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Plasticity of T_H17 cells in Peyer's patches is responsible for the induction of T cell–dependent IgA responses

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Intestinal Peyer's patches are essential lymphoid organs for the generation of T cell–dependent immunoglobulin A (IgA) for gut homeostasis. Through the use of interleukin 17 (IL-17) fate-reporter mice, we found here that endogenous cells of the T_H17 subset of helper T cells in lymphoid organs of naive mice 'preferentially' homed to the intestines and were maintained independently of IL-23. In Peyer's patches, such T_H17 cells acquired a follicular helper T cell (T_{FH} cell) phenotype and induced the development of IgA-producing germinal center B cells. Mice deficient in T_H17 cells failed to generate antigen-specific IgA responses, which provides evidence that T_H17 cells are the crucial subset required for the production of high-affinity T cell–dependent IgA.

Disruption of mucosal homeostasis can lead not only to infections but also to chronic inflammatory diseases and cancer. Intestinal homeostasis is maintained by the immune system and the barrier function of epithelial cells. Many cells of the innate and adaptive immune systems reside in mucosal tissues and establish an immunological network to maintain healthy conditions. Among the cells of the adaptive immune system, B cells that produce immunoglobulin A (IgA) are important participants in the maintenance of homeostasis and mucosal host defense[1], and the lamina propria of the small intestine is home to a substantial proportion of the T_H17 subset of helper T cells present in nonimmune mice.

IgA in its dimeric form is the dominant immunoglobulin isotype secreted into the intestinal lumen. The differentiation of B cells that secrete T cell–dependent IgA occurs in the Peyer's patches (PP) of the small intestine. Selective deficiency in IgA is the most common form of primary immunodeficiency, with an incidence of approximately 1 in 600 people in the Western world. Although its symptoms are rarely severe, symptomatic selective IgA deficiency can result in recurrent pulmonary and gastrointestinal infections[2]. T_H17 cells serve a crucial role in the mucosal host defense as well as in the development of autoimmune diseases[3]. Under steady-state conditions, T_H17 cells are usually found in the lamina propria of the small intestine, where their development depends on the presence of commensal microbiota, in particular segmented filamentous bacteria[4]. Notably, stimulation by those bacteria is also linked to the production of a large amount of total intestinal IgA[5].

In the PP, the main function of cells of the immune system is surveillance of the intestinal lumen, which involves the induction of IgA antibody responses. IgA is important for the neutralization of toxins and response to pathogens but is also critically involved in shaping the diversity of the commensal microbiota[6,7]. After activation of B cells in the context of cognate T cell help, germinal centers (GCs) are generated, and induction of the cytidine deaminase AID in GC B cells promotes somatic hypermutation and class-switch recombination of genes encoding immunoglobulins. Most B cells in the PP differentiate into IgA-producing cells in the presence of help from T cells, whereas B plasma cells with T cell–independent production of IgA, which are $B220^-$, can differentiate in the gut lamina propria without the generation of GCs[8–10]. IgA-producing B cells in GCs undergo extensive somatic hypermutation[10], which results in higher antibody affinity.

Here we found that most T_H17 cells in lymphoid organs of nonimmune mice were dependent on gut microbiota and had a natural 'preference' for the small intestine, as after adoptive transfer they selectively homed to that site. Intestinal T_H17 cells underwent deviation toward a follicular helper T cell (T_{FH} cell) phenotype in PP, where they induced GCs and the development of host-protective IgA responses. In contrast to pathogenic T_H17 cells developing in the course of experimental autoimmune encephalomyelitis (EAE), which are highly dependent on interleukin 23 (IL-23)[11,12], intestinal T_H17 cells did not require IL-23 for their maintenance or for their plasticity in deviating toward a T_{FH} cell profile. Mice deficient in T_H17 cells had a considerable deficiency in antigen-specific intestinal IgA after immunization with cholera toxin, which emphasized that T_H17 cells were the helper T cell subset responsible for inducing the switch of GC B cells toward the production of high-affinity, T cell–dependent IgA.

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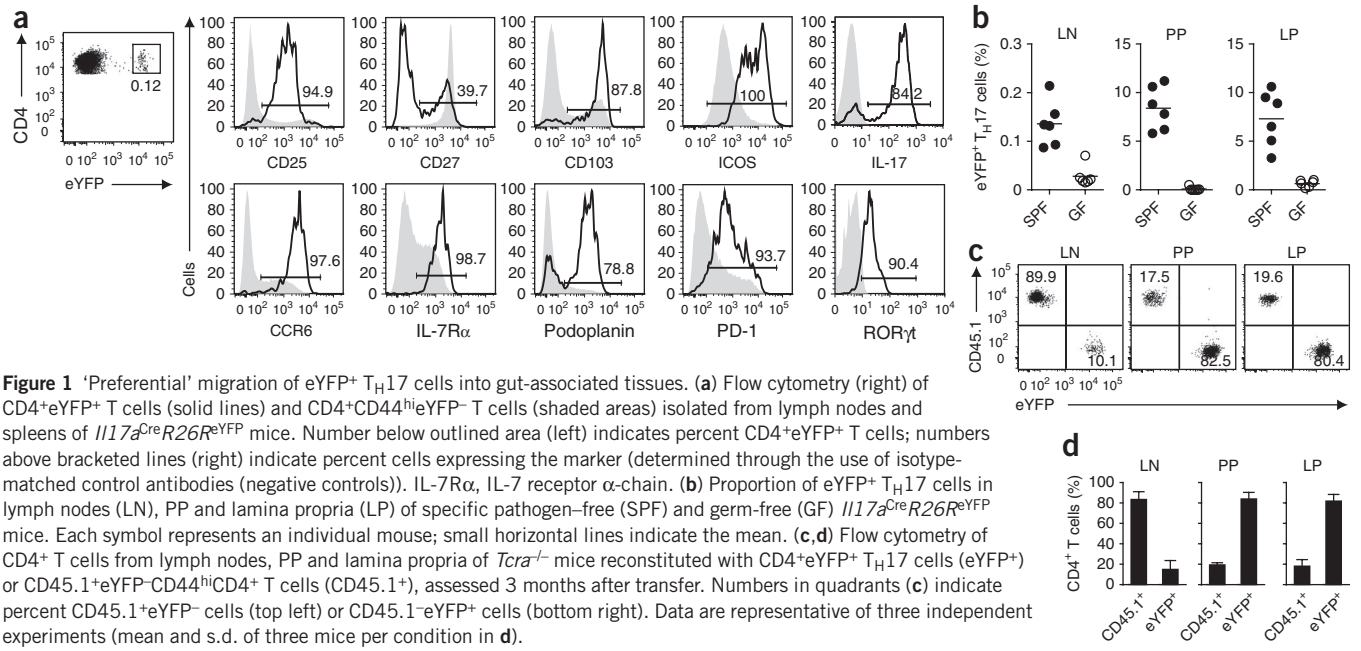


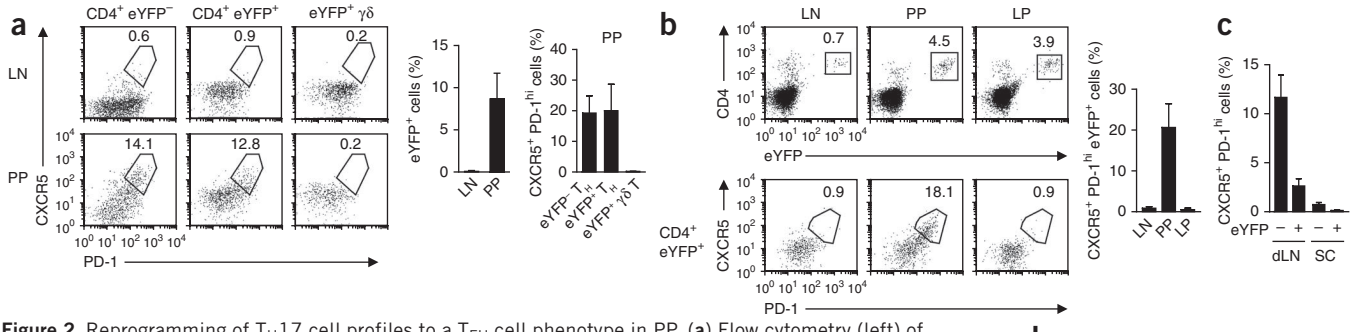
Figure 1 'Preferential' migration of eYFP$^+$ T_H17 cells into gut-associated tissues. (**a**) Flow cytometry (right) of CD4$^+$eYFP$^+$ T cells (solid lines) and CD4$^+$CD44^{hi}eYFP$^-$ T cells (shaded areas) isolated from lymph nodes and spleens of *Il17a*^{Cre}*R26R*^{eYFP} mice. Number below outlined area (left) indicates percent CD4$^+$eYFP$^+$ T cells; numbers above bracketed lines (right) indicate percent cells expressing the marker (determined through the use of isotype-matched control antibodies (negative controls)). IL-7Rα, IL-7 receptor α-chain. (**b**) Proportion of eYFP$^+$ T_H17 cells in lymph nodes (LN), PP and lamina propria (LP) of specific pathogen–free (SPF) and germ-free (GF) *Il17a*^{Cre}*R26R*^{eYFP} mice. Each symbol represents an individual mouse; small horizontal lines indicate the mean. (**c**,**d**) Flow cytometry of CD4$^+$ T cells from lymph nodes, PP and lamina propria of *Tcra*$^{-/-}$ mice reconstituted with CD4$^+$eYFP$^+$ T_H17 cells (eYFP$^+$) or CD45.1$^+$eYFP$^-$CD44^{hi}CD4$^+$ T cells (CD45.1$^+$), assessed 3 months after transfer. Numbers in quadrants (**c**) indicate percent CD45.1$^+$eYFP$^-$ cells (top left) or CD45.1$^-$eYFP$^+$ cells (bottom right). Data are representative of three independent experiments (mean and s.d. of three mice per condition in **d**).

RESULTS

Gut-homing properties of T_H17 cells in nonimmune mice

T_H17 cells constitute approximately 0.1% of CD4$^+$ helper T cells in the peripheral lymph nodes and spleens of nonimmune IL-17 fate-reporter (*Il17a*^{Cre}*R26R*^{eYFP}) mice, which express Cre recombinase from *Il17a*, linked to enhanced yellow fluorescent protein (eYFP) expressed from the ubiquitous *Rosa26* locus, and thus IL-17-producing cells are permanently marked as eYFP$^+$ cells[12]. This system is a powerful tool with which to track T_H17 cells and to investigate their potential plasticity toward alternative effector functions, as detection of T_H17 cells with this system does not depend on staining for intracellular IL-17.

Flow cytometry of eYFP$^+$ T_H17 cells from lymph nodes of *Il17a*^{Cre}*R26R*^{eYFP} mice showed almost uniform surface expression of the chemokine receptor CCR6, the IL-7 receptor α-chain, the IL-2 receptor α-chain (CD25), integrin $\alpha_E\beta_7$ (CD103) and the inducible costimulator ICOS, as well as expression of the signature cytokine IL-17 and the transcription factor RORγt (**Fig. 1a**). Expression of CCR6 and CD103 suggested gut-homing ability, because the CCR6 ligand CCL20 is known to be expressed in the small intestine[13]. As intestinal T_H17 cells are dependent on the gut microbiota and are absent from germ-free mice[4], we compared the proportion of eYFP$^+$ T_H17 cells in lymph nodes, PP and lamina propria of specific



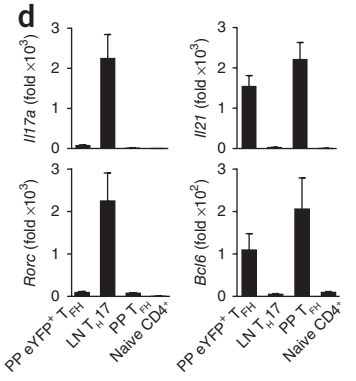


Figure 2 Reprogramming of T_H17 cell profiles to a T_{FH} cell phenotype in PP. (**a**) Flow cytometry (left) of CD4$^+$CD44^{hi}eYFP$^-$ T cells (left column), CD4$^+$eYFP$^+$ cells (middle column) and eYFP$^+$ γδ T cells (right column) in lymph nodes and PP from *Il17a*^{Cre}*R26R*^{eYFP} mice, showing expression of CXCR5 and PD-1; frequency of eYFP$^+$ cells among T_{FH} cells in lymph nodes and PP (middle); and frequency of CXCR5$^+$PD-1^{hi} cells among various subsets (horizontal axis) in PP (right). T_H, helper T cell. (**b**) Flow cytometry (left) of T cells from lymph nodes, PP and lamina propria of *Tcra*$^{-/-}$ mice 3 months after transfer of eYFP$^+$ T_H17 cells. Numbers above outlined areas indicate percent CD4$^+$eYFP$^+$ cells (top row) or CD4$^+$eYFP$^+$ T cells expressing CXCR5 and PD-1 (bottom row). Right, frequency of CXCR5$^+$PD-1^{hi}eYFP$^+$ cells in those tissues. (**c**) Frequency of CXCR5$^+$PD-1^{hi} cells among eYFP$^-$CD44^{hi}CD4$^+$ T cells (eYFP –) or eYFP$^+$CD4$^+$ T cells (eYFP +) from draining lymph nodes (dLN) and spinal cords (SC) of *Il17a*^{Cre}*R26R*^{eYFP} mice 20 d after immunization with myelin oligodendrocyte glycoprotein in complete Freund's adjuvant. (**d**) Quantitative PCR analysis of *Il17a*, *Il21*, *Rorc* and *Bcl6* mRNA in flow cytometry–purified CXCR5$^+$eYFP$^+$ T cells isolated from PP (PP eYFP$^+$ T_{FH}), CXCR5$^-$eYFP$^+$ T_H17 cells from lymph nodes (LN T_H17), non-T_H17 CXCR5$^+$eYFP$^-$ cells from PP (PP T_{FH}) and naive CD4$^+$ T cells (Naive CD4$^+$) from *Il17a*^{Cre}*R26R*^{eYFP} mice; results are presented relative to expression of the housekeeping gene *Hprt*. Data are representative of at least three independent experiments (mean and s.d.).

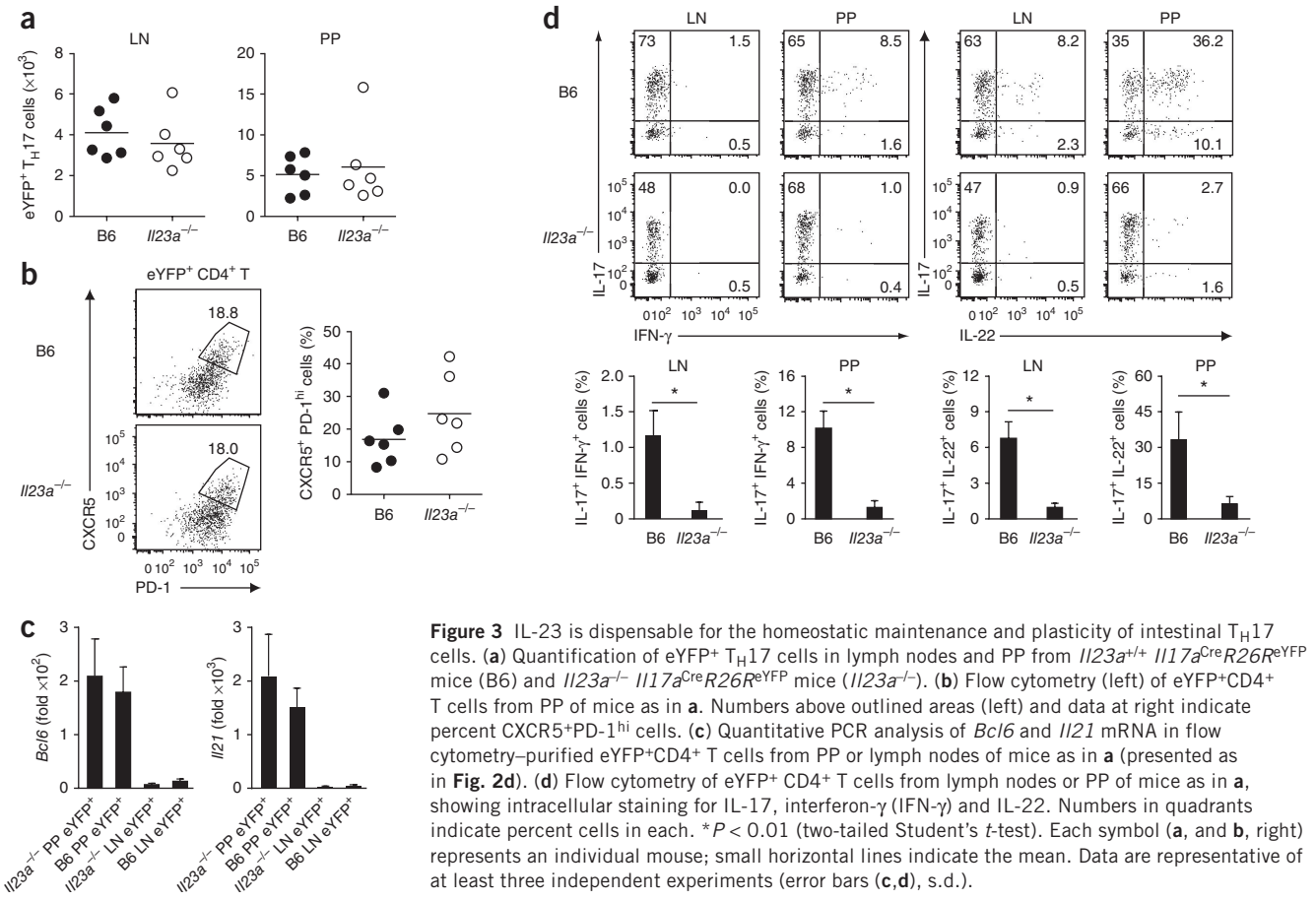


Figure 3 IL-23 is dispensable for the homeostatic maintenance and plasticity of intestinal T_H17 cells. (**a**) Quantification of eYFP$^+$ T_H17 cells in lymph nodes and PP from *Il23a*$^{+/+}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (B6) and *Il23a*$^{-/-}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (*Il23a*$^{-/-}$). (**b**) Flow cytometry (left) of eYFP$^+$CD4$^+$ T cells from PP of mice as in **a**. Numbers above outlined areas (left) and data at right indicate percent CXCR5$^+$PD-1^{hi} cells. (**c**) Quantitative PCR analysis of *Bcl6* and *Il21* mRNA in flow cytometry–purified eYFP$^+$CD4$^+$ T cells from PP or lymph nodes of mice as in **a** (presented as in **Fig. 2d**). (**d**) Flow cytometry of eYFP$^+$ CD4$^+$ T cells from lymph nodes or PP of mice as in **a**, showing intracellular staining for IL-17, interferon-γ (IFN-γ) and IL-22. Numbers in quadrants indicate percent cells in each. *$P < 0.01$ (two-tailed Student's *t*-test). Each symbol (**a**, and **b**, right) represents an individual mouse; small horizontal lines indicate the mean. Data are representative of at least three independent experiments (error bars (**c**,**d**), s.d.).

pathogen–free and germ-free *Il17a*^{Cre}*R26R*^{eYFP} mice. We found that eYFP$^+$ T_H17 cells were undetectable in PP and lamina propria and were also almost completely absent from lymph nodes of germ-free *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 1b**). To assess the homing properties of T_H17 cells and those of other memory-type T cells from nonimmune mice, we sorted eYFP$^+$ T_H17 cells and eYFP$^-$ CD4$^+$ T cells with an activated phenotype (CD44^{hi}) from lymph nodes of *Il17a*^{Cre}*R26R*^{eYFP} mice (distinguished by expression of the allotypic marker CD45.1) and adoptively transferred them together at a ratio of 1:1 into host mice deficient in the gene encoding the α-subunit of the T cell antigen receptor (*Tcra*$^{-/-}$; these mice are CD45.2$^+$), which lack conventional CD4$^+$ T cells and CD8$^+$ T cells. We found that eYFP$^+$ T_H17 cells 'preferentially' reconstituted gut-associated tissues, such as the lamina propria and PP of the small intestine, but not the peripheral lymph nodes in which the cells had originally resided (**Fig. 1c**,**d**). In contrast, eYFP$^-$CD44^{hi}CD45.1$^+$ non-T_H17 cells 'preferentially' seeded peripheral lymph nodes (**Fig. 1c**,**d**). Thus, most T_H17 cells in the lymphoid organs of nonimmune mice had gut-homing properties.

Intestinal T_H17 cells deviate to T_{FH} cells in Peyer's patches

The 'preferential' accumulation of T_H17 cells in PP prompted us to assess the possibility that they might have a role in helping B cells differentiate in GCs. T_{FH} cells reside in GCs and have an essential role in the differentiation of B cells in GCs; their distinguishing feature is expression of the chemokine receptor CXCR5, the costimulatory molecule PD-1, IL-21, ICOS and the transcription factor Bcl-6 (refs. 14–16). We found that ~13–20% of eYFP$^+$ T_H17 cells, as well as a similar proportion of eYFP$^-$ cells, present in the PP of nonimmune *Il17a*^{Cre}*R26R*^{eYFP} mice expressed CXCR5 and PD-1, whereas eYFP$^+$ γδ T cells in the PP did not express those T_{FH} cell markers (**Fig. 2a**).

To verify the developmental origin of those cells, we sorted CXCR5$^-$ eYFP$^+$ T_H17 cells from the lymph nodes of nonimmune *Il17a*^{Cre}*R26R*^{eYFP} mice, adoptively transferred them into *Tcra*$^{-/-}$ hosts and then assessed expression of CXCR5 and PD-1 in various tissues of the recipient mice. Although eYFP$^+$ T_H17 cells homed to both PP and lamina propria of the recipient mice, the conversion of eYFP$^+$ cells to a T_{FH} phenotype occurred exclusively in the environment of the PP (**Fig. 2b**). The small proportion of eYFP$^-$ CD4$^+$ T cells detected in the host mice after adoptive transfer were not donor-derived T cells that had lost eYFP expression but were CD4$^+$ non-T cells present in the host mice.

To determine the extent to which eYFP$^+$ T_H17 cells demonstrated plasticity toward a T_{FH} profile in other tissues as a consequence of immunization, we induced EAE in *Il17a*^{Cre}*R26R*^{eYFP} mice by immunizing them with myelin oligodendrocyte glycoprotein in complete Freund's adjuvant. Such immunization induces an antibody response to myelin oligodendrocyte glycoprotein during EAE, and T_H17 cells are thought to be involved in the development of ectopic lymphoid follicles in the central nervous system during EAE[17]. Analysis of lymph nodes and spinal cords from *Il17a*^{Cre}*R26R*^{eYFP} mice with EAE showed that about 4–7% of CD4$^+$ T cells in the lymph nodes and 60% of CD4$^+$ T cells in the spinal cord were eYFP$^+$. However, about 2–4% of eYFP$^+$ cells in the lymph nodes, and none in the spinal cord, had a T_{FH} cell signature (CXCR5$^+$PD-1^{hi}). In contrast, a substantial proportion (10–15%) of eYFP$^-$ CD4$^+$ T cells had a T_{FH} cell profile (**Fig. 2c**). These observations suggested that T_H17 cells showed plasticity in deviating toward the T_{FH} phenotype 'preferentially' in the environment of the PP.

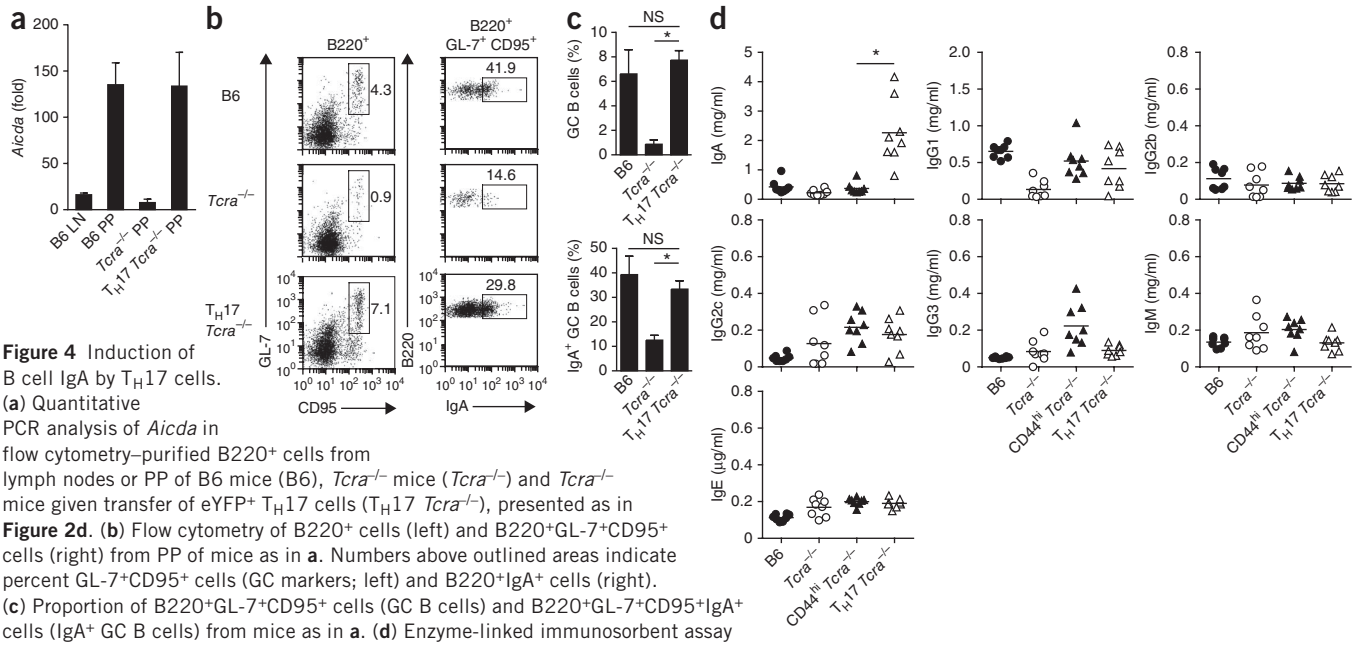


Figure 4 Induction of B cell IgA by T_H17 cells. (**a**) Quantitative PCR analysis of *Aicda* in flow cytometry–purified B220$^+$ cells from lymph nodes or PP of B6 mice (B6), *Tcra*$^{-/-}$ mice (*Tcra*$^{-/-}$) and *Tcra*$^{-/-}$ mice given transfer of eYFP$^+$ T_H17 cells (T_H17 *Tcra*$^{-/-}$), presented as in **Figure 2d**. (**b**) Flow cytometry of B220$^+$ cells (left) and B220$^+$GL-7$^+$CD95$^+$ cells (right) from PP of mice as in **a**. Numbers above outlined areas indicate percent GL-7$^+$CD95$^+$ cells (GC markers; left) and B220$^+$IgA$^+$ cells (right). (**c**) Proportion of B220$^+$GL-7$^+$CD95$^+$ cells (GC B cells) and B220$^+$GL-7$^+$CD95$^+$IgA$^+$ cells (IgA$^+$ GC B cells) from mice as in **a**. (**d**) Enzyme-linked immunosorbent assay of immunoglobulin isotypes in serum from B6 mice, *Tcra*$^{-/-}$ mice, and *Tcra*$^{-/-}$ mice given transfer of CD4$^+$eYFP$^-$CD44^{hi} cells (CD44^{hi} *Tcra*$^{-/-}$) or eYFP$^+$ T_H17 cells (T_H17 *Tcra*$^{-/-}$). Each symbol represents an individual mouse; small horizontal lines indicate the mean. *$P < 0.01$ (two-tailed Student's *t*-test). Data are representative of three independent experiments (**a**–**c**; mean and s.d. of three mice in **b**) or are pooled from three experiments (**d**).

We next analyzed T_H17 and T_{FH} gene signatures in sorted CXCR5$^+$eYFP$^+$ T cells isolated from PP, lymph node eYFP$^+$ T_H17 cells and non-T_H17 eYFP$^-$ CXCR5$^+$ T cells (T_{FH} cells) isolated from PP, as well as naive CD4$^+$ T cells, all from nonimmune *Il17a*^{Cre}*R26R*^{eYFP} mice. The eYFP$^+$ CD4$^+$ T cells with a T_{FH} cell surface phenotype had downregulated their expression of *Rorc* mRNA (which encodes RORγt) and *Il17a* mRNA (which encodes IL-17A) and upregulated their expression of the T_{FH} signature genes *Bcl6* and *Il21*, similar to non-T_H17 T_{FH} cells isolated from PP (**Fig. 2d**). Together these data demonstrated that the plasticity of T_H17 cells in deviating toward a T_{FH} cell–like phenotype was continuous in the environment of the PP under steady-state conditions.

IL-23 independence of intestinal T_H17 cell homeostasis

The plasticity of T_H17 cells in autoimmune settings is very dependent on IL-23 (ref. 12). To determine whether IL-23 is similarly involved in the plasticity of intestinal T_H17 cells in developing into T_{FH} cells, we analyzed *Il17a*^{Cre}*R26R*^{eYFP} mice crossed onto a background with genetic deficiency in the p19 subunit of IL-23 (an *Il23a*-deficient (*Il23a*$^{-/-}$) background). First, and in contrast to the well-defined role of IL-23 in the maintenance of T_H17 cells in autoimmune settings, IL-23 was dispensable for the survival of intestinal T_H17 cells, as similar numbers of T_H17 cells were present in the lymph nodes and PP of *Il23a*$^{+/+}$ and *Il23a*$^{-/-}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 3a**). Furthermore, the phenotypic conversion to a T_{FH} cell phenotype occurred to the same extent in *Il23a*$^{+/+}$ and *Il23a*$^{-/-}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 3b**), and T_H17 cells with the T_{FH} phenotype in *Il23a*$^{-/-}$ *Il17a*^{Cre}*R26R*^{eYFP} mice upregulated the expression of *Bcl6* and *Il21* similar to T_H17 cells from *Il23a*$^{+/+}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 3c**). In contrast, in accordance with published observations[12], T_H17 cells were unable to deviate toward expression of interferon-γ and did not express IL-22 in *Il23a*$^{-/-}$ *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 3d**). These data indicated that the steady-state population of T_H17 cells in the intestine had features distinct from those of T_H17 cells elicited by immunization in the periphery.

'Ex-T_H17' cells in PP induce IgA production by GC B cells

The dependence of intestinal T_H17 cells on commensal bacteria raised the possibility that T_{FH} cells developing from gut-homing former T_H17 cells may be specialized for helping B cell IgA responses in PP GCs. We therefore analyzed B cell expression of *Aicda*, which encodes AID, the cytidine deaminase required for somatic hypermutation, gene conversion and class-switch recombination of genes encoding immunoglobulins. There was little *Aicda* expression in lymph node B cells of C57BL/6 (B6) mice, in line with the absence of GCs in mice kept under specific pathogen–free conditions. B cells in PP, however, are continuously stimulated by the commensal flora and had high expression of *Aicda* (**Fig. 4a**). In absence of T cells in *Tcra*$^{-/-}$ hosts, *Aicda* expression was very low (**Fig. 4a**), as no GC B cells develop in the absence of T cell help. Transfer of eYFP$^+$ T_H17 cells, however, reconstituted *Aicda* expression to the amount in B6 mice (**Fig. 4a**).

Furthermore, B cell expression of the GC markers GL-7 and CD95, as well as expression of IgA, which we detected on B cells from B6 mice but not those from *Tcra*$^{-/-}$ mice, was induced in B cells in PP of *Tcra*$^{-/-}$ mice after transfer of T_H17 cells (**Fig. 4b**,**c**). Immunohistochemistry of tissue from the PP of a *Tcra*$^{-/-}$ mouse given transfer of eYFP$^+$ T_H17 cells showed that eYFP$^+$ formerly T_H17 cells were indeed situated in the GC (**Supplementary Fig. 1**). As a result, the concentration of IgA, but not that of other immunoglobulin isotypes, was much higher in serum from *Tcra*$^{-/-}$ mice that had received T_H17 cells than in *Tcra*$^{-/-}$ mice that had not received adoptively transferred T_H17 cells or those that had received non-T_H17 (eYFP$^-$CD44^{hi}) effector cells (**Fig. 4d**). These data suggested that intestinal T_H17 cells deviating toward a T_{FH} profile in PP may have been responsible for the induction of T cell–dependent IgA responses.

Regulatory T cells are not associated with induction of IgA

Published reports have suggested that regulatory T cells (T_{reg} cells) expressing the transcirption factor Foxp3 might adopt a T_{FH} cell phenotype in PP[8,18–20]. As those studies focused on T_{reg} cells isolated from lymphoid organs, we first compared the homing of, as well as

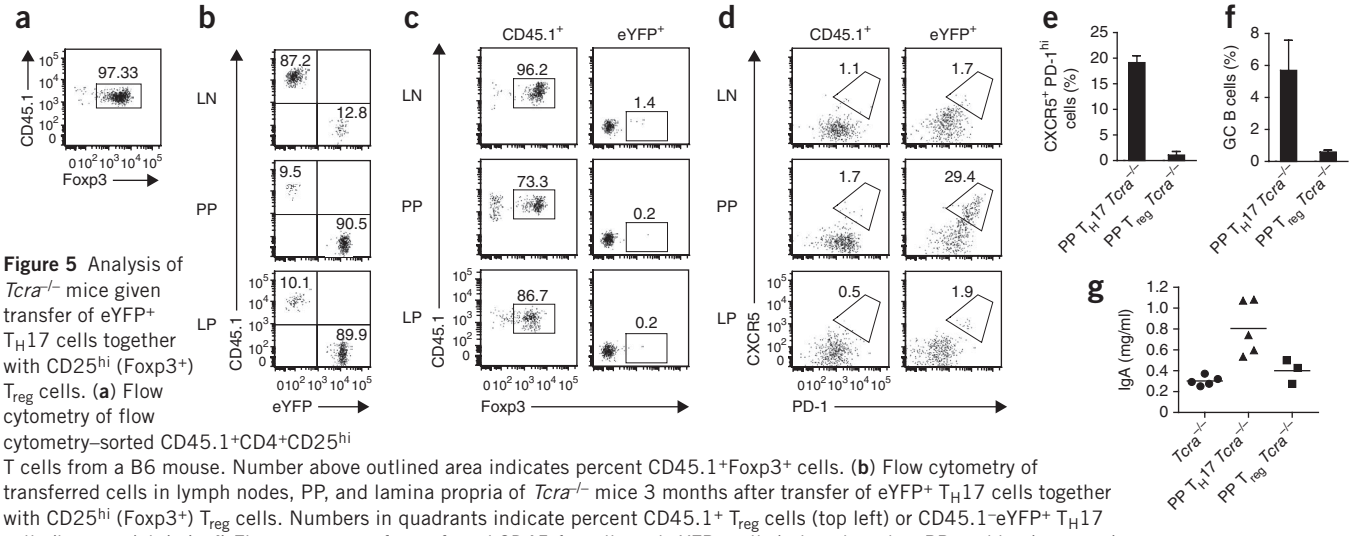


Figure 5 Analysis of *Tcra*$^{-/-}$ mice given transfer of eYFP$^+$ T_H17 cells together with CD25^{hi} (Foxp3$^+$) T_{reg} cells. (**a**) Flow cytometry of flow cytometry–sorted CD45.1$^+$CD4$^+$CD25^{hi} T cells from a B6 mouse. Number above outlined area indicates percent CD45.1$^+$Foxp3$^+$ cells. (**b**) Flow cytometry of transferred cells in lymph nodes, PP, and lamina propria of *Tcra*$^{-/-}$ mice 3 months after transfer of eYFP$^+$ T_H17 cells together with CD25^{hi} (Foxp3$^+$) T_{reg} cells. Numbers in quadrants indicate percent CD45.1$^+$ T_{reg} cells (top left) or CD45.1$^-$eYFP$^+$ T_H17 cells (bottom right). (**c**,**d**) Flow cytometry of transferred CD45.1$^+$ cells and eYFP$^+$ cells in lymph nodes, PP, and lamina propria of mice as in **b**. Numbers above outlined areas indicate percent CD45.1$^+$Foxp3$^+$ cells (**c**, left) or CD45.1$^-$Foxp3$^+$ eYFP$^+$ cells (**c**, right), or CXCR5$^+$PD-1^{hi} cells (**d**). (**e**,**f**) Proportion of CXCR5$^+$PD-1^{hi} cells (**e**) or B220$^+$GL-7$^+$CD95$^+$ (GC) B cells (**f**) in PP of *Tcra*$^{-/-}$ mice 3 months after transfer of eYFP$^+$ T_H17 cells (PP T_H17 *Tcra*$^{-/-}$) or CD45.1$^+$CD4$^+$RFP^{hi} T cells (PP T_{reg} *Tcra*$^{-/-}$). (**g**) IgA concentration in serum from *Tcra*$^{-/-}$ mice (*Tcra*$^{-/-}$) and from mice as in **e**,**f**. Each symbol represents an individual mouse; small horizontal lines indicate the mean. Data are representative of three independent experiments (**a**–**d**) or two experiments (**e**–**g**; mean and s.d. of three mice per condition in **e**,**f**).

the adoption of a T_{FH} cell phenotype by, T_{reg} cells and T_H17 cells isolated from lymph nodes and spleen. We isolated T_{reg} cells from B6 (CD45.1$^+$) mice on the basis of high CD25 expression, which correlated well with Foxp3 expression (**Fig. 5a**). We cotransferred equal numbers of CD45.1$^+$ T_{reg} cells and eYFP$^+$ T_H17 cells into *Tcra*$^{-/-}$ hosts and, 3 months later, found transferred T_{reg} cells mainly in lymph nodes, whereas transferred eYFP$^+$ cells had homed to the lamina propria and PP of the small intestine (**Fig. 5b**). Donor CD45.1$^+$ T_{reg} cells retained their Foxp3 expression, but there was no indication that donor eYFP$^+$ T_H17 cells acquired Foxp3 expression in any location in the adoptive host (**Fig. 5c**). Furthermore, whereas 15–30% of T_H17 cells deviated to a T_{FH} cell profile in PP, T_{reg} cells did not acquire a T_{FH} cell profile in any of the tissues examined (**Fig. 5d**). As the poor homing of lymph node–derived T_{reg} cells to intestinal tissues might have precluded acquisition of a T_{FH} cell phenotype in PP, we isolated T_{reg} cells with high expression of red fluorescent protein (RFP) from the lamina propria and PP of *Foxp3*^{RFP} mice (which have sequence encoding an RFP reporter knocked into the *Foxp3* locus) and transferred those cells into *Tcra*$^{-/-}$ hosts. Although we observed efficient homing of donor RFP^{hi} T_{reg} cells into PP, those cells did not acquire a T_{FH} cell profile in the adoptive hosts (**Fig. 5e**). Furthermore, adoptive transfer of T_{reg} cells did not induce GC B cells or IgA production (**Fig. 5f**,**g**). Together these data suggested that the promotion of class switching to IgA in GC B cells in PP was a function of T_{FH} cells derived from former T_H17 cells, whereas T_{reg} cells neither adopted a T_{FH} cell profile nor supported IgA production.

Class switching to IgA in intact mice depends on T_H17 cells

After transfer into *Tcra*$^{-/-}$ hosts, transferred eYFP$^+$ T_H17 cells expanded their populations substantially, which resulted in IgA production that far exceeded that seen in B6 (*Tcra*-sufficient) mice at steady state (**Fig. 4d**). In *Tcra*$^{-/-}$ hosts, lymphopenia may have resulted in unimpeded recognition of and response to the commensal flora by transferred T_H17 cells. However, adoptive transfer of eYFP$^+$ T_H17 cells into intact wild-type hosts, which have full niches of intestinal T_H17 cells and T_{FH} cells, does not lead to efficient engraftment of the small number of donor cells that can be isolated for transfer from unmanipulated mice. The minimal difference between

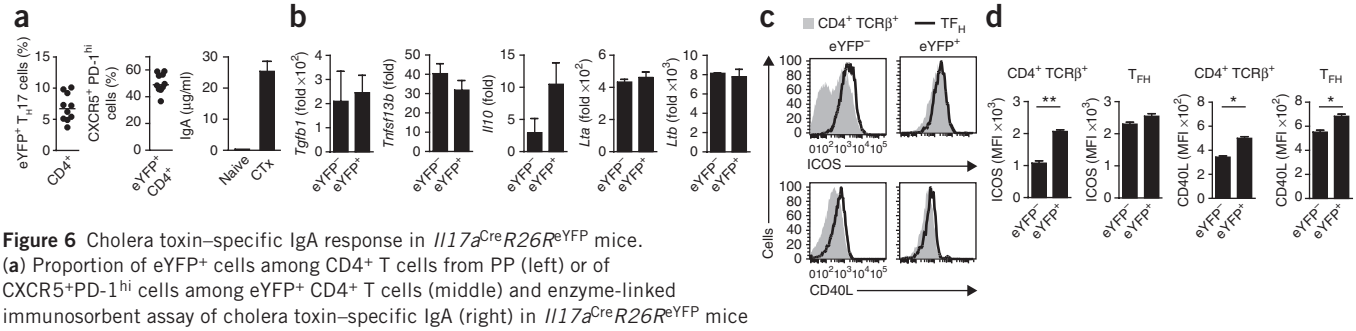


Figure 6 Cholera toxin–specific IgA response in *Il17a*^{Cre}*R26R*^{eYFP} mice. (**a**) Proportion of eYFP$^+$ cells among CD4$^+$ T cells from PP (left) or of CXCR5$^+$PD-1^{hi} cells among eYFP$^+$ CD4$^+$ T cells (middle) and enzyme-linked immunosorbent assay of cholera toxin–specific IgA (right) in *Il17a*^{Cre}*R26R*^{eYFP} mice 7–10 d after immunization with cholera toxin (CTx). Naive, unimmunized mice. (**b**) Quantitative PCR analysis of mRNA (vertical axes) in flow cytometry–purified eYFP$^+$ and eYFP$^-$ CD4$^+$ T cells from PP of *Il17a*^{Cre}*R26R*^{eYFP} mice 10 d after immunization with cholera toxin (presented as in **Fig. 2d**). (**c**) Flow cytometry of eYFP$^+$ or eYFP$^-$ CD4$^+$TCRβ$^+$ T cells and T_{FH} cells from PP of *Il17a*^{Cre}*R26R*^{eYFP} mice 10 d after immunization with cholera toxin. (**d**) Mean fluorescent intensity (MFI) of staining as in **c**. $^*P < 0.05$ and $^{**}P < 0.005$ (two-tailed Student's *t*-test). Data are representative of three independent experiments (**a**,**b**) or three experiments (**c**,**d**; mean and s.e.m. of three mice per condition in **d**).

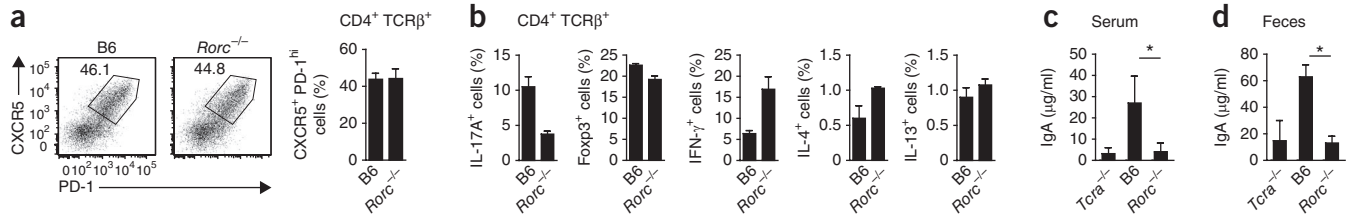


Figure 7 The cholera toxin–specific IgA response requires T_H17 cells. (**a**) Flow cytometry (left) of PP CD4+ T cells from *Tcra*$^{-/-}$ mice reconstituted with B6 bone marrow (B6) or *Rorc*-deficient bone marrow (*Rorc*$^{-/-}$) and challenged with cholera toxin 3 months later, assessed 10 d after challenge. Numbers above outlined areas indicate percent CXCR5+PD-1^{hi} cells. Right, summary of results at left. (**b**) Proportion of PP CD4+ T cells expressing Foxp3 or producing various cytokines (vertical axes) in *Tcra*$^{-/-}$ mice treated as in **a**. (**c**,**d**) Enzyme-linked immunosorbent assay of cholera toxin–specific IgA in serum (**c**) and feces (**d**) from *Tcra*$^{-/-}$ mice given no cells (*Tcra*$^{-/-}$) and in *Tcra*$^{-/-}$ mice given cell transfer as in **a**, assessed 10 d after challenge with cholera toxin. $*P \leq 0.01$ (two-tailed Student's *t*-test). Data are representative of three experiments (**a**,**b**) or two experiments (**c**,**d**; mean and s.e.m. of four mice per condition).

T cell–deficient *Tcra*$^{-/-}$ mice and nonimmune wild-type (B6) mice in serum IgA concentration (**Fig. 4d**) suggested that under steady-state conditions, most IgA expression was T cell independent. To investigate T cell–dependent IgA immune responses in intact mice, we immunized *Il17a*^{Cre}*R26R*^{eYFP} mice with cholera toxin and evaluated the proportion of total eYFP+ cells and eYFP+ T_{FH} cells in the PP, as well as antigen-specific IgA responses in serum and feces. The proportion of eYPF+ cells (5–10%) among total CD4+ T cells in the PP of cholera toxin–immunized *Il17a*^{Cre}*R26R*^{eYFP} mice was similar that observed in nonimmunized *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 6a**). However, 40–60% of the eYPF+ cells in the PP had acquired a T_{FH} cell phenotype, compared with 13% at steady state (**Figs. 2a** and **6a**). We also observed a strong cholera toxin–specific IgA response in the serum of immunized *Il17a*^{Cre}*R26R*^{eYFP} mice (**Fig. 6a**). We used quantitative PCR to assess markers associated with class switching to IgA in eYFP− and eYFP+ T cells from PP of cholera toxin–immunized mice, but did not detect substantial differences between the two populations analyzed (**Fig. 6b**). However, expression of ICOS and CD40L (CD154; the ligand for the costimulatory molecule CD40) was consistently higher on eYFP+ T cells than on eYFP− T cells, even before the eYFP+ cells had acquired the CXCR5+PD-1^{hi} T_{FH} profile (**Fig. 6c**,**d**).

To address whether the induction of T cell–dependent IgA required T_H17 cells in an otherwise intact mouse, we generated bone marrow chimeras of *Tcra*$^{-/-}$ hosts reconstituted with whole bone marrow from RORγt-deficient (*Rorc*$^{-/-}$) donor mice[21] (*Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras); these chimeras do not develop T_H17 cells[22]. Although RORγt is required for the development of lymphoid architecture in the mucosal immune system[21,23,24], the mucosal environment of these chimeras is not disturbed, as *Rorc*-expressing innate lymphoid cell types are present in the *Tcra*$^{-/-}$ hosts. We also reconstituted *Tcra*$^{-/-}$ mice with bone marrow from B6 wild-type donors as a control (B6 *Tcra*$^{-/-}$ chimeras). Flow cytometry of PP showed a similar proportion of T_{FH} cells in *Rorc*$^{-/-}$*Tcra*$^{-/-}$ and B6 *Tcra*$^{-/-}$ chimeras after immunization with cholera toxin (**Fig. 7a**). *Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras had fewer IL-17-producing CD4+ T cells than did B6 *Tcra*$^{-/-}$ chimeras, whereas the proportion of T_{reg} cells was similar in both chimeras, and the abundance of interferon-γ-, IL-4- or IL-13-producing CD4+ T cells was similar in both or was even higher in *Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras than in B6 *Tcra*$^{-/-}$ chimeras (**Fig. 7b**). The serum isotype profiles in the two sets of chimeras before immunization were similar (**Supplementary Fig. 2**). To assess the production of T cell–dependent IgA, we immunized *Rorc*$^{-/-}$*Tcra*$^{-/-}$ and B6 *Tcra*$^{-/-}$ chimeras with cholera toxin and measured serum and fecal IgA 10 d later. B6 *Tcra*$^{-/-}$ chimeras mounted a strong cholera toxin–specific IgA response detectable in serum (**Fig. 7c**) and feces (**Fig. 7d**). In contrast, *Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras had very low concentrations of cholera toxin–specific IgA, similar to those observed in *Tcra*$^{-/-}$ mice (**Fig. 7c**,**d**). Thus, these results showed that T_H17 cells were required for the GC switch to IgA production in PP.

DISCUSSION

T_H17 cells are known to diversify their effector profile in response to various environmental conditions[25]. Here we have described the consequences of the plasticity of T_H17 cells in developing toward a T_{FH} cell program in the environment of the small intestine PP, a process that promoted T cell–dependent IgA responses. Although published studies have demonstrated that T_H17 cells can be reprogrammed to obtain T_{FH} cell characteristics *in vitro*[26], an *in vivo* demonstration of this phenomenon would not have been possible without an IL-17 fate-reporter mouse (the *Il17a*^{Cre}*R26R*^{eYFP} mouse), as this allows the identification of a T_H17 cell origin regardless of production of the signature cytokine IL-17. Here we used that fate-reporter mouse to demonstrate the deviation of T_H17 cells toward a T_{FH} cell phenotype under the influence of the environment of the PP, which resulted in substantial phenotypic and functional changes.

We found that expression of IL-17 and RORγt was extinguished in T_{FH} cells derived from former T_H17 cells. However, the expression of IL-21 and Bcl-6 was upregulated in those cells. Although IL-21 has been associated with T_H17 cells generated *in vitro*[3], expression of IL-21 was not detectable in T_H17 cells from the intestine or lymphoid organs of nonimmune mice.

T_H17 cells are naturally found in the small intestine of nonimmune pathogen-free mice. Under steady-state conditions, these cells are thought to contribute to gut barrier function by stimulating the formation of tight junctions and antimicrobial peptides[27,28]. Our analysis of *Il17a*^{Cre}*R26R*^{eYFP} mice indicated that most of the few T_H17 cells found in peripheral lymphoid organs probably had their developmental origin in the gut, a proposal also supported by the finding that germ-free *Il17a*^{Cre}*R26R*^{eYFP} mice lacked both intestinal T_H17 cells and most T_H17 cells from lymphoid organs. It was notable that the cytokine IL-23, a key factor for the development of T_H17 responses with pathogenic features[11,12,29], was dispensable for the maintenance of intestinal T_H17 cells and their deviation toward a T_{FH} cell program. That confirmed published suggestions that T_H17 cells might develop toward having either protective functions or pathogenic functions[30].

Our data have expanded the functional repertoire of intestinal T_H17 cells to include induction of the GC B cell IgA response. In *Tcra*$^{-/-}$ mice given transfer of eYFP+ T_H17 cells, serum concentrations of IgA were much higher regardless of deliberate immunization.

That was presumably due to the exaggerated population expansion of the transferred cells in the lymphopenic hosts and recall responses to commensal microbiota that may be less well controlled in T cell–deficient hosts. In fact, immunization with cholera toxin did not result in an antigen-specific IgA response in these mice. In contrast, immunization of *Il17a*^{Cre}*R26R*^{eYFP} mice with cholera toxin resulted in a pronounced cholera toxin–specific IgA response and more switching of eYFP$^+$ cells toward a T_{FH} cell phenotype.

In *Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras, serum concentrations of IgA before immunization with cholera toxin were similar to those seen in *Tcra*$^{-/-}$ mice as well as in wild-type B6 mice (data not shown), which suggests that basal concentrations of IgA in specific pathogen–free mice may be mostly T cell independent. T_H17 cells are involved in upregulating expression of the polymeric immunoglobulin receptor that transports IgA across the intestinal epithelium[31]. However, it seems that role can be attributed to IL-17 itself rather than to T_H17 cells. One possibility that remains to be addressed is whether RORγt$^+$ innate lymphoid cells contribute to the induction of T cell–independent IgA and/or upregulation of expression of the IgA transporter. As T_{FH} cells derived from former T_H17 cells switched off their production of IL-17, it is unlikely that they participated in this process, and we have not detected changes in expression of polymeric immunoglobulin receptor in our various models, none of which were devoid of IL-17. Nevertheless, IgA responses to challenge with cholera toxin depended on the presence of T_H17 cells.

The plasticity of T_H17 cells in developing toward a T_{FH} cell fate was restricted to the environment of the PP and was not evident in peripheral lymph nodes. As intestinal IgA fulfils important roles in maintaining equilibrium with the commensal flora and efficient mucosal host defense[7], this newly identified function of T_H17 cells provides another example of their crucial role in mucosal immunity. It is notable that segmented filamentous bacteria, which are important stimulators of the T_H17 cell development, also drive GC formation and IgA production in PP[4,5]. IgA deficiency causes aberrant expansion of segmented filamentous bacteria [4,32,33], and a deficiency in T_H17 cells might result in the same features.

The developmental relationship between T_{FH} cells and other CD4$^+$ T cell subsets remains a matter of debate[15]. Our data are compatible with a nonexclusive CD4$^+$ T cell program that obtains input from multiple T cell subsets. T helper type 2 cells are able to acquire CXCR5 expression, which results in the induction or inhibition of B cell differentiation and class switching in GCs[18,34–36]. IL-12–mediated activation of the transcription factor STAT4 transiently induces a T_{FH} cell transcriptional profile, followed by repression of the T_{FH} cell gene signature by the T helper type 1–specific transcription factor T-bet[37]. Induction of Bcl-6 requires ICOS[38], which has high expression on T_H17 cells. Temporal and spatial regulation of the expression of CXCR5 and Bcl-6 in interactions with dendritic cells and B cells promotes the development of T_{FH} cells[38–40], but it remains unclear whether the T_{FH} cell state resembles a terminal effector status or whether such cells can be redirected toward other T cell programs. Thus, it remains to be determined whether the extinction of a previous effector profile is complete after the acquisition of a T_{FH} cell phenotype or whether each effector T cell subset contributes a unique feature of its original signature to the functional helper response in the GC reaction. Elucidation of these possibilities would be facilitated by fate-reporter mice for each T cell subset, which would allow analysis of functional profiles regardless of expression of the signature cytokines that now define their subset allocation.

The role of T_{reg} cells in GC reactions in PP remains controversial. In some reports it has been argued that T_{reg} cells convert into T_{FH} cells to promote intestinal IgA responses[18,41], whereas other studies have suggested that T_{reg} cells that express markers of T_{FH} cells are essential for control, rather than promotion, of the GC reaction[19,20]. Notably, depletion of T_{reg} cell through the use of antibody to CD25, as used in a published study of the role of T_{reg} cells in the induction of intestinal IgA[41], would also result in the depletion of T_H17 cells, which are homogenously CD25$^+$. Our data did not confirm the plasticity of T_{reg} cells in developing toward a T_{FH} cell profile or a role for T_{reg} cells in promoting IgA responses either in the transfer model or in bone marrow chimeras. For transfer, we isolated the T_{reg} cell population on the basis of either high CD25 expression, which has been shown to mark stable T_{reg} cells[42], or high RFP (Foxp3) expression in the *Foxp3*^{RFP} mouse[43]. The discrepancy in T_{reg} cell plasticity might be due to technical issues with the Foxp3 reporter model used before[18], showing particularly prominent plasticity of cells with lower expression of Foxp3 or after transfer of mixtures of Foxp3$^+$ and Foxp3$^-$ cells. Given the reciprocal relationship between Foxp3 and RORγt in the development of T_{reg} cells and T_H17 cells[44], it is conceivable that Foxp3^{lo} T_{reg} cells may have deviated toward a T_H17 cell fate, thus mimicking their unique function in the intestinal immune response.

Our data emphasize another facet of the host-protective function of T_H17 cells in mucosal tissues. At present it remains unclear what particular features former T_H17 cells contribute to their interaction with B cells to promote IgA responses. Genes encoding molecules known to affect IgA have wide expression in the environment of the PP, and we did not detect differences in the expression of such markers in eYFP$^+$ and eYFP$^-$ T cells in PP. Notably, eYFP$^+$ T cells had much higher expression of ICOS and CD40L, which might facilitate 'preferential' contact with B cells. B cells in the environment of the PP are characterized by expression of the transcription factor RORα[45] and compete for help from T cells before entering GCs. The eYFP$^+$ T cells may have had 'preferential' access. However, this does not explain why T_{FH} cells in the PP of *Rorc*$^{-/-}$*Tcra*$^{-/-}$ chimeras, which did not face competition by former T_H17 cells, were still not able to induce a switch to IgA. Given the prominent role of T_H17 cells in autoimmunity, these cells seem obvious targets for therapeutic intervention. However, understanding of their role in maintaining intestinal barrier integrity is needed to avoid disturbance of these beneficial functions.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

K.H. and B.S. conceived of the project, designed the experiments and wrote the paper; K.H. did most of the experiments; M.V., J-E.T. and J.H.D. did specific experiments; J.D. established the germ-free colony of reporter mice; and O.M.S. supplied bone marrow from *Rorc*$^{-/-}$ mice.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. *Il17a*^{Cre}*R26R*^{eYFP} mice[12] and *Foxp3*^{RFP} mice (with a bicistronic RFP reporter knocked into the *Foxp3* locus)[43], as well as TCRα-deficient (*Tcra*$^{-/-}$) mice on a B6 background[46] and p19-deficient (*Il23a*$^{-/-}$) mice (from D. Cua), were bred in the National Institute for Medical Research animal facility under specified pathogen–free conditions. All animal experiments were done according to the National Institute for Medical Research Ethical Review committee and Home Office regulations. Some *Il17a*^{Cre}*R26R*^{eYFP} mice were raised in germ-free conditions by rederivation from caesarean section, as described at The European Mouse Mutant Archive (http://www.emmanet.org/protocols/GermFree_0902.pdf). At day 20 after coitus, uteri from donor females were transferred through a reservoir containing 1% VirkonS to the isolator housing the germ-free surrogate mothers. The microbiological status of the isolator was monitored every 3 weeks. Bones from *Rorc*(γt)$^{GFP/GFP}$ mice (with sequence encoding green fluorescent protein in exon 1γt (the alternative first exon used in expression of RORγt) of *Rorc*)[21] were from O. Steinmetz.

Antibodies. Antibody to CCR6 (anti-CCR6; 140708), anti-CXCR5 (2G8), anti-CD95 (Jo2) and anti-IgA (C10-3) were from BD Biosciences. Anti-GL-7 (GL7) and anti-RORγt (AFKJS-9) were from eBioscience. Anti-CD4 (GK1.5), anti-CD25(PC61), anti-CD27 (LG.3A10), anti-CD44 (IM7), anti-CD45.1 (A20), anti-CD103 (2E7), anti-ICOS (C398.4A), anti-IL-7Ra (A7R34), anti-podoplanin (8.1.1), anti-PD-1 (29F.1A12), anti-B220 (RA3-6B2), anti-IL-17 (TC11-18H10.1), anti-IL-22 (AM22.3), anti-interferon-γ (XMG1.2) and anti-TCRβ (H57-597) were from Biolegend.

Preparation of lymphocytes in tissues. Lymphocytes in the lamina propria were prepared by cutting of the small intestine into pieces 1 cm in length pieces after removal of Peyer's patches, followed by shaking for 20 min at 37 °C in 10 ml IEL buffer (PBS supplemented with 10% FCS, 1 mM pyruvate, 20 μM HEPES, 10 mM EDTA, penicillin-streptomycin mix and 10 ug/ml polymyxin B) for removal of epithelial and intraepithelial cells and then digestion of the remaining tissue for 1 h at 37 °C with 1 mg/ml collagenase D (Roche) and 10 U/ml DNase1 (Sigma), followed by separation with 36.5% Percoll.

Real-time PCR. RNA was extracted from flow cytometry–sorted CD4$^+$ T cells with TRIzol and was reverse-transcribed with Omniscript (Qiagen) according to the manufacturer's protocol. The cDNA served as template for the amplification of genes of interest (Applied Biosystems identifiers (https://bioinfo.appliedbiosystems.com/genome-database/gene-expression.html): *Aicda*, Mm00507774_m1; *Bcl6*, Mm00477633_m1; *Il17a*, Mm00439619_m1; *Il21*, Mm00517640_m1; *Il22*, Mm00444241_m1; and *Rorc*, Mm01261019_g1) and the housekeeping gene *Hprt* (Mm00446968_m1) by real-time PCR, with ABI TaqMan Gene Expression Assays, universal PCR Master Mix and the ABI-PRISM 7900 Sequence detection system (all from Applied Biosystems). Target-gene expression was calculated by the comparative method for relative quantification after normalization to *Hprt* expression.

Immunization with cholera toxin. *Tcra*$^{-/-}$ mice were sublethally irradiated (500 rads) and then were reconstituted with B6 or *Rorc*$^{-/-}$ bone morrow. Before immunization with cholera toxin, mice were deprived of food for 2 h and then were given 0.25 ml of a solution containing eight parts HBSS and two parts 7.5% sodium bicarbonate by oral gavage to neutralize stomach acidity. After 30 min, mice were immunized by oral gavage with 25 μg cholera toxin (List Biological Laboratories). Concentrations of antibodies specific for cholera toxin were determined by enzyme-linked immunosorbent assay with cholera toxin (List Biological Laboratories) as the capture agent.

Statistical analysis. The two-tailed Student's *t*-test was used for statistical analyses.

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