Th17 Cells Upregulate Polymeric Ig Receptor and Intestinal IgA and Contribute to Intestinal Homeostasis

Anthony T. Cao, Suxia Yao, Bin Gong, Charles O. Elson and Yingzi Cong

*J Immunol* published online 19 September 2012
http://www.jimmunol.org/content/early/2012/09/19/jimmunol.1200955

Why *The JI*?

- **Rapid Reviews! 30 days*** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Speedy Publication!** 4 weeks from acceptance to publication

*average

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Th17 Cells Upregulate Polymeric Ig Receptor and Intestinal IgA and Contribute to Intestinal Homeostasis

Anthony T. Cao,* Suxia Yao,* Bin Gong,† Charles O. Elson,‡ and Yingzi Cong*,†

Although CD4+ Th17 cells are enriched in normal intestines, their role in regulation of the host response to microbiota, and whether and how they contribute to intestinal homeostasis, is still largely unknown. It is also unclear whether Th17 cells regulate intestinal IgA production, which is also abundant in the intestinal lumen and has a crucial role as the first defense line in host response to microbiota. In this study, we found that intestinal polymeric Ig receptor (pIgR) and IgA production was impaired in T cell-deficient TCR-βx6−/− mice. Repletion of TCR-βx6−/− mice with Th17 cells from CBir1 flagellin TCR transgenic mice, which are specific for a commensal Ag, increased intestinal pIgR and IgA. The levels of intestinal pIgR and IgA in B6.IL-17R (IL-17R−/−) mice were lower than wild type mice. Treatment of colonic epithelial HT-29 cells with IL-17 increased pIgR expression. IL-17R−/− mice demonstrated systemic antimicrobial Ab response. Consistently, administering dextran sulfate sodium (DSS) to C57BL/6 mice after treatment with IL-17–neutralizing Ab resulted in more severe intestinal inflammation compared with control Ab. Administering DSS to IL-17R−/− mice resulted in increased weight loss and more severe intestinal inflammation compared with wild type mice, indicating a protective role of Th17 cells in intestinal inflammation. Individual mice with lower levels of pIgR and intestinal-secreted IgA correlated with increased weight loss at the end of DSS administration. Collectively, our data reveal that microbiota-specific Th17 cells contribute to intestinal homeostasis by regulating intestinal pIgR expression and IgA secretion. The Journal of Immunology, 2012, 189: 000–000.

T helper 17 cells, a subset of CD4+ T cells that primarily secrete IL-17A (also referred to as IL-17), IL-17F, IL-21, and IL-22, have been shown to be present in the intestinal lamina propria (LP), where they encounter a large number and diverse array of microbiota, commensal fungi, and food Ags (1). Although accumulating evidence demonstrates that Th17 cells play a pathogenic role in a variety of inflammatory conditions (2), there is considerable controversy as to whether they also contribute to the maintenance of intestinal immune homeostasis. Both protective and pathogenic functions of the Th17 cytokine IL-17 have been reported in patients with inflammatory bowel diseases (IBD) and in experimental colitis. IBD patients often have increased levels of IL-17 in inflamed tissues (3, 4). Specific inhibition of IL-17–producing Th17 cells by anti–IL-23p19 mAb prevents, as well as treats, colitis in an adoptive T cell transfer model, further confirming a role for the IL-23/Th17 pathway in the pathogenesis of colitis (5). Furthermore, IL-17 deficiency results in resistance to TNBS-induced colitis (4). However, IL-17 or IL-17F deficiency does not prevent colitis mediated by transfer of naive CD4+ T cells. Adoptive transfer of IL-17−/−CD45RBhi T cells, compared with wild type counterparts, induced a more severe wasting disease when transferred into RAG−/− mice, indicating a protective role of IL-17 (6). Dextran sulfate sodium (DSS)-induced colitis has also provided conflicting reports of IL-17 involvement in intestinal inflammation (7, 8). However, whether and how Th17 cells protect against chronic intestinal inflammation is still not understood.

