T$_H$17 cell plasticity in Peyer's patches is responsible for induction of T cell-dependent IgA responses

Keiji Hirota[1,2], **Jan-Eric Turner**[1,*], **Matteo Villa**[1,*], **Joao H Duarte**[1], **Jocelyn Demengeot**[3], **Oliver M. Steinmetz**[4], and **Brigitta Stockinger**[1]

[1]Division of Molecular Immunology, MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, UK

[3]Department of Lymphocyte Physiology, IGC , P-2781-901 Oeiras, Portugal

[4]III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Germany

Abstract

Intestinal Peyer's patches are essential lymphoid organs for the generation of T cell-dependent immunoglobulin (Ig) A production for gut homeostasis. Using IL-17 fate reporter mice we show here that endogenous T$_H$17 cells in lymphoid organs of naïve mice home preferentially to the intestine and are maintained independently of IL-23. In Peyer's patches such T$_H$17 cells acquire a T follicular helper (T$_{FH}$) phenotype and induce the development of IgA-producing germinal center B cells. Mice deficient in T$_H$17 cells fail to generate antigen specific IgA responses, providing evidence that T$_H$17 cells are the crucial subset required for high affinity T cell-dependent IgA production.

Disruption of mucosal homeostasis can lead not only to infections, but also chronic inflammatory diseases and cancer. Intestinal homeostasis is maintained by the immune system and the barrier function of epithelial cells. A large number of innate and adaptive immune cells reside in mucosal tissues and establish an immunological network to maintain healthy conditions. Amongst the adaptive immune cells, B cells producing IgA are an important player in maintenance of homeostasis and mucosal host defense[1] and the lamina propria (LP) of the small intestine (SI) is home to a substantial proportion of T$_H$17 cells present in non-immune mice.

IgA in the dimeric form is the dominant immunoglobulin isotype secreted into the intestinal lumen. The differentiation of T cell-dependent IgA-secreting B cells occurs in the Peyer's patches (PP) of the small intestine. Selective deficiency of IgA is the most common form of primary immunodeficiency, with an incidence of approximately 1 in 600 individuals in the western world. Although symptoms are rarely severe, individuals with symptomatic selective IgA deficiency can suffer from recurrent pulmonary and gastrointestinal infections[2]. T$_H$17 cells play a crucial role in the mucosal host defence as well as in the development of autoimmune diseases[3]. Under steady-state conditions T$_H$17 cells are preferentially found in the lamina propria of the small intestine where their development

[2]Present affiliation: Department of Experimental Immunology, Immunology Frontier Research Center, Osaka University, Suita565-0871, Japan
*these authors contributed equally

depends on the presence of commensal microbiota, in particular on segmented filamentous bacteria (SFB)[4]. Interestingly SFB also stimulate a large amount of total intestinal IgA[5].

The primary function of immune cells in the PP 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 also critically involved in shaping the diversity of the commensal microbiota[6-7]. Upon activation of B cells in the context of cognate T cell help, germinal centres (GCs) are generated and the induction of the activation-induced cytidine deaminase (AID) in GC B cells promotes somatic hypermutation and class-switch recombination of immunoglobulin genes. The majority of B cells in the PP differentiate into IgA-producing cells in the presence of T cell help, whereas T-independent IgA-producing B plasma cells, which are B220⁻ can differentiate in the gut lamina propria without the generation of the germinal centers[8-10]. IgA-producing B cells in germinal centers undergo extensive somatic hypermutation[10], resulting in higher antibody affinity.

Here we show that the majority of T_H17 cells found in lymphoid organs of non-immune mice were dependent on gut microbiota and had a natural preference for the small intestine as upon adoptive transfer they selectively homed to this site. Intestinal T_H17 cells underwent deviation towards a follicular helper T cell phenotype (T_{FH}) in Peyer's patches where they induced germinal centers (GC) and the development of host protective IgA responses. In marked contrast to pathogenic T_H17 cells developing in the course of EAE, which are highly dependent on IL-23[11-12], intestinal T_H17 cells did not require IL-23 for their maintenance or for plasticity towards a T_{FH} profile. Mice deficient in T_H17 cells had a pronounced deficiency of antigen-specific intestinal IgA following immunization with cholera toxin, emphasising that T_H17 cells are the helper T cell subset responsible for inducing the germinal center B cell switch towards high affinity, T cell-dependent IgA.

Results

T_H17 cells in non-immune mice have gut-homing properties

T_H17 cells constitute approximately 0.1 % of CD4⁺ T helper cells in the peripheral lymph nodes (LN) and spleen in non-immune IL-17 fate reporter mice (*Il17a*^Cre^*R26R*^eYFP^ mice) in which IL-17-producing cells are permanently marked as eYFP⁺ cells[12]. This system is a powerful tool to track T_H17 cells and investigate potential plasticity towards alternative effector functions, as detection of these cells does not depend on staining for intracellular IL-17. Flow cytometric analysis of eYFP⁺ T_H17 cells from LN of *Il17a*^Cre^*R26R*^eYFP^ mice showed almost uniform surface expression of CCR6, IL-7Rα, CD25, CD103 and 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 capacity, 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 absent in germfree mice[4], we compared the proportions of eYFP⁺ T_H17 cells in LN, PP and LP of SPF or germfree *Il17a*^Cre^*R26R*^eYFP^ mice. eYFP⁺ T_H17 cells were undetectable in PP and LP and also almost completely absent from LN of germfree *Il17a*^Cre^*R26R*^eYFP^ mice (Fig.1b). To test the homing properties of T_H17 cells compared with other memory type T cells from non-immune mice, we sorted eYFP⁺ T_H17 cells and eYFP⁻ CD4⁺ T cells with an activated phenotype (CD44^high^) from LN of *Il17a*^Cre^*R26R*^eYFP^ mice (distinguished by expression of the allotypic marker CD45.1) and adoptively co-transferred them in a 1:1 ratio into *Tcra*-deficient hosts (CD45.2), which lack conventional CD4⁺ and CD8⁺ T cells. eYFP⁺ T_H17 cells preferentially reconstituted the gut-associated tissues, such as the LP and PP of the small intestine, but not the peripheral lymph nodes where the cells had originally resided (Fig.1c,d). In contrast, eYFP⁻ CD44^high^ CD45.1⁺ non-T_H17 cells preferentially seeded peripheral LN (Fig.1c,d). Thus, the majority of T_H17 cells found in lymphoid organs of non-immune mice have gut-homing properties.

Intestinal T$_H$17 cells deviate to T$_{FH}$ in Peyer's patches

The preferential accumulation of T$_H$17 cells in PP prompted us to examine the possibility that they might play a role in helping germinal center B cell differentiation. T follicular helper (T$_{FH}$) cells reside in germinal centers and play an essential role in germinal center B cells differentiation and their distinguishing features are the expression of CXCR5, PD-1, IL-21, ICOS and the transcription factor Bcl6[14-16]. We determined that about ~13-20% of eYFP$^+$ T$_H$17 cells as well as a similar proportion of eYFP$^−$ cells present in the PP of non-immune *Il17a*^{Cre}*R26R*^{eYFP} mice expressed CXCR5 and PD-1, whereas PP eYFP$^+$ γδ T cells did not express these T$_{FH}$ markers (Fig. 2a).

In order to verify the developmental origin of these cells, we sorted CXCR5$^−$ eYFP$^+$ T$_H$17 cells from LN of non-immune *Il17a*^{Cre}*R26R*^{eYFP} mice, adoptively transferred them into *Tcra*-deficient hosts and subsequently determined expression of CXCR5 and PD-1 in different tissues of the adoptive hosts. Although eYFP$^+$ T$_H$17 cells homed to both PP and lamina propria of the adoptive hosts, conversion of eYFP$^+$ cells to a T$_{FH}$ phenotype occurred exclusively in the PP environment (Fig. 2b). It should be noted that the small proportion of CD4$^+$ eYFP$^−$ cells detected in the hosts after adoptive transfer are not donor derived T cells that lost eYFP expression, but CD4$^+$ non-T cells present in the host.

In order to test to what extent plasticity of eYFP$^+$ T$_H$17 cells towards a T$_{FH}$ profile could occur in other tissues as a consequence of immunization, we induced EAE in *Il17a*^{Cre}*R26R*^{eYFP} mice by immunizing with MOG+CFA. This induces an antibody response to MOG in addition to EAE and T$_H$17 cells are thought to be involved in development of ectopic lymphoid follicles in the CNS[17]. Analysis of LN and spinal cord from *Il17a*^{Cre}*R26R*^{eYFP} mice with EAE showed that about 4-7% of CD4$^+$ T cells in the LN and 60% of CD4$^+$ T cells in the spinal cord were eYFP$^+$. However, about 2-4% of eYFP$^+$ cells in LN, and none in the spinal cord, had a T$_{FH}$ signature (CXCR5$^+$ PD-1^{high}). In contrast, a substantial proportion (10-15%) of eYFP$^−$ CD4$^+$ T cells had a T$_{FH}$ profile (Fig. 2c). These observations suggest that T$_H$17 plasticity towards T$_{FH}$ preferentially takes place in the PP environment.

We next analysed T$_H$17 gene signatures in sorted CXCR5$^+$ eYFP$^+$ T cells isolated from PP (PP eYFP$^+$ T$_{FH}$), LN eYFP$^+$ T$_H$17 cells (LN T$_H$17), non-T$_H$17 PP eYFP$^−$ CXCR5$^+$ T$_{FH}$ cells (PP T$_{FH}$), as well as naïve CD4$^+$ T cells, all isolated from non-immune *Il17a*^{Cre}*R26R*^{eYFP} mice. eYFP$^+$ CD4$^+$ T cells with a T$_{FH}$ surface phenotype had down-regulated *Rorc* and *Il17a* mRNA and up-regulated the T$_{FH}$ signature genes *Bcl6* and *Il21*, similar to non-T$_H$17 related PP TF$_H$ cells (Fig.2d). Taken together, these data demonstrate that plasticity of T$_H$17 towards a T$_{FH}$-like phenotype takes place continuously in the PP environment under steady state conditions.

Maintenance of intestinal T$_H$17 and deviation to T$_{FH}$ are IL-23-independent

T$_H$17 cell plasticity in autoimmune settings is strongly dependent on IL-23[12]. In order to test whether IL-23 is similarly involved in plasticity of intestinal T$_H$17 cells towards T$_{FH}$, we analysed *Il17a*^{Cre}*R26R*^{eYFP} mice crossed onto a *p19* (*Il23a*)-deficient background. Firstly, and in contrast to the well-defined role of IL-23 in T$_H$17 maintenance in autoimmune settings, IL-23 was dispensable for the survival of intestinal T$_H$17 cells, as similar numbers of T$_H$17 cells were present in LN and PP of *Il23a*-deficient and wild-type *Il17a*^{Cre}*R26R*^{eYFP} mice (Fig.3a). Furthermore, phenotypic conversion to a T$_{FH}$ phenotype occurred to the same extent in IL-23-deficient and wild-type *Il17a*^{Cre}*R26R*^{eYFP} mice (Fig.3b) and T$_H$17 cells with the T$_{FH}$ phenotype in *Il23a*$^{−/−}$ reporter mice upregulated *Bcl6* and *Il21* expression similar to T$_H$17 cells from wild-type reporter mice (Fig.3c). In contrast, in accordance with previous observations[12], T$_H$17 cells were unable to deviate towards IFN-γ expression and did not

express IL-22 in *Il23a*-deficient mice (Fig.3d). These data indicate that the intestinal, steady-state population of T_H17 cells has distinct features from the T_H17 cells elicited by immunization in the periphery.

PP ex-T_H17 cells induce IgA production by GC B cells

The dependency of intestinal T_H17 cells on commensal bacteria raises the possibility that T_{FH} cells developing from gut homing ex-T_H17 cells may be specialized for helping B cell IgA responses in PP germinal centers. We therefore analysed B cell expression of *Aicda*, which is required for somatic hypermutation, gene conversion and class-switch recombination of immunoglobulin genes. There was little *Aicda* expression in LN B cells of B6 mice, in line with the absence of germinal centers in mice that are kept under specific pathogen free (SPF) conditions. B cells in PP on the other hand are continuously stimulated by the commensal flora and expressed high levels of *Aicda* (Fig.4a). In absence of T cells in *Tcra*-deficient hosts, *Aicda* expression was very low (Fig.4a), as no germinal center B cells develop in the absence of T cell help. Transfer of eYFP$^+$ T_H17 cells, however, reconstituted *Aicda* expression to the level seen in B6 mice (Fig.4a).

Furthermore, B cell expression of the germinal center markers GL-7 and CD95, as well as expression of IgA, which was detected on B cells from wild-type, but not *Tcra*-deficient mice, was induced in PP B cells of *Tcra*-deficient mice upon T_H17 transfer (Fig.4b,c). As a result, the concentration of IgA, but not that of other Ig isotypes, was substantially increased in serum of *Tcra*-deficient mice that had received T_H17 cells, whereas *Tcra* -deficient mice that had not received adoptively transferred T_H17 cells, as well as those which had received non-T_H17 effector cells (eYFP$^-$ CD44^{high}) showed no change in their IgA expression (Fig. 4d). These data strongly suggest that intestinal T_H17 cells deviating to a T_{FH} profile in the PP may be responsible for the induction of T cell-dependent IgA responses.

Ex-T_H17 do not acquire Foxp3 and T_{reg} do not induce IgA

Previous reports suggested that Foxp3$^+$ regulatory T (T_{reg}) cells might adopt a T_{FH} phenotype in PP[8, 18-20]. Since these studies had focused on T_{reg} isolated from lymphoid organs, we first compared the homing as well as the adoption of a T_{FH} phenotype in T_{reg} and T_H17 cells isolated from LN and spleen. T_{reg} from B6.CD45.1 mice were isolated on the basis of high CD25 expression, which correlated well with Foxp3 expression (Fig.5a), and co-transferred with equal numbers of eYFP$^+$ T_H17 cells into *Tcra*-deficient hosts. Three months later, transferred T_{reg} were preferentially found in LN, whereas transferred eYFP$^+$ cells homed to LP and PP of the small intestine (Fig.5b). CD45.1$^+$ donor T_{reg} retained their Foxp3 expression and there was no indication that donor eYFP$^+$ T_H17 cells acquired Foxp3 expression in any location within the adoptive host (Fig.5c). Furthermore, while 15-30% of T_H17 cells deviated to a T_{FH} profile in PP, T_{reg} cells did not acquire a T_{FH} profile in any of the tissues examined (Fig.5d). As the poor homing of LN derived T_{reg} to intestinal tissues might have precluded acquisition of a T_{FH} phenotype in PP, we isolated RFP^{high} Treg from LP and PP of *Foxp3^{RFP}* and transferred them into *Tcra*-deficient hosts. Although we observed efficient homing of donor RFP^{high} T_{reg} into PP, these cells did not acquire a T_{FH} profile in the adoptive hosts (Fig.5e). Furthermore, adoptive T_{reg} transfer did not induce germinal center B cells and IgA production (Fig.5f,g). Taken together these data strongly suggest that the promotion of IgA class switching in GC B cells in the PP is a function of ex-T_H17 derived T_{FH} cells, whereas T_{reg} neither adopt a T_{FH} profile nor support IgA production.

IgA class switching in intact mice is dependent on T_H17 cells

After transfer into *Tcra*-deficient host, transferred eYFP$^+$ T_H17 cells expanded substantially, resulting in IgA production that far exceeded that seen in wild-type mice in steady-state. In

Tcra-deficient hosts, lymphopenia may have resulted in unimpeded expansion to the commensal flora by transferred T_H17 cells. However, adoptive transfer into intact wild-type hosts, which contain full niches of intestinal T_H17 and T_{FH} cells, does not lead to efficient engraftment of the small number of donor cells that can be isolated for transfer from nonmanipulated mice. The minimal differences in serum IgA concentration between T cell-deficient *Tcra*^{−/−} mice and non-immune wild-type mice (see Fig.4d) suggest that under steady-state conditions most IgA expression is T cell-independent. In order to investigate a T cell-dependent IgA immune response 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 non-immunized *Il17a*[Cre]*R26R*[eYFP] mice (Fig.6a). However, 40-60% of the eYPF^{+} cells in the PP had acquired a T_{FH} phenotype, compared with 13% at steady-state (Fig.6a and Fig.2a). We also observed a strong cholera toxin specific IgA response in the serum of immunized *Il17a*[Cre]*R26R*[eYFP] mice (Fig.6a).

We used qPCR to assess markers associated with IgA switching in eYFP[−] and eYFP[+] PP T cells from cholera toxin-immunized mice, but could not detect statistically significant differences in the two populations analyzed (Fig.6b). However, expression of ICOS and CD40L was consistently higher on eYFP^{+} cells than on eYFP^{−} T cells, even before eYFP^{+} cells had acquired the CXCR5^{+} PD-1^{high} T_{FH} profile (Fig.6c,d).

To address if T cell-dependent IgA induction requires T_H17 cells in an otherwise intact mouse, we generated bone marrow chimeras in which *Tcra*^{−/−} hosts were reconstituted with whole bone marrow from *Rorc*^{−/−} mice[21] (*Rorc*^{−/−} *Tcra*^{−/−} chimeras), which do not develop T_H17 cells[22]. Although *Rorc* is required for the development of lymphoid architecture in the mucosal immune system[21, 23-24], the mucosal environment of these chimeric mice is not disturbed, as *Rorc*-expressing innate lymphoid cell types are present in the *Tcra*^{−/−} hosts. Control *Tcra*^{−/−} chimeras were reconstituted with bone marrow from wild-type hosts. Flow cytometric analysis of PP showed similar proportions of T_{FH} cells in *Rorc*^{−/−} and control *Tcra*^{−/−} chimeras (Fig.7a). *Rorc*^{−/−} *Tcra*^{−/−} chimeras had a reduction of IL-17-producing CD4^{+} T cells compared to control *Tcra*^{−/−} chimeras, whereas the proportion of T_{reg} was comparable and IFNγ-, IL-4- and IL-13-producing CD4^{+} T cells were similar or even higher in *Rorc*^{−/−} *Tcra*^{−/−} chimeras compared to control *Tcra*^{−/−} chimeras (Fig.7b). Serum isotype profiles in the two sets of chimeras prior to immunization were similar (Suppl.Fig.2). In order to test for T cell-dependent IgA production, we immunized *Rorc*^{−/−} and control *Tcra*^{−/−} chimeras with cholera toxin and analysed serum and fecal IgA amounts 10 days later. Control *Tcra*^{−/−} chimeras mounted a strong cholera toxin-specific IgA response detectable in serum (Fig.7c) and faeces (Fig.7d). In contrast, *Rorc*^{−/−} *Tcra*^{−/−} chimeras had very low levels of cholera toxin-specific IgA, which were comparable to those observed in *Tcra*^{−/−} mice (Fig.7c,d). Thus, these results show that T_H17 cells are required for the germinal center switch to IgA production in PP.

DISCUSSION

T_H17 cells are known to diversify their effector profile in response to different environmental conditions[25]. Here we describe the consequences of T_H17 cell plasticity towards a T_{FH} program in the small intestine PP environment, a process that promoted T cell-dependent IgA responses. Although previous studies demonstrated that T_H17 cells could be reprogrammed to obtain T_{FH} characteristics *in vitro*[26], an *in vivo* demonstration of this phenomenon would not have been possible without an IL-17 fate reporter mouse (*Il17a*[Cre]*R26R*[eYFP] mouse), as this allows identification of a T_H17 origin irrespective of the

production of the signature cytokine IL-17. Here we used the fate reporter mouse to demonstrate the deviation of T_H17 cells towards a T_{FH} phenotype under the influence of the PP environment, which results in substantial phenotypic and functional changes.

Thus, IL-17 -as well as RORγt expression, was extinguished in ex-T_H17 cell-derived T_{FH}, whereas IL-21 and Bcl-6 were strongly upregulated. Although IL-21 has been associated with *in vitro* generated T_H17 cells[3], expression of this cytokine was not detectable in T_H17 cells from the intestine or lymphoid organs of non-immune mice.

T_H17 cells are naturally found in the small intestine of non-immune pathogen free mice. Under steady-state conditions they are thought to contribute to gut barrier function via the stimulation of tight junction formation and anti-microbial peptides[27-28]. Our analysis of the *Il17a*[Cre]*R26R*[eYFP] mice indicated that the majority of the few T_H17 cells found in peripheral lymphoid organs probably have their developmental origin in the gut, which is also supported by the fact that germfree *Il17a*[Cre]*R26R*[eYFP] mice lack both intestinal and most of the T_H17 cells from lymphoid organs. It was particularly interesting to observe that the cytokine IL-23, a key factor for the development of T_H17 responses with pathogenic features[11-12, 29], is dispensable for the maintenance of intestinal T_H17 cells and their deviation towards a T_{FH} program. This confirms earlier suggestions that T_H17 cells might develop towards either protective or pathogenic functions[30].

Our data expand the functional repertoire of intestinal T_H17 cells to include the induction of the GC B cell IgA response. In *Tcra*-deficient mice transferred with eYFP+ T_H17 cells serum IgA levels were highly increased irrespective of deliberate immunization. This was presumably due to the exaggerated 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 these mice (data not shown). In contrast, immunization of *Il17a*[Cre]*R26R*[eYFP] mice with cholera toxin resulted in a pronounced cholera toxin specific IgA response and more pronounced switching of eYFP+ towards a T_{FH} phenotype.

In *Rorc*^{−/−} *Tcra*^{−/−} chimeras, serum IgA levels prior to immunization with cholera toxin were comparable to those seen in *Tcra*^{−/−} mice as well as wild-type B6 mice (data not shown), suggesting that basal IgA levels in SPF mice may be mostly T cell-independent. T_H17 cells were shown to be involved in upregulating the expression of the polymeric Ig receptor that transports IgA across the intestinal epithelium[31]. However, it seems this role can be attributed to IL-17 rather than to T_H17 cells. An interesting possibility that remains to be addressed is whether RORγt+ ILCs contribute to T cell-independent IgA induction and/or upregulation of the IgA transporter. Since ex-T_H17 derived T_{FH} switch off IL-17, it is unlikely that they participate in this process and we have not detected changes in expression of polymeric Ig receptor in our various models, none of which were devoid of IL-17. Nevertheless, IgA responses to challenge with cholera toxin strongly depended on the presence of T_H17 cells.

Plasticity of T_H17 cells towards a T_{FH} fate was restricted to the PP environment and not evident in peripheral LN. As intestinal IgA fulfils important roles in maintaining equilibrium with the commensal flora and efficient mucosal host defence[7], this novel T_H17 function provides another example of their crucial role in mucosal immunity. It is interesting to note that segmented filamentous bacteria, which are important stimulators of T_H17 cell development, also drive germinal center formation and IgA production in PP[4-5]. IgA deficiency causes aberrant expansion of SFB[4, 32-33] and one might speculate that a deficiency in T_H17 cells would result in the same features.

The developmental relationship between T_{FH} cells and other CD4$^+$ T cell subsets remains a matter of debate (reviewed in[15]). Our data are compatible with a non-exclusive CD4$^+$ T cell program that obtains input from multiple T cell subsets. T_H2 cells have been shown able to acquire CXCR5 expression, resulting in the induction or inhibition of B cell differentiation and class switching in GC[18, 34-36]. IL-12-mediated STAT4 activation transiently induces a T_{FH} transcriptional profile, followed by repression of the T_{FH} gene signature by the T_H1-specific transcription factor T-bet[37]. Induction of Bcl-6 requires ICOS[38], which is highly expressed on T_H17 cells. Temporal and spatial regulation of CXCR5 and Bcl-6 expression in interaction with dendritic cells and B cells promote T_{FH} development[38-40], but it remains unclear whether the T_{FH} state resembles a terminal effector status or whether such cells can be redirected towards other T cell programs. Thus, it remains to be determined whether the extinction of a previous effector profile is complete following the acquisition of a T_{FH} phenotype or whether each effector T cell subset contributes a unique feature of its original signature to the functional helper response in the germinal center reaction. Elucidation of these possibilities would be facilitated by fate reporter mice for each T cells subset, which would allow analysis of functional profiles irrespective of the expression of signature cytokines that currently defines their subset allocation.

The role of T_{reg} in PP germinal center reactions remains controversial. In some cases it was argued that T_{reg} converted to T_{FH} to promote intestinal IgA responses[41,18], whereas other studies suggested that T_{reg}, expressing markers of T_{FH} cells are essential to control, rather than promote the germinal center reaction[19-20]. It should be noted that depletion of T_{reg} via antibodies to CD25 as used in a previous study on the role of T_{reg} in intestinal IgA induction[41], would also have depleted T_H17 cells, which are homogenously CD25$^+$. Our data did not confirm plasticity of T_{reg} towards a T_{FH} profile, nor a role in promoting IgA responses either in the transfer model or in bone marrow chimeras. For transfers we isolated the T_{reg} population based either on high CD25 expression, which was shown to mark stable Treg[42] or based on high RFP-Foxp3 expression in the *Foxp3^{RFP}* mouse[43]. The discrepancy regarding T_{reg} plasticity might be explained by technical issues with the Foxp3 reporter model that was previously used[18], where plasticity was particularly prominent in cells expressing lower levels of Foxp3 or in transfers of mixtures of Foxp3$^+$ and Foxp3$^-$ cells. Given the reciprocal relationship between Foxp3 and RORγt in T_{reg} and T_H17 cell development[44], it is conceivable that Foxp3^{low} T_{reg} cells may have deviated towards a T_H17 fate, thus mimicking their unique function in the intestinal immune response.

Our data highlight another facet of the host protective function of T_H17 cells in mucosal tissues. At present it remains unclear what particular features ex-T_H17 cells contribute to their interaction with B cells to promote IgA responses. Currently known genes that affect IgA are widely expressed in the PP environment and we did not detect differential expression in eYFP$^+$ and eYFP$^-$ PP T cells for these markers. Interestingly, eYFP$^+$ T cells display substantially higher levels of ICOS and CD40L, which might facilitate preferential contact with B cells. B cells in the PP patch environment are characterized by expression of the transcription factor RORα[45] and compete for T cell help before entry into germinal centres. eYFP$^+$ T cells may have preferential access. This, however, does not explain why T_{FH} in PP of the *Rorc$^{-/-}$ Tcra$^{-/-}$* chimeras, which do not face competition by ex-T_H17 cells are still not competent to induce an IgA switch.

Given the prominent role of T_H17 cells in autoimmunity, these cells seem as obvious targets for therapeutic intervention. However, understanding their role in maintaining intestinal barrier integrity is required in order to avoid disturbance of these beneficial functions.

Online METHODS

Mice

IL-17 fate reporter (*Il17a^CreR26R^eYFP*) mice[12] and *Foxp3^RFP* mice with a bicistronic red fluorescent protein (RFP) reporter knocked into the Foxp3 locus[43], as well as TCRα-deficient on a B6 background[46] and *p19^−/−* (*Il23a^−/−*) mice (obtained from Dan Cua,Merck Research laboratories USA) were bred in the NIMR animal facility under specified pathogen free conditions. All animal experiments were done according to the NIMR Ethical Review committee and Home Office regulations. Some IL-17 fate reporter mice were raised in germ free (GF) conditions using caesarean section rederivation, as described at The European Mouse Mutant Archive http://www.emmanet.org/protocols/GermFree_0902.pdf. Briefly, day 20 post coitum 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 which are knockout for *Rorc (γt)*[21] were obtained from Oliver Steinmetz (Hamburg).

Antibodies

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

Preparation of lymphocytes in tissues

Briefly, lymphocytes in the lamina propria were prepared by cutting the small intestine into 1cm pieces after removing Peyer's patches, shaking for 20min at 37°C in 10ml IEL buffer (PBS supplemented with 10% FCS, 1mM pyruvate, 20μM HEPES, 10mM EDTA and penicillin /streptomycin mix, 10ug/ml Polymyxin B) to remove epithelial and intraepithelial cells and then digesting the remaining tissue using 1mg/ml collagenase D (Roche) and 10U/ml DNase1 (Sigma) at 37°C for 1 hr followed by 36.5% Percoll separation.

Real time PCR

RNA was extracted from FACS sorted CD4 T cells using Trizol and reverse transcribed with Omniscript (Qiagen) according to the manufacturer's protocol. The cDNA served as template for the amplification of genes of interest and the housekeeping gene (Hprt) by real-time PCR, using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA); Hprt1 (Mm00446968_m1), Aicda (Mm00507774_m1), Bcl6 (Mm00477633_m1), Il17a (Mm00439619_m1), Il21 (Mm00517640_m1), Il22 (Mm00444241_m1), Prdm1 (Mm00476128_m1), Rorc (Mm01261019_g1), universal PCR Master Mix (Applied Biosystems, Warrington, UK) and the ABI-PRISM 7900 Sequence detection system (Applied Biosystems, Foster City, CA). Target gene expression was calculated using the comparative method for relative quantification upon normalisation to *Hprt* gene expression.

Cholera toxin immunisation

TCRα^−/− mice were sublethally irradiated (500 rad) and reconstituted with B6 or Rorc^−/− bone morrow. Before immunization with cholera toxin mice were deprived of food for 2 h and then given 0.25 ml of a solution containing eight parts HBSS and two parts 7.5% sodium bicarbonate by oral gavage (o.g.) to neutralize stomach acidity. After 30 min, mice were o.g. immunized with 25μg cholera toxin (List Biological Laboratories, USA).

Concentrations of antibodies specific for cholera toxin were determined by ELISA with cholera toxin (List Biological Laboratories) as the capture agent.

Statistics were performed using a two-tailed Student T test.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

1. Shulzhenko N, et al. Crosstalk between B lymphocytes, microbiota and the intestinal epithelium governs immunity versus metabolism in the gut. Nat Med. 2011; 17:1585–1593. [PubMed: 22101768]

2. Castigli E, et al. TACI is mutant in common variable immunodeficiency and IgA deficiency. Nat Genet. 2005; 37:829–834. [PubMed: 16007086]

3. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. Annu Rev Immunol. 2009; 27:485–517. [PubMed: 19132915]

4. Ivanov II, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell. 2009; 139:485–498. [PubMed: 19836068]

5. Talham GL, Jiang HQ, Bos NA, Cebra JJ. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. Infect Immun. 1999; 67:1992–2000. [PubMed: 10085047]

6. Fagarasan S, Kawamoto S, Kanagawa O, Suzuki K. Adaptive immune regulation in the gut: T cell-dependent and T cell-independent IgA synthesis. Annu Rev Immunol. 2010; 28:243–273. [PubMed: 20192805]

7. Macpherson AJ, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. Mucosal Immunol. 2008; 1:11–22. [PubMed: 19079156]

8. Fritz JH, et al. Acquisition of a multifunctional IgA+ plasma cell phenotype in the gut. Nature. 2012; 481:199–203. [PubMed: 22158124]

9. Tezuka H, et al. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. Nature. 2007; 448:929–933. [PubMed: 17713535]

10. Bergqvist P, Stensson A, Lycke NY, Bemark M. T cell-independent IgA class switch recombination is restricted to the GALT and occurs prior to manifest germinal center formation. J Immunol. 2010; 184:3545–3553. [PubMed: 20207993]

11. McGeachy MJ, et al. The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. Nat Immunol. 2009; 10:314–324. [PubMed: 19182808]

12. Hirota K, et al. Fate mapping of IL-17-producing T cells in inflammatory responses. Nat Immunol. 2011; 12:255–263. [PubMed: 21278737]

13. Esplugues E, et al. Control of TH17 cells occurs in the small intestine. Nature. 2011; 475:514–518. [PubMed: 21765430]

14. Vinuesa CG, Cyster JG. How T cells earn the follicular rite of passage. Immunity. 2011; 35:671–680. [PubMed: 22118524]

15. Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol. 2011; 29:621–663. [PubMed: 21314428]

16. Kawamoto S, et al. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Science. 2012; 336:485–489. [PubMed: 22539724]

17. Peters A, et al. Th17 cells induce ectopic lymphoid follicles in central nervous system tissue inflammation. Immunity. 2011; 35:986–996. [PubMed: 22177922]

18. Tsuji M, et al. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. Science. 2009; 323:1488–1492. [PubMed: 19286559]

19. Linterman MA, et al. Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med. 2011; 17:975–982. [PubMed: 21785433]

20. Chung Y, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nat Med. 2011; 17:983–988. [PubMed: 21785430]

21. Eberl G, et al. An essential function for the nuclear receptor RORgamma(t) in the generation of fetal lymphoid tissue inducer cells. Nature immunology. 2004; 5:64–73. [PubMed: 14691482]

22. Ivanov II, et al. The orphan nuclear receptor RORgammat directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell. 2006; 126:1121–1133. [PubMed: 16990136]

23. Sun Z, et al. Requirement for RORgamma in thymocyte survival and lymphoid organ development. Science. 2000; 288:2369–2373. [PubMed: 10875923]

24. Kurebayashi S, et al. Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. Proceedings of the National Academy of Sciences of the United States of America. 2000; 97:10132–10137. [PubMed: 10963675]

25. Lee YK, Mukasa R, Hatton RD, Weaver CT. Developmental plasticity of Th17 and Treg cells. Curr Opin Immunol. 2009; 21:274–280. [PubMed: 19524429]

26. Lu KT, et al. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. Immunity. 2011; 35:622–632. [PubMed: 22018472]

27. Kinugasa T, Sakaguchi T, Gu X, Reinecker HC. Claudins regulate the intestinal barrier in response to immune mediators. Gastroenterology. 2000; 118:1001–1011. [PubMed: 10833473]

28. Ishigame H, et al. Differential roles of interleukin-17A and −17F in host defense against mucoepithelial bacterial infection and allergic responses. Immunity. 2009; 30:108–119. [PubMed: 19144317]

29. Codarri L, et al. RORgammat drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nature immunology. 2011; 12:560–567. [PubMed: 21516112]

30. O'Connor W Jr. Zenewicz LA, Flavell RA. The dual nature of T(H)17 cells: shifting the focus to function. Nature immunology. 2010; 11:471–476. [PubMed: 20485275]

31. Cao AT, Yao S, Gong B, Elson CO, Cong Y. Th17 cells upregulate polymeric Ig receptor and intestinal IgA and contribute to intestinal homeostasis. J Immunol. 2012; 189:4666–4673. [PubMed: 22993206]

32. Suzuki K, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc Natl Acad Sci U S A. 2004; 101:1981–1986. [PubMed: 14766966]

33. Gaboriau-Routhiau V, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity. 2009; 31:677–689. [PubMed: 19833089]

34. King IL, Mohrs M. IL-4-producing CD4+ T cells in reactive lymph nodes during helminth infection are T follicular helper cells. J Exp Med. 2009; 206:1001–1007. [PubMed: 19380638]

35. Reinhardt RL, Liang HE, Locksley RM. Cytokine-secreting follicular T cells shape the antibody repertoire. Nat Immunol. 2009; 10:385–393. [PubMed: 19252490]

36. Zaretsky AG, et al. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. J Exp Med. 2009; 206:991–999. [PubMed: 19380637]

37. Nakayamada S, et al. Early Th1 cell differentiation is marked by a Tfh cell-like transition. Immunity. 2011; 35:919–931. [PubMed: 22195747]

38. Choi YS, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity. 2011; 34:932–946. [PubMed: 21636296]

39. Kerfoot SM, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity. 2011; 34:947–960. [PubMed: 21636295]

40. Kitano M, et al. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. Immunity. 2011; 34:961–972. [PubMed: 21636294]

41. Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:19256–19261. [PubMed: 19889972]

42. Komatsu N, et al. Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proceedings of the National Academy of Sciences of the United States of America. 2009; 106:1903–1908. [PubMed: 19174509]

43. Wan YY, Flavell RA. Identifying Foxp3-expressing suppressor T cells with a bicistronic reporter. Proceedings of the National Academy of Sciences of the United States of America. 2005; 102:5126–5131. [PubMed: 15795373]

44. Zhou L, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgammat function. Nature. 2008; 453:236–240. [PubMed: 18368049]

45. Wang NS, et al. Divergent transcriptional programming of class-specific B cell memory by T-bet and RORalpha. Nature immunology. 2012; 13:604–611. [PubMed: 22561605]

46. Philpott KL, et al. Lymphoid development in mice congenitally lacking T cell receptor alpha beta-expressing cells. Science. 1992; 256:1448–1452. [PubMed: 1604321]
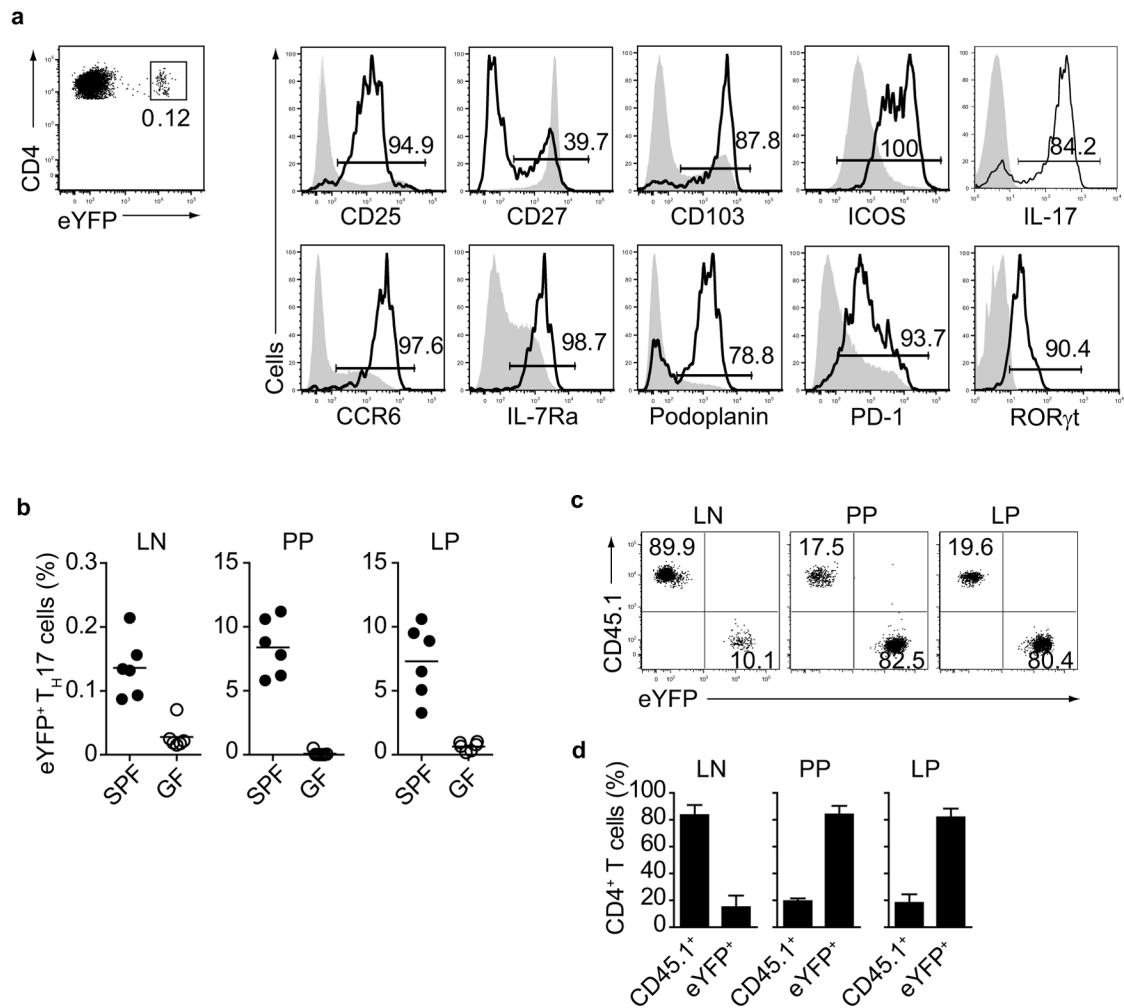
Figure 1. Preferential migration of eYFP$^+$ T$_H$17 cells into gut-associated tissues
a) Flow cytometry of CD4$^+$ eYFP$^+$ T cells (solid line) and CD4$^+$ CD44^{high} eYFP$^-$ T cells (shaded line) isolated from LN and spleen of $Il17a^{Cre}R26R^{eYFP}$ mice. The percentage of cells expressing the indicated marker is shown in the histogram. Isotype controls were used as negative controls indicated by placement of the bars. **(b)** Proportion of eYFP$^+$ T$_H$17 cells in LN, PP and LP of SPF and germfree (GF) $Il17a^{Cre}R26R^{eYFP}$ mice. **(c,d)** Flow cytometry of CD4$^+$ T cells in LN, PP and LP cells of $Tcra^{-/-}$ mice reconstituted with CD4$^+$ eYFP$^+$ T$_H$17 cells and CD45.1$^+$ eYFP$^-$ CD44^{high} CD4$^+$ T cells, as assessed three months after transfer. Mean values +/− SD for three individual mice are shown. Data are representative of three independent experiments.
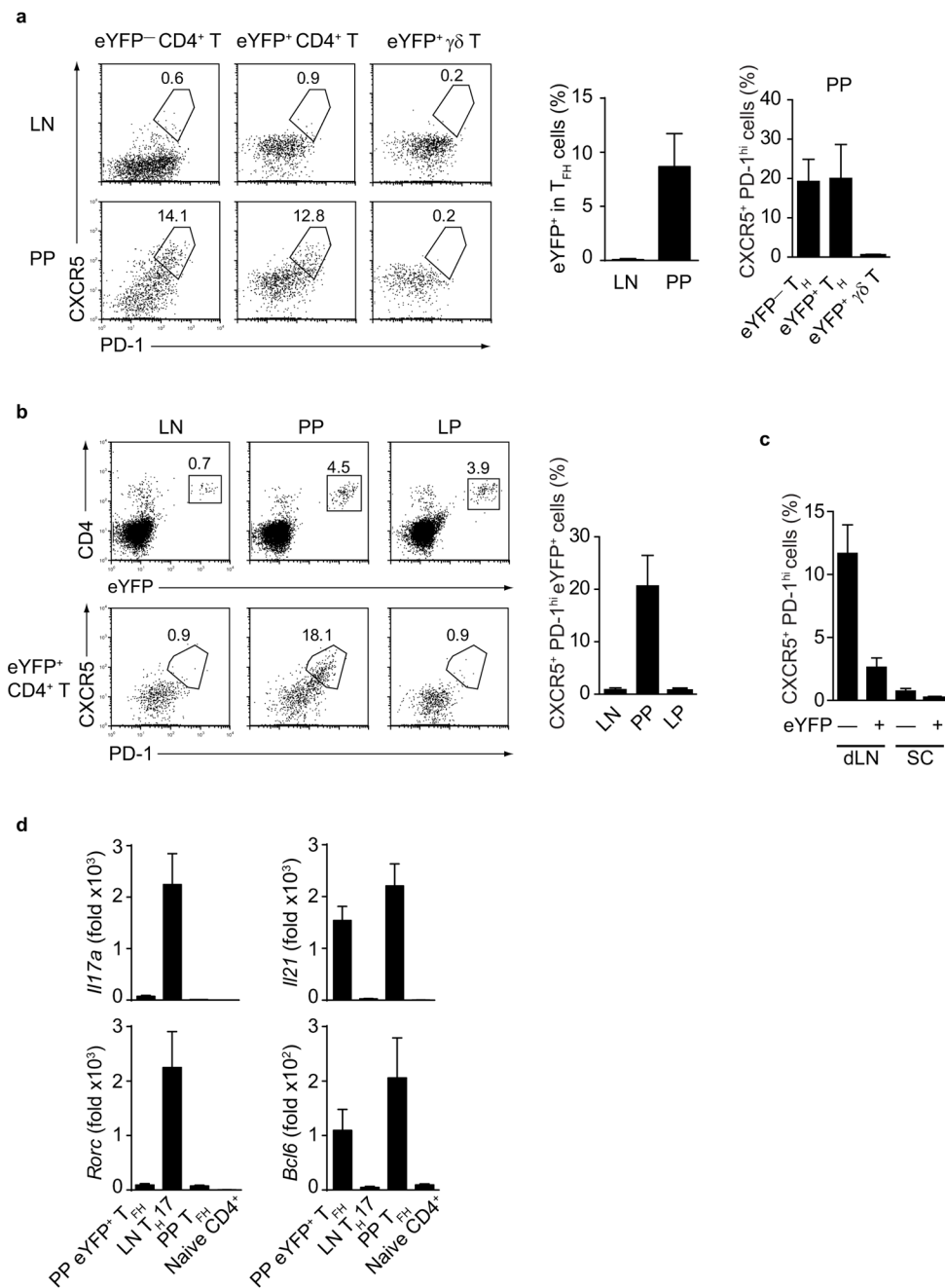
Figure 2. Reprogramming of T_H17 profiles to T_{FH} phenotype in the Peyer's patches
a) Flow cytometry of CD4+ CD44high eYFP− T cells (left panel), CD4+ eYFP+ cells (middle panel) and eYFP+ γδ T cells (right panel) showing expression of CXCR5 and PD-1 in LN (upper row) or PP (lower row) of $Il17a^{Cre}R26R^{eYFP}$ mice. Mean values +/− SD of CXCR5+ PD-1high cells are given in histograms. **b)** Flow cytometry of CD4+ eYFP+ T cells (upper panel) and CD4+ eYFP+ T cells expressing CXCR5 and PD-1 (lower panel) in LN, PP, and LP of $Tcra^{−/−}$ mice transferred with eYFP+ T_H17 cells and analysed three months after transfer. Mean values of CXCR5+ PD-1high eYFP+ cells +/− SD are shown in histograms. **c)** Proportion of CXCR5+ PD-1high eYFP− CD44high CD4+ or eYFP+ CD4+ T cells in draining

LN or spinal cord of $Il17a^{Cre}R26R^{eYFP}$ mice 20d after immunization with MOG+CFA. **d**) Quantitative PCR analysis for expression of indicated mRNA in FACS purified CXCR5$^+$ eYFP$^+$ (PP eYFP$^+$ T$_{FH}$), CXCR5$^-$ eYFP$^+$ (LN T$_H$17), CXCR5$^+$ eYFP$^-$ (PP T$_{FH}$) and naïve CD4$^+$ T cells from $Il17a^{Cre}R26R^{eYFP}$ mice. mRNA expression is relative to $Hprt$. Data are representative of at least three independent experiments.
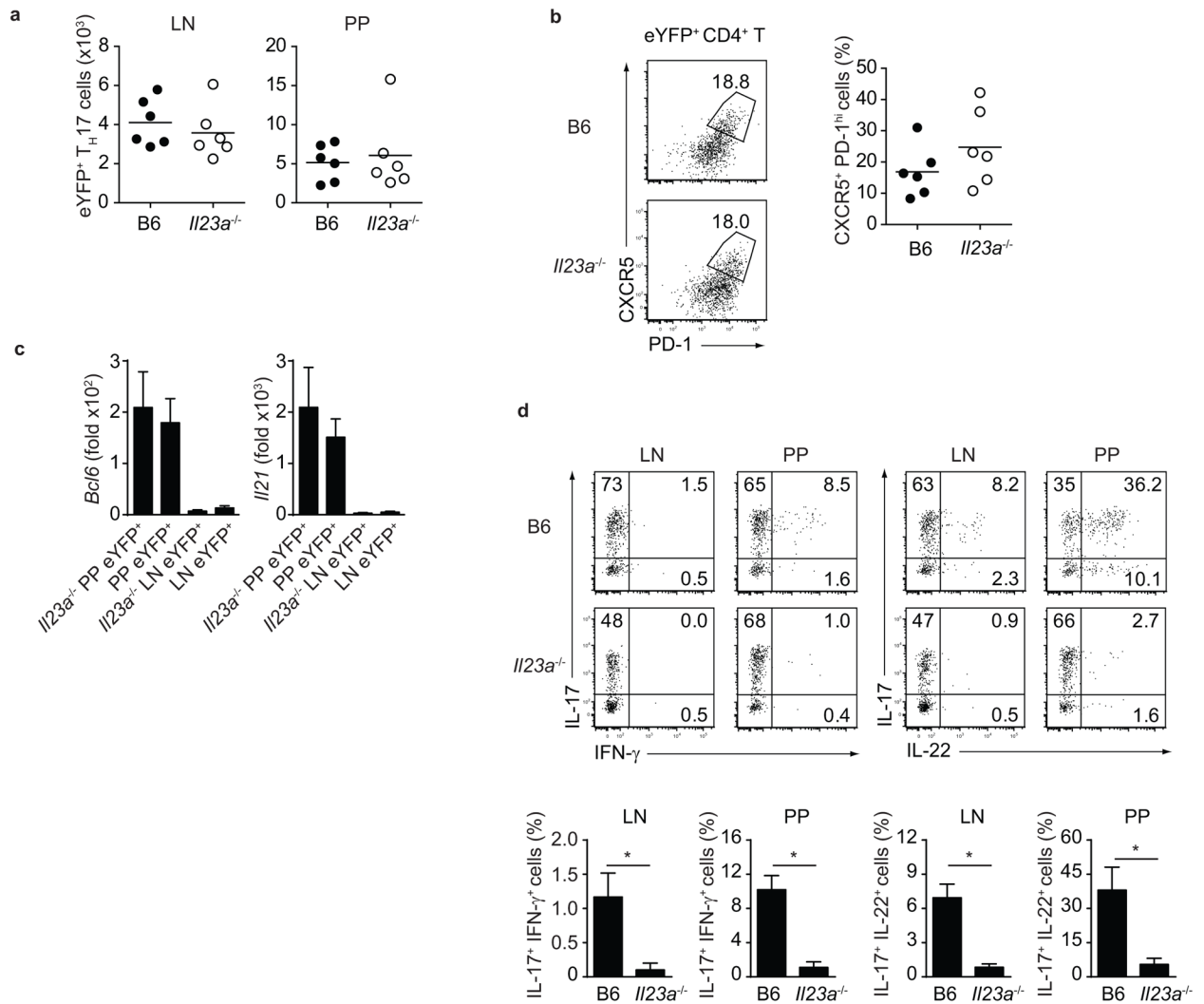
Figure 3. IL-23 is dispensable for homeostatic maintenance and plasticity of intestinal T_H17
a) Number of eYFP$^+$ T_H17 cells in LN and PP of $Il17a^{Cre}R26R^{eYFP}$ (B6, closed dots) and $Il17a^{Cre}R26R^{eYFP}Il23a$-deficient ($Il23a^{-/-}$, open dots) mice. **b)** Flow cytometry of eYFP$^+$ CD4$^+$ T cells showing expression of CXCR5 and PD-1 in PP of B6 and $Il23a^{-/-}$ mice. **c)** Quantitative PCR analysis for expression of indicated mRNA in FACS purified PP or LN eYFP$^+$ CD4$^+$ T cells from B6 and $Il23a^{-/-}$ mice. **d)** Flow cytometry of eYFP$^+$ CD4$^+$ T cells from LN or PP of B6 and $Il23a^{-/-}$ mice showing intracellular staining for IL-17, IFNγ and IL-22. Data are representative of at least three independent experiments. *, p-value < 0.01.
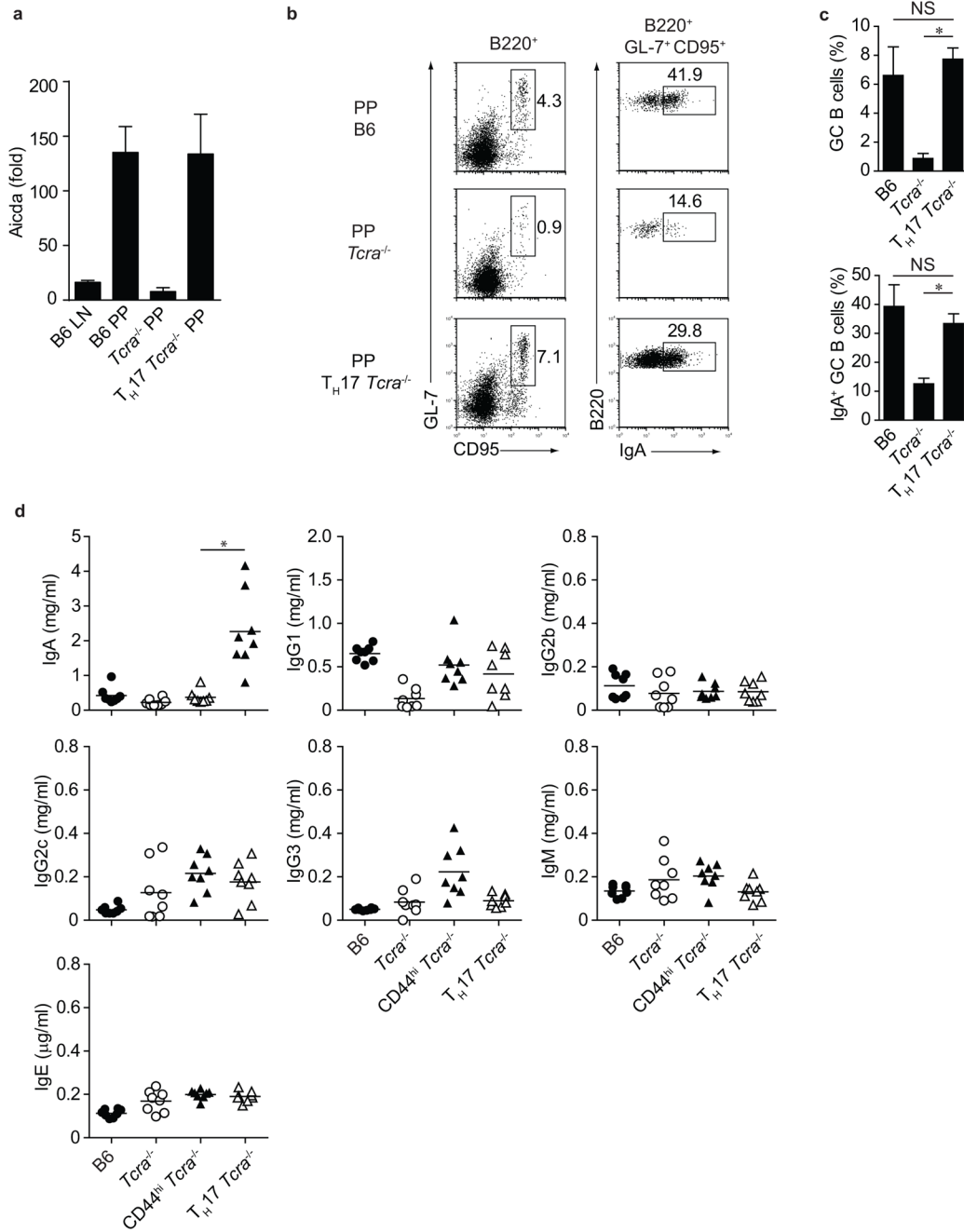
Figure 4. Induction of B cell IgA by T$_H$17 cells

a) Quantitative PCR analysis for expression of *Aicda* in FACS purified B220$^+$ cells from LN or PP of C57Bl/6 (B6), *Tcra$^{-/-}$* or *Tcra$^{-/-}$* mice transferred with eYFP$^+$ T$_H$17 cells (T$_H$17 *Tcra$^{-/-}$*). **b)** Flow cytometry of B220$^+$ cells (left panel) and B220$^+$ GL-7$^+$ CD95$^+$ cells (right panel) from PP of B6, *Tcra$^{-/-}$* or *Tcra$^{-/-}$* mice transferred with eYFP$^+$ T$_H$17 cells showing expression of germinal center markers CD95 and GL-7 (left) and IgA (right). **c)** Proportion of B220$^+$ GL-7$^+$ CD95$^+$ and B220$^+$ GL-7$^+$ CD95$^+$ IgA$^+$ cells from B6, *Tcra$^{-/-}$* and *Tcra$^{-/-}$* mice transferred with eYFP$^+$ T$_H$17 cells. Mean values +/− SD for three individual mice are shown. Data in **a**, **b** and **c** are representative of three independent

experiments. **d)** ELISA quantification of serum immunoglobulin isotypes from B6, $Tcra^{-/-}$ and $Tcra^{-/-}$ mice transferred with CD4$^+$ CD44^{high} eYFP$^-$ (CD44^{high} $Tcra^{-/-}$) or with eYFP$^+$ T$_H$17 cells (T$_H$17 $Tcra^{-/-}$). *, p-value < 0.01.
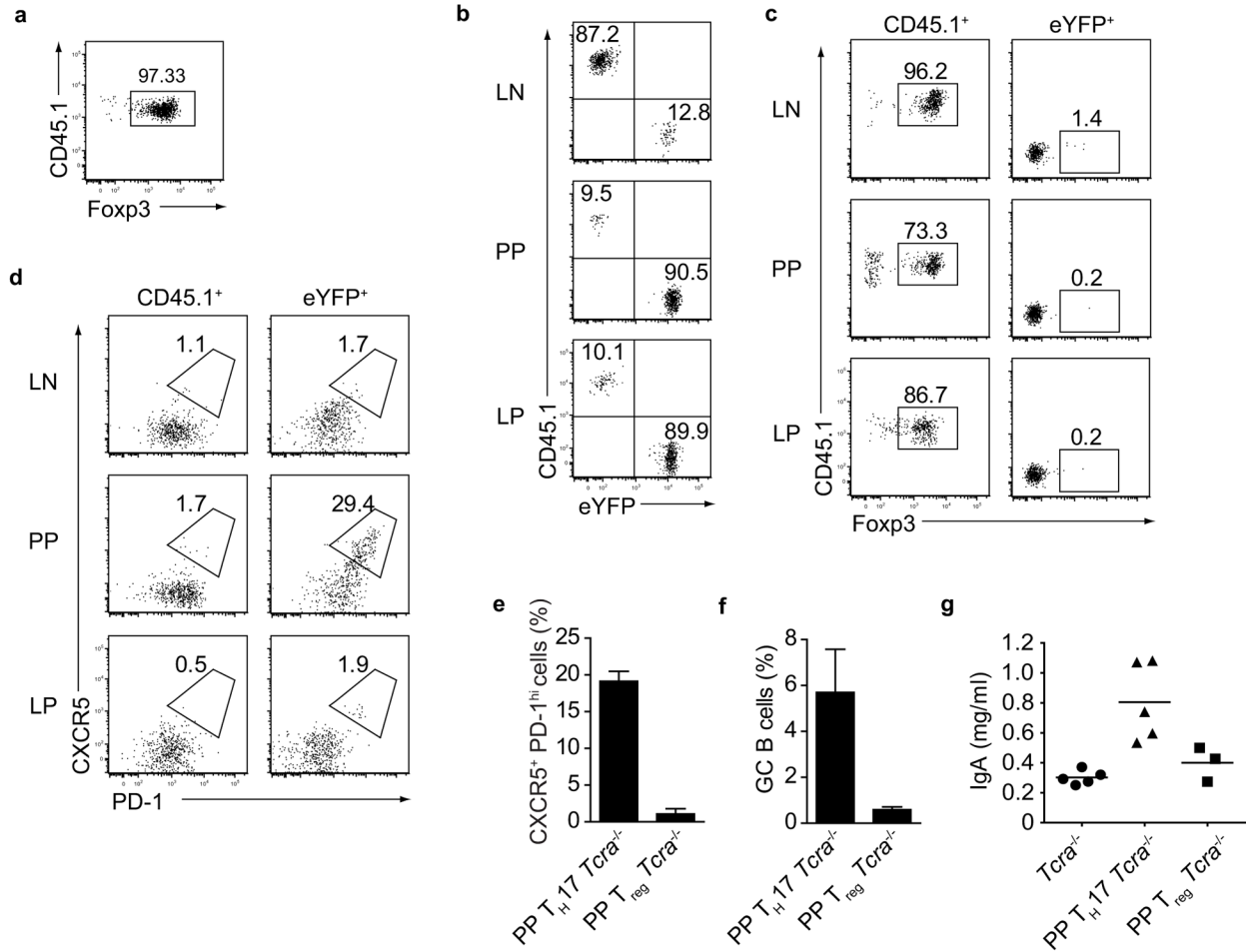
Figure 5. Co-transfer of eYFP$^+$ T$_H$17 with CD25^{high} (Foxp3$^+$) T$_{reg}$ cells
a) Flow cytometry of FACS sorted CD45.1$^+$ CD4$^+$ CD25^{high} T cells showing Foxp3 staining **b)** Flow cytometry of transferred CD45.1$^+$ and eYFP$^+$ cells in LN, PP, and LP of *Tcra$^{-/-}$* mice three months after transfer. **c)** Flow cytometry of transferred CD45.1$^+$ (left panel) and eYFP$^+$ (right panel) cells in LN, PP, and LP of *Tcra$^{-/-}$* mice three months after transfer showing Foxp3 staining. **d)** Flow cytometry of transferred CD45.1 (left panel) and eYFP$^+$ (right panel) cells in LN, PP, and LP three months after transfer showing CXCR5 and PD-1 expression. Data in **a**, **b**, **c** and **d** are representative of three independent experiments. **e)** Proportion of CXCR5$^+$ PD-1^{high} cells and **f)** proportion of B220$^+$ GL-7$^+$ CD95$^+$ (GC) B cells in PP of *Tcra$^{-/-}$* mice three months after transfer with eYFP$^+$ T$_H$17 (PP T$_H$17 *Tcra$^{-/-}$*) or CD45.1$^+$ CD4$^+$ RFP^{high} T cells (PP T$_{reg}$ *Tcra$^{-/-}$*). Mean values +/− SD for three individual mice are shown. **g)** Serum IgA levels from *Tcra$^{-/-}$*, PP T$_H$17 *Tcra$^{-/-}$* and PP T$_{reg}$ *Tcra$^{-/-}$* mice 3 months after transfer.
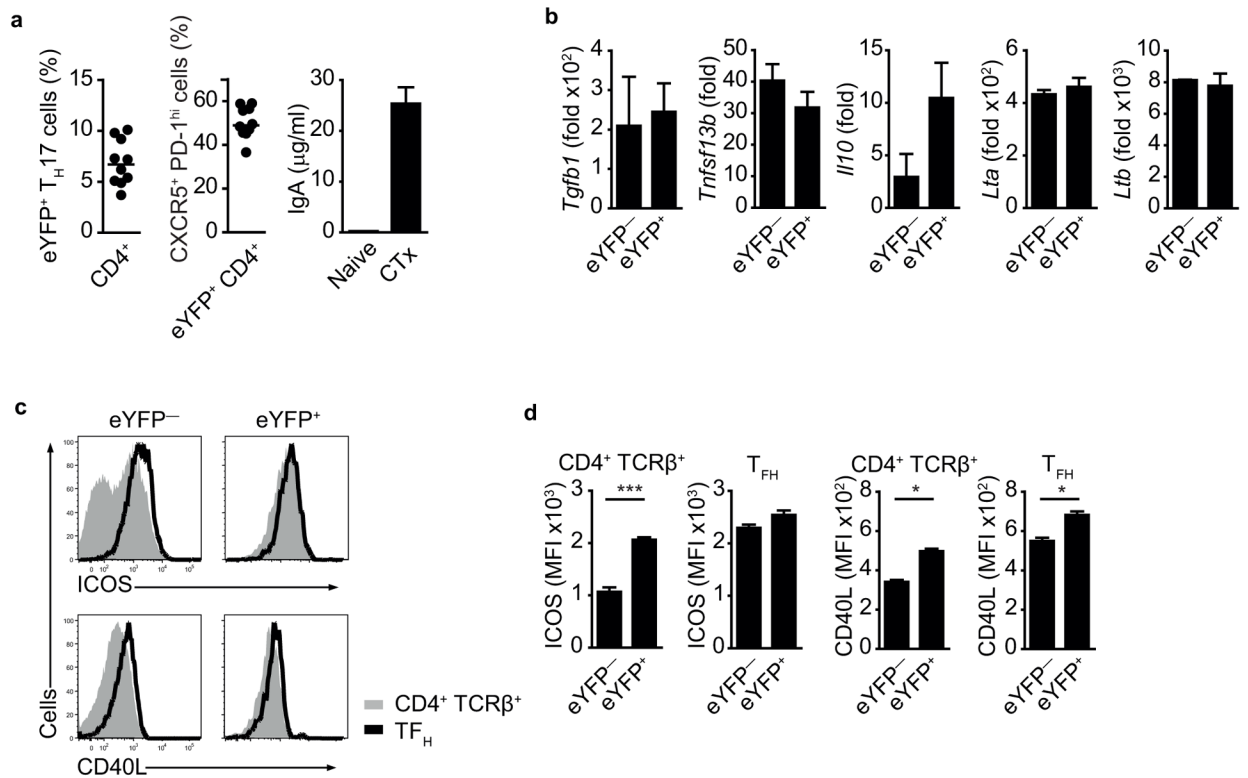
Fig.6. Cholera toxin specific IgA response in *Il17a^Cre R26R^eYFP* mice
a) Proportion of eYFP$^+$ cells in PP CD4$^+$ T cells (left panel), proportion of CXCR5$^+$
PD-1^{high} cells in eYFP$^+$ CD4$^+$ T cells (middle panel) and ELISA quantification of cholera
toxin-specific IgA (right panel) in cholera toxin-immunized *Il17a^CreR26R^eYFP* mice 7-10
days after challenge. **b)** Quantitative PCR analysis for expression of indicated mRNA in
FACS purified eYFP$^+$ and eYFP$^-$ CD4$^+$ T cells from PP of cholera toxin-immunized
Il17a^CreR26R^eYFP mice 10 days after challenge. mRNA expression is relative to *Hprt*. Data
in **a** and **b** are representative of three independent experiments. **c)** Flow cytometry of eYFP$^+$
or eYFP$^-$ CD4$^+$ TCRβ$^+$ T (shaded line) and T$_{FH}$ (solid line) cells from PP of cholera toxin-
immunized *Il17a^CreR26R^eYFP* mice 10 days after challenge. **d)** MFI values of cell types as
in c). Mean values +/− SEM are shown for three individual mice. *, *p*-value < 0.05; **, *p*-
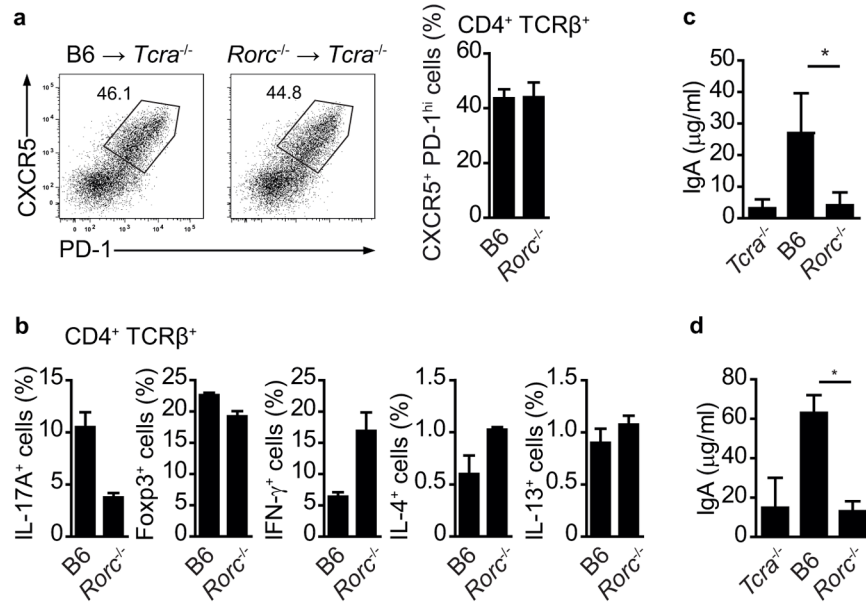value < 0.005.

Figure 7. Cholera toxin specific IgA response requires T_H17 cells

a) Flow cytometry of PP CD4$^+$ T cells from $Tcra^{-/-}$ mice three months after reconstitution with C57Bl/6 (B6) or $Rorc$-deficient ($Rorc^{-/-}$) bone marrow 10 days after cholera toxin challenge, showing expression of CXCR5 and PD-1. **b)** Proportion of PP CD4$^+$ T cells expressing Foxp3 or producing indicated cytokines in $Tcra^{-/-}$ mice three months after reconstitution with C57Bl/6 or $Rorc$-deficient bone marrow 10 days after cholera toxin challenge. **c, d)** ELISA quantification of serum (c) and feces (d) cholera toxin-specific IgA in $Tcra^{-/-}$ and $Tcra^{-/-}$ mice three months after reconstitution with C57Bl/6 or $Rorc$-deficient bone marrow 10 days after cholera toxin challenge. Mean values +/− SEM for four individual mice are shown. *, p-value 0.01.