic model for T helper cell differentiation whereby the bacterial context of cognate antigen delivery dictates the fate of the antigen-specific T cells (Fig. 4c). Our work opens the way towards elucidating the mechanisms of T\(_{h17}\) cell induction by microbiota and of how gut-induced T\(_{h17}\) cells can contribute to distal organ-specific autoimmune disease. In addition, it serves as a guide for future studies of human commensal-specific pro-inflammatory T cells that are thought to contribute to autoimmune diseases such as rheumatoid arthritis\(^{26}\). Finally, the demonstration of controlled polarized T-cell responses towards commensal bacteria offers the potential for novel approaches towards mucosal vaccination.

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**Figure 3** | SFB-specific T cells become T\(_{h17}\) cells in the SILP. a, 7B8Tg cells (Ly5.2) were transferred into SFB-colonized mice (Ly5.1), and SILP T cells were analysed after 8–15 days. Left, representative FACS plots. Right, analysis of multiple animals (one symbol per animal). b, I-A\(^\beta\)/3340-A6 tetramer stain of SILP T cells from SFB-colonized B6 mice. Left, representative FACS plots. Right, analysis of multiple animals (one symbol per animal). C, 7B8Tg cells (Ly5.1) were transferred into Ly5.2 congenic hosts orally colonized with Listeria-3340 or SFB. Seven days after transfer, donor-derived cells in the SILP were analysed. Analyses of cells from different mice are shown in the composite FACS plot. The results are representative of three experiments.
METHODS SUMMARY

Mice. All mice were housed in the animal facility of The Skirball Institute of Molecular Medicine at the New York University School of Medicine. Experimental protocols were approved by the Institutional Animal Care and Use Committee. C57BL/6 mice were purchased from Taconic Farm (B6 Taconic) or the Jackson Laboratory (B6 Jackson). IL23−/− mice, a gift from M. Oukka, were maintained by breeding with B6 Taconic mice. 7B8Tg, 1A2Tg and 5A11Tg SFB-specific TCR transgenic (Tg) mice were generated as previously described and kept with SFB-minus flora. 6 mice were purchased from Taconic Farm (B6 Taconic) or the Jackson Laboratory (B6 Jackson). 7B8Tg, 1A2Tg and 5A11Tg SFB-specific TCR transgenic (Tg) mice were generated as previously described and kept with SFB-minus flora. For adoptive transfer, naive Tg T cells (CD62Lhi CD44lo Vb+ cells) were added to a co-culture of APCs and hybridomas. For adoptive transfer, naive Tg T cells (CD62L hi CD44 lo Vb+ cells) were added to a co-culture of APCs and hybridomas. For adoptive transfer, naive Tg T cells (CD62L hi CD44 lo Vb+ cells) were added to a co-culture of APCs and hybridomas. For adoptive transfer, naive Tg T cells (CD62L hi CD44 lo Vb+ cells) were added to a co-culture of APCs and hybridomas.

Construction and screen of whole-genome shotgun library of SFB. The shotgun library was prepared with a procedure modified from a previous study. The library is estimated to contain 106 clones. The expression of exogenous proteins was induced by isopropylthigalactoside for 4h. For antigen screening, pools of heat-killed bacteria (~30 clones per pool) were added to a co-culture of APCs and hybridomas. MHCI tetramer production and staining. MHCI tetramers were produced as previously described. SILP T cells were incubated at room temperature for 60 min with fluorochrome-labelled tetramer (10 nM) before staining with rel.

Heterologous expression of SFBNYU_00340 in Listeria monocytogenes. The entire coding region of SFBNYU_00340, including its predicted signal sequence, was sub-cloned into the Listeria expression vector pMK2. The resultant plasmid was transformed into electrocompetent Listeria monocytogenes strain 10403S-inlA and plated on selective medium containing kanamycin (50 μg ml−1). The plasmid was transformed into electrocompetent Listeria monocytogenes strain 10403S-inlA and plated on selective medium containing kanamycin (50 μg ml−1). The plasmid was transformed into electrocompetent Listeria monocytogenes strain 10403S-inlA and plated on selective medium containing kanamycin (50 μg ml−1). The plasmid was transformed into electrocompetent Listeria monocytogenes strain 10403S-inlA and plated on selective medium containing kanamycin (50 μg ml−1). The plasmid was transformed into electrocompetent Listeria monocytogenes strain 10403S-inlA and plated on selective medium containing kanamycin (50 μg ml−1).

Online Content Any additional Methods, Extended Data display items and Source Data are available in the online version of the paper; references unique to these sections appear only in the online paper.

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1. Bettelli, E., Korn, T., Oukka, M. & Kuchroo, V. K. Induction and effector functions of Th17 cells. Nature 453, 1051–1057 (2008).

2. McGeachy, M. J. & Cua, D. J. The link between IL-23 and Th17 cell-mediated immune pathologies. Semin. Immunol. 19, 372–376 (2007).

3. Littman, D. R. & Rudensky, A. Y. Th17 and regulatory T cells in mediating and restraining inflammation. Cell 140, 845–858 (2010).

4. Ivanov, I. I. et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 126, 1121–1133 (2006).

5. Ivanov, I. I. et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. Cell Host Microbe 4, 337–349 (2008).

6. Atarashi, K. et al. ATP drives lamina propria Th17 cell differentiation. Nature 455, 808–812 (2008).

7. Atarashi, K. et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500, 232–236 (2013).

8. Gaboriau-Routhiau, V. et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 31, 677–689 (2009).

9. Wu, H. J. et al. Gut-residing segmented filamentous bacteria drive autoimmune arthritis via T helper 17 cells. Immunity 32, 815–827 (2010).

10. Lee, Y. K., Menezes, J. S., Umesaki, Y. & Mazmanian, S. K. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc. Natl Acad. Sci. USA 108 (Suppl 1), 4615–4622 (2011).

11. Ivanov, I. I. & honda, K. Intestinal commensal microbes as immune modulators. Cell Host Microbe 12, 496–508 (2012).

12. Hooper, L. V., Littman, D. R. & Macpherson, A. J. Interactions between the microbiota and the immune system. Science 336, 1268–1273 (2012).

13. Schnupf, P., Gaboriau-Routhiau, V. & Cerf-Bensussan, N. Host interactions with segmented filamentous bacteria: An unusual trade-off that drives the post-natal maturation of the gut immune system. Semin. Immunol. 25, 342–351 (2013).

14. Lochner, M. et al. Restricted microbiota and absence of cognate TCR antigen leads to an unbalanced generation of Th17 cells. J. Immunol. 186, 1531–1537 (2011).

15. Awashti, A. et al. Cutting edge: IL-23 receptor GFP reporter mice reveal distinct populations of IL-17-producing cells. J. Immunol. 182, 5904–5908 (2009).

16. isee, W. et al. CTLA-4 suppresses the pathogenicity of self antigen-specific T cells by cell-intrinsic and cell-extrinsic mechanisms. Nature Immunol. 11, 129–135 (2010).
18. Sanderson, S., Campbell, D. J. & Shastri, N. Identification of a CD4⁺ T cell-stimulating antigen of pathogenic bacteria by expression cloning. *J. Exp. Med.* 182, 1751–1757 (1995).

19. Szczesań, A. et al. The genome of Th17 cell-inducing segmented filamentous bacteria reveals extensive auxotrophy and adaptations to the intestinal environment. *Cell Host Microbe* 10, 260–272 (2011).

20. Prakash, T. et al. Complete genome sequences of rat and mouse segmented filamentous bacteria, a potent inducer of Th17 cell differentiation. *Cell Host Microbe* 10, 273–284 (2011).

21. Kouskoff, V., Signorelli, K., Benoist, C. & Mathis, D. Cassette vectors directing expression of T cell receptor genes in transgenic mice. *J. Immunol. Methods* 180, 273–280 (1995).

22. Kearney, E. R., Pape, K. A., Loh, D. Y. & Jenkins, M. K. Visualization of peptide-specific T cell immunity and peripheral tolerance induction in vivo. *Immunity* 1, 327–339 (1994).

23. Moon, J. J. et al. Naive CD4⁺ T cell frequency varies for different epitopes and predicts repertoire diversity and response magnitude. *Immunity* 27, 203–213 (2007).

24. Hsieh, C. S. et al. Development of TH1 CD4⁺ T cells through IL-12 produced by Listeria-induced macrophages. *Science* 260, 547–549 (1993).

25. Hand, T. W. et al. Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses. *Science* 337, 1553–1556 (2012).

26. Scher, J. U. et al. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *eLife* 2, e01202 (2013).

27. Monir, I. R., Gahan, C. G. & Hill, C. Tools for functional postgenomic analysis of *Listeria monocytogenes*. *Appl. Environ. Microbiol.* 74, 3921–3934 (2008).

28. Xayarath, B., Marquis, H., Port, G. C. & Freitag, N. E. *Listeria monocytogenes* CtaP is a multifunctional cysteine transport-associated protein required for bacterial pathogenesis. *Mol. Microbiol.* 74, 956–973 (2009).

**Supplementary Information** is available in the online version of the paper.

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**Author Contributions** Y.Y. and D.R.L. designed the experiments and wrote the manuscript with input from the co-authors. Y.Y., M.B.T., M.X., C.N., A.C., X.L. and J.-J.L. performed most analyses. M.B.T. constructed TCR hybridomas. M.X. developed SFB-specific antibodies. M.G., H.X. and J.J.L. did TCR pyrosequencing analysis. J.L.L. and M.K.J. developed tetramers. F.A. and V.J.T. generated transgenic *Listeria*. A.S. performed RNA-seq analysis of SFB.

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METHODS
Mice. C57/BL/6 mice were purchased from Taconic Farm (B6 Taconic) or Jackson Laboratory (B6 Jackson). IL23−/− mice were provided by M. Oukka and maintained by breeding with B6 Taconic mice. Ly5.1 mice (B6.SJL-Ptprc Pepck−/−) and MHCII-deficient mice (B6.129S2-H2-K10/10) were from Jackson Laboratory.

Antibodies and flow cytometry. The following antibodies were from eBioscience, BD Pharmingen or BioLegend: Vβ1 (R20.6), Vβ3 (K25), Vβ4 (KT4), Vβ5 (MR9-4), Vβ6 (RR4-7), Vβ7 (TR310), Vβ8 (F31.3), Vβ8.1/8.2 (MR5-2), Vβ8.3 (BC1), Vβ10 (B21.5), Vβ11 (CTVB11), Vβ12 (MR1-1), Vβ14 (14-2), CD3 (145-2C11), CD4 (RM4-5), CD25 (PC61), Ly5.1 (A20), Ly5.2 (104), MHCII (M5/114), ROByt (AFK5-9 or B2D), FOX3 (FKJ-16a), T-bet (eBioB10), GATA3 (TWA1), IL-17A (eBio17B) and IFN-γ (XM6.12). Flow cytometric analysis was performed on an LSR II (BD Biosciences) or an Aria II (BD Biosciences) and analysed using FlowJo software (Treestar). DAPI (Sigma) was used to exclude dead cells.

T-cell preparation and staining. Small intestine lamina propria were minced and then incubated for 30 min at 37 °C with collagenase D (1 mg ml−1; Roche), dispase (0.05 U ml−1; Worthington) and DNsase I (100 μg ml−1; Sigma). Lymphocytes were collected at the interface of a 40%/80% Percoll gradient (GE Healthcare). Cells were stained for surface markers, followed by fixation and permeabilization (eBioscience).

Calculating enrichment scores. An enrichment score for a given Vβ in IL-23R (GFP)− cells is defined as the equation of (per cent of Vβ+ cells in the GFP-positive fraction)/(per cent of Vβ− cells in the GFP-negative fraction). For example, for Extended Fig. 2b, Vβ14 enrichment was calculated as (7.45/(7.45 + 6.26))/(4.48/(4.86 + 6.18)) or 3.3. A score > 1 means a positive enrichment and a score = 1 means no enrichment.

High-throughput TCR sequencing. The SILP cells from IL23−/− mice were stained for surface markers and Vβ14+ CD4+ T cells were sorted on the Aria II. For each sample, we collected about 2 × 10^5 cells (2.17 ± 0.43 ± 10^5 cells for GFP+ Tψ17 cells and 2.38 ± 0.54 ± 10^5 cells for GFP− non-Tψ17 cells). Cells were lysed in TRizol reagent (Invitrogen) and RNA was extracted following the manufacturer’s instruction. RNA precipitation was aided with GlycoBlue (Invitrogen). Complemen- tary DNAs were prepared with a reverse transcription kit (USB). Vβ were performed using barcoded oligonucleotides. PCR products from 16 samples were quantified on NanoDrop. Equal amounts of barcoded PCR product were mixed and sequenced using a 454 GS Junior system (Roche). The raw sequencing data was first aligned using the high-throughput analysis tool provided by IMGT. We obtained 6.647 ± 954 reads for Tψ17 cells and 5.573 ± 889 reads for non-Tψ17 cells. CD3 usage was further computed with Perl-based scripts developed in-house. The Tψ17 samples had 340–772 unique Vβ14 CD3 sequences and the non-Tψ17 samples had 849–2,148 unique Vβ14 CD3 sequences.

Single-cell TCR sequencing. The SILP cells from IL23−/− mice were stained for surface markers. GFP+ and GFP− Vβ14+ CD4+ T cells were sorted on the BD Aria II and deposited at one cell per well into 96-well PCR plates preloaded with nuclease-free TE. Immediately after sorting, whole plates were incubated at 50 °C for 60 min for cDNA synthesis. Half of the cDNA was used for PCR amplification for 16 cycles using a mix of twenty-one reagent (USB) and Sanger sequencing was performed by Macrogen. In nearly all cases, for cells with the same Vβ14 sequence, we retrieved a single unique Vβ sequence. The analysis using these cells was clone-matched using the NEAT−/− mouse and the other half of cDNA was amplified for 16 cycles with a mix of twenty-forward primers (each modified by adding a 5' extended anchor sequence: TAATAAGCAGTCACTATAGGG and reverse primer 5'-CAGTGCAGAGGTAGCGTCT-3'). To retrieve sequences, we first ran Vβ23 sequencing PCR, which amplifies the first 100 nucleotides from the Met-α bacteria and Human/mouse/rat kits. An Illumina RNA-seq library was prepared from these samples using a previously described strand-specific Nextera protocol. The resulting reads were aligned to the SRFBYU (Genome Assembly) with Bowtie2 and transcript abundance was estimated using Cufflinks26 with default parameters.

Production of anti-SFB antibody and immunostaining. The cDNA fragments corresponding to amino acids 43–359 (3340N) and 734–1060 (3340C) of SRFBYU 003340 were cloned into the pGEX6p1 expression vector. Recombinant proteins fused to N-terminal glutathione-S-transferase (GST) were expressed in E. coli BL21, purified with glutathione Sepharose 4B (GE), and were prepared for Western blotting (GE). The flow-through fractions containing polyepitopes without the GST tag were collected as immunogen. Rabbit polyclonal antibodies against both polyepitopes were raised by Covance. For immunostaining, antibodies were fixed with 2% paraformaldehyde, followed by washing with 0.01 M Tris/0.1% BSA and blocked sequentially with primary antibody (1:1 mix of the two rabbit anti-3340 antibodies) and phycocyanin-conjugated goat anti-rabbit antibody.

Activation of polyclonal SLP Tψ17 cells. GFP+ and GFP− SLP CD4+ T cells sorted from IL23−/− mice were incubated with 2 × 10^5 APCs (CD11c+ cells purified from the spleen) and stimulated in complete RPMI medium supplemented with IL-2 (10 U ml−1) and IL-7 (5 ng ml−1) for 2–3 days. Cells were collected and stained with Vβ-specific antibodies. Forward scatter increment, as readout for cell activation, was analysed by FACS.

IL-17A ELISPot assay. IL-17A ELISPot was performed with a mouse/rat IL-17A ELISPot Ready-SET-Go! kit (eBioscience). Dots were automatically enumerated with Immunospot software (Version 5.0.0).

MHCII tetramer production and staining. 1A*3340–A6 tetramer was produced as previously described27. Briefly, QFSGAVYPKTD, an immunodominant epitope from SRFBYU 003340, covalently linked to I-A* via a flexible linker, was produced in Drosophila S2 cells. Soluble pMHCII monomers were purified, biotinylated,
and tetramerized with phycoerythrin- or allophycocyanin-labelled streptavidin. To stain endogenous cells, SILP cells were first resuspended in FACS buffer with FcR block, 2% mouse serum and 2% rat serum. Then tetramer was added (10 nM) and incubated at room temperature for 60 min. Cells were washed and followed by regular staining at 4°C. I-Ab/2W and I-Ab/LLO tetramers were previously described.23

**Generation of Th17-TCRtg mice.** TCR sequences of 7B8, 1A2 and 5A11 were cloned into the pTa and pTb vectors kindly provided by D. Mathis.21 TCR transgenic animals were generated by the Rodent Genetic Engineering Core at the New York University School of Medicine. Positive pups were genotyped by PCR and kept on SFB-minus flora.

**Adoptive transfer.** Spleens from 7B8Tg mice were collected and disassociated. Red blood cells were lysed using ACK lysis buffer (Lonza). Naive Tg T cells (CD62Lhi CD44lo Vb141 CD41 CD31) were sorted on a BD Aria II. Cells were transferred into congenic Ly5.1 recipient mice by retro-orbital injection. In some experiments, we used Ly5.1/Ly5.2 TCRTg mice as donor and transferred naive Tg T cells to congenic Ly5.2 recipient mice.

**Heterologous expression of SFBNYU_003340 in Listeria monocytogenes.** To generate strains of *L. monocytogenes* that express the SFBNYU_003340 antigen, the entire coding region including its predicted signal sequence was PCR-amplified from a plasmid containing the SFBNYU_003340 gene. The resultant PCR product was digested and sub-cloned into the *Listeria* expression vector pIMK2 (provided by C. Hill), allowing the gene to be expressed under the synthetic promoter Phelp, (High expression promoter in *L. monocytogenes*)27. The resultant plasmid designated pIMK2-3340 was transformed into electrocompetent *Listeria monocytogenes* strain 10403S-inlAm (provided by N. E. Freitag) and plated on selective medium containing kanamycin (50 µg ml⁻¹).28 pIMK2 is a derivative of the plasmid pPL2 and stably integrates in single copy within the tRNAArg gene following electro-poration.34 The integrity of the SFBNYU_003340 gene was validated by PCR and expression confirmed by Coomassie staining of *L. monocytogenes* exoproteins.

**Oral infection with SFB and *L. monocytogenes***. For SFB colonization, we dissolved in sterile PBS fresh faecal pellets collected from Il23GFP/GFP Rag2⁻/⁻ mice that have highly elevated levels of SFB, and infected mice by oral gavage. For *L. monocytogenes* colonization, we grew *Listeria-3340* and *Listeria-empty* in brain heart infusion medium and infected mice orally with 10⁹ colony forming units.

**Bioinformatic analysis.** Protein predictions were made by bioinformatic tools, including Paart (Version 3.0)33 and Cello (Version 2.3)36 for localization prediction, and IEDB (Immune Epitope Database) for MHCII binding affinity prediction. **Statistical analysis.** All analyses were performed using GraphPad Prism (Version 6.0). Differences were considered to be significant at *P* values <0.05.

29. Alamyar, E., Giudicelli, V., Li, S., Duroux, P. & Lefranc, M. P. IMGT/HighV-QUEST: the IMGT(R) web portal for immunoglobulin (IG) or antibody and T cell receptor (TR) analysis from NGS high throughput and deep sequencing. *Immunome Res.* 8, 26 (2012).
30. Currier, J. R. & Robinson, M. A. Spectratype/immunoscope analysis of the expressed TCR repertoire. *Curr. Protocols Immunol. Chapter 10*, Unit 10.28 (2001).
31. Langmead, B., Trapnell, C., Pop, M. & Salzberg, S. L. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol.* 10, R25 (2009).
32. Roberts, A., Pimentel, H., Trapnell, C. & Pachter, L. Identification of novel transcripts in annotated genomes using RNA-Seq. *Bioinformatics* 27, 2325–2329 (2011).
33. Tubo, N. J. et al. Single naive CD4+ T cells from a diverse repertoire produce different effector cell types during infection. *Cell* 153, 785–796 (2013).
34. Lauer, P., Chow, M. Y., Loessner, M. J., Portnoy, D. A. & Calendar, R. Construction, characterization, and use of two *Listeria monocytogenes* site-specific phage integration vectors. *J. Bacteriol.* 184, 4177–4186 (2002).
35. Yü, N. Y. et al. PSORTb 3.0: improved protein subcellular localization prediction with refined localization subcategories and predictive capabilities for all prokaryotes. *Bioinformatics* 26, 1608–1615 (2010).
36. Yü, C.-S., Chen, Y.-C., Lu, C.-H. & Hwang, J. K. Prediction of protein subcellular localization. *Proteins* 64, 643–651 (2006).
Extended Data Figure 1 | Stimulation of SILP T<sub>H17</sub> cells requires intestinal microbiota antigen presentation. 

**a**, Intestinal GFP<sup>+</sup> CD4<sup>+</sup> T cells from Il23rGFP/Il23rGFP<sup>+</sup> mice stimulated with faecal material from Jackson and Taconic mice in the presence of syngeneic splenic APCs. Forward scatter was evaluated after 2 days. 

**b**, TH17 cell activation by faecal material from SFB-monoassociated mice in the presence of APCs sufficient (WT) or deficient (KO) for MHC class II. 

**c**, Evaluation of potential activation of bystander CD4<sup>+</sup> T cells upon stimulation with SFB antigen. SILP CD4<sup>+</sup> T cells from mice with Jackson flora (Ly5.1) and Taconic flora (Ly5.2) were co-cultured or stimulated separately with APCs and SFB-monoassociated faecal material, and FSC was evaluated.
Extended Data Figure 2 | Microbiota-dependent TCR usage bias among SILP T\textsubscript{H17} cells. a, SILP CD\textsuperscript{+} T cells from Il23r\textsuperscript{GFP\textsuperscript{+/+}} mice were analysed for utilization of V\textbeta\textsubscript{14} in T\textsubscript{H17} cells versus non-T\textsubscript{H17} cells. Ratios of the percentage of each TCR V\textbeta in GFP\textsuperscript{+} vs GFP\textsuperscript{−} cells are shown. Each symbol represents one mouse. b, Relative expression of V\textbeta\textsubscript{14} and V\textbeta\textsubscript{6} TCRs by SILP T\textsubscript{H17} versus non-T\textsubscript{H17} CD\textsuperscript{4+} T cells from Il23r\textsuperscript{GFP\textsuperscript{+/+}} mice. Left, representative FACS plots; Right, analysis of multiple animals. Each symbol represents one mouse. c, Specific enrichment of V\textbeta\textsubscript{14} TCRs in CD\textsuperscript{4+} T cells expressing ROR\gamma\textsuperscript{t} and IL-17A, but not FOXP3 or IFN\gamma. Left, representative FACS plots; Right, analysis of multiple animals. d, Correlation of V\textbeta\textsubscript{14} enrichment in T\textsubscript{H17} cells with the presence of specific commensal microbiota. B6 Jackson mice were housed alone or cohoused with B6 Taconic mice for two weeks. Left, representative FACS analyses; Right, analysis of multiple animals.
Extended Data Figure 3 | TH17 TCR repertoire analysis by pyrosequencing.

a, Numbers of unique \( \text{V}_{14} \) CDR3 sequences of individual SILP TH17 and non-TH17 samples. The sequences were normalized for numbers of cells and total reads.

b, Preferential expansion of \( \text{V}_{14} \) clones in the TH17 compartment in the SILP. The proportions of the 10 most abundant \( \text{V}_{14} \) CDR3 sequences from TH17 and non-TH17 cells from 8 mice are shown.

c, TH17-non TH17 bias of unique \( \text{V}_{14} \) CDR3 sequences in the SILP of multiple mice.
Extended Data Figure 4 | Single-cell TCR cloning and TCR hybridoma screen.

a, Efficiency of single-cell Vβ14 cloning from SILP TβR17 and non-TβR17 cells of multiple mice. b, Distributions of unique Vβ14 sequences in TβR17 and non-TβR17 cells within the SILP. Each plot represents one mouse shown in a. y and x axes represent numbers of TβR17 cells and non-TβR17 cells for each unique Vβ14 sequence. Numbers of unique sequences are shown in coloured circles. c, Responses of TβR17 and non-TβR17 TCR hybridomas to small intestinal luminal contents from B6 Taconic and B6 Jackson mice. d, Stimulation of TβR17 TCR hybridomas by SFB-monoassociated antigens in the presence of APCs sufficient (WT) or deficient (KO) for MHC class II.
Extended Data Figure 5 | Identification of SFBNYU_003340 epitopes recognized by a subset of the Th17 TCR hybridomas.  

**a** Schematic representation of the antigen screen using a whole-genome shotgun SFB library.  

**b** Stimulation of the 7B8 hybridoma by bacterial pool 3F12.  

**c** Reactivity of 7B8 and four other TCR hybridomas with bacterial clone 3F12-E8.  

**d** Diversity of the CDR3 sequences of TCRs specific for 3F12-E8. Note that they belong to different Vα subsets and have distinct Vβ14 CDR3 sequences.  

**e** Responses of the 3F12-E8-specific TCR hybridomas to core epitopes encoded by minigenes expressed in E. coli.

| CLONE | CDR3 (Vβ14) | Vα subset | CDR3 (Vα) |
|-------|-------------|-----------|-----------|
| 5A5   | AWSLVNYNSPLY | α11       | AAGGNNKLT |
| 5B5   | AWSLVYGGHERL | α5        | AVSAGTQVVGQLT |
| 7B8   | AWRSSP5QNTLY | α15       | AEGNMGYKLT |
| 1A2   | AWSGQGREEQY  | α18       | ATVMNYNQGKLI |
| 2A6   | AWGSGTGGASPLY| α4        | ALGENSEGTYQR |

3F12-E8
Extended Data Figure 6 | Identification of SFBNYU_004990 epitopes recognized by related TCRs. a, Top, the distribution in Th17 and non-Th17 cells of four TCRs that share an identical TCRα chain. Bottom, amino acid alignment of the Vβ14 CDR3 sequences. The green box highlights the sequence differences. b, Stimulation of the 5A11 hybridoma by bacterial pool 2D10 in the SFB antigen screen. c, Responses of 4 TCR hybridomas, including a non-Th17 hybridoma, to bacterial clone 2D10-A10. d, Responses of the 2D10-A10-specific TCR hybridomas to core epitopes encoded by minigenes expressed in E. coli. e, TCR hybridoma responses to titrated synthetic peptide (IRWFGSSVQKV) in the presence of APCs.
Extended Data Figure 7 | SFB epitopes recognized by diverse T\(_{H17}\) cell TCRs. a, The epitopes recognized by the V\(_{\beta}^{14+}\) TCR hybridomas stimulate only V\(_{\beta}^{14+}\) T\(_{H17}\) cells from the SILP. T\(_{H17}\) cells sorted from Il23rGFP/1 mice were stimulated with indicated peptides (listed in d) in the presence of APCs. Left, representative IL-17A ELISPOT assay with triplicates. Right, normalized peptide-specific T\(_{H17}\) responses. Each dot represents one mouse. b, Polyclonal responses of V\(_{\beta}^{14+}\) and V\(_{\beta}^{14-}\) SILP T\(_{H17}\) cells to SFB antigens. Representative FACS plots from five experiments are shown. c, Bioinformatics filtering approach to select candidate SFB epitopes. d, Summary of newly selected and the known A6 and A15 SFB peptides. e, IL-17A ELISPOT screen for indicated peptides using SILP T\(_{H17}\) cells sorted from SFB-colonized Il23rGFP/1 mice. The A6 peptide from SFBNYU_003340 and anti-CD3 served as positive controls. f, V\(_{\beta}^{14+}\) usage in TH17 cells specific for peptide N5. Left, representative IL-17A ELISPOT assay with triplicates for peptide N5, using V\(_{\beta}^{14+}\) and V\(_{\beta}^{14-}\) SILP T\(_{H17}\) cells sorted from Il23rGFP/1 mice. Right, normalized N5-specific T\(_{H17}\) responses. Each dot represents one mouse.
Extended Data Figure 8 | SFB-specific T cells become T<sub>H17</sub> cells in SFB-colonized mice. a, SFB-dependent 7B8Tg T cell accumulation in the SILP. 2 × 10<sup>6</sup> naive 7B8Tg T cells were transferred into congenic Ly5.1 recipient mice that were SFB-colonized or SFB-free. CD4<sup>+</sup> T cells in the SILP were examined for donor and recipient isotype markers after 13 days. b, Top, strategy for co-transfer of congenic 1A2Tg and 5A11Tg T cells into SFB-colonized recipient mice. Bottom, FACS analysis of ROR<gamma> expression in host- and donor-derived CD4<sup>+</sup> T cells in the SILP at 7 days after transfer. c, FACS analysis of transcription factors in host- and donor-derived SILP CD4<sup>+</sup> T cells after transfer of naive 7B8Tg T cells as in a. d, FACS analysis of SILP T cells from Il23r<sup>GFP<sup>+</sup></sup> mice, stained with I-<sub>A</sub>3340-A6 tetramer and control tetramer (2W). e, FACS analysis of SILP T cells of B6 mice from colonies with different microbiota, stained with I-<sub>A</sub>3340-A6 tetramer and intracellular ROR<gamma> antibody. f, Expansion of 7B8Tg T cells in mice colonized with <i>Listeria monocytogenes</i> expressing SFRNYU_003340. Top, immunofluorescence microscopic visualization of the expression of SFB protein by <i>L. monocytogenes</i>. Listeria-3340 and Listeria-empty were stained with anti-3340 rabbit polyclonal antibody. Red, anti-3340 antibody staining. Blue, DAPI staining. Bottom, naive Ly5.1<sup>+</sup> 7B8Tg cells were transferred into congenic mice infected with Listeria-3340 or Listeria-empty. Seven days after transfer, donor-derived CD4<sup>+</sup> T cells in the SILP were analysed by FACS.
Extended Data Figure 9 | Transcription factor expression in SFB-specific and *Listeria*-specific T cells in co-infected mice. Representative of data plotted in Fig. 4b. 

**a** Experimental design for tracking both SFB- and *Listeria*-specific CD4^+^ T cells following intestinal colonization with both bacteria. Ly5.2 B6 mice were colonized with *Listeria monocytogenes*, SFB, or both bacteria, and 7B8Tg T cells from Ly5.1 mice were injected intravenously. Expression of T_{H1} and T_{H17} transcription factors in the SFB-specific 7B8Tg cells and LLO tetramer-specific recipient T cells was evaluated. 

**b** Intracellular stain for RORγt. 

**c** Intracellular stain for T-bet.
Extended Data Figure 10 | SFB-specific T_{H}17 cells are present in both SILP and large intestine lamina propria (LILP) of SFB-colonized mice. T cells were stained with 1-A3/3340-A6 tetramer and antibody to intracellular RORγt. 

a, Representative FACS plots (gated on CD4^+ T cells). b, Analysis of multiple animals. Left, per cent of tetramer-positive cells among total CD4^+ T cells in each region of the intestine. Right, per cent of RORγt^+ cells among the tetramer-positive cells. Each symbol represents cells from a separate animal.