IgA is enriched in mucosal secretions of the intestine (9). Both T cell-dependent and T cell-independent mechanisms regulate intestinal IgA production (10). IgA functions in the neutralization and clearance of extracellular pathogens by preventing adherence and access to epithelial surfaces (9). Notably, germ-free mice that lack microbiota exhibit very low levels of intestinal IgA. Colonization with commensal microbiota restores IgA production. In particular, colonization with segmented filamentous bacteria (SFB) selectively increases IgA production and secretion (11, 12). It has been separately reported that colonization of germ-free mice with SFB also selectively increases levels of Th17 cells in the intestines (13, 14). The observations that SFB can induce both Th17 cells and IgA indicate that there could be a link between Th17 cells and IgA production and secretion. Produced by plasma cells in the mucosa, IgA secretion relies on transport across the intestinal epithelium, which is mediated by the polymeric Ig receptor (pIgR) expressed on the basolateral surface of epithelial cells (15). After translocation, a portion of the pIgR is covalently linked to IgA and secreted in the form of secretory IgA (sIgA), thereby improving stability of the complex (16). Expression of the pIgR is vital to IgA-mediated innate protection (17). The rate of IgA secretion is limited by the rate in which IgA binds to the pIgR, and is therefore ultimately dictated by the expression levels of the pIgR (15). Reductions in pIgR expression lead to decreased IgA-mediated protection against luminal Ags (17). Previous studies inflicting epithelial injury and colitis revealed that secretory Abs significantly contributed to protection of the intestinal mucosa and that mice deficient in the pIgR displayed greater...
Th17 REGULATION OF INTESTINAL IgA

disease than did wild type mice (18). A recent study also demonstrated that Th17 cells increase plgR expression in the bronchial epithelium in response to inhaled Ag (19). However, it is unknown whether and how Th17 cells regulate intestinal IgA and plgR expression and whether the Th17-IgA axis contributes to intestinal homeostasis. In this report, we demonstrate that Th17 cells contribute to the maintenance of host immune homeostasis against microbiota at least partially via IL-17 induction of epithelial plgR expression, thereby increasing IgA secretion into the lumen. In the context of intestinal inflammation, mice that lack IL-17 signaling displayed more severe inflammation than their counterparts, correlating with decreased plgR expression and subsequent IgA secretion.

Materials and Methods

**Mice**
C57BL/6 and TCR-βΔ5/− mice were obtained from the Jackson Laboratory. IL-17R−/− mice were provided by Amgen. CBir1 flagellin-specific TCR transgenic (CBir1-Tg) mice were maintained in the Animal Facilities at University of Texas Medical Branch. Eight- to 12-wk-old mice were used for all experiments. All experiments were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Texas Medical Branch. All the mice strains were bred in the University of Texas Medical Branch animal facility and housed together from 3 wk of age. All mice contain SPF as verified via PCR.

**Abs and reagents**
Abs against IL-17A, CD45.2, and avidin were purchased from BioLegend. Neutralizing Ab to IL-17A was provided by Merck. Mouse recombinant IL-6, IL-12, and human recombinant IL-17A, TNF-α, TGF-β1 were purchased from R&D Systems. Abs against IgA were purchased from Kirkegaard and Perry Labs. Abs against plgR and Actin were purchased from Santa Cruz Laboratories. Abs against phosphorylated NF-κB–p65 were purchased from Cell Signaling. NF-κB inhibitor Bayl11-7082, P38 inhibitor LY294002, and all-trans retinoic acid were purchased from Sigma-Aldrich.

**Polarization of Th17 and Th1 cells**
CD4+ T cells were isolated from spleens of CBir1 Tg mice using anti-mouse CD4-magnetic beads (BD Biosciences) as described previously (20). To polarize Th17 cells, CBir1-Tg CD4+ T cells were cultured with 10 ng/ml TGF-β1, 20 ng/ml IL-6, 10 µg/ml anti-IFN-γ, and 10 µg/ml anti-IL-4 (21) with irradiated splenic APCs. After 5 d, cells were stimulated with PMA (50 ng/ml) and ionomycin (750 ng/ml) and were isolated with a capture complex of avidin, biotinylated-CD45.2, and biotinylated–IL-17A. Abs were counterstained with fluorescence-labeled Abs for IL-17A, CD4, and CD45.2, and sorted by flow cytometry with >97% purity. To polarize Th1 cells, CBir1-Tg CD4+ T cells were cultured with 10 ng/ml IL-12 and 10 µg/ml anti-IL-4.

**Fecal pellet preparation**
Fecal pellets were homogenized in PBS containing 0.04 mg/ml soybean trypsin inhibitor, 20 mM EDTA, and 2 mM PMSF and centrifuged to remove bacteria and insoluble debris as described previously (22). Commensal bacterial lysate was prepared by homogenizing cecal contents and centrifuging to remove insoluble debris as described previously (22).

**ELISA**
Ninety-six-well plates (Nunc) were coated with 1 µg/ml anti-IgA (Kirkegaard and Perry Labs) or 0.5 µg/ml anti-plgR (R&D Systems) or 1 µg/ml of commensal bacterial lysate overnight at 4°C. The plates were washed in PBS/Tween and blocked in PBS with 1% BSA. Fecal samples were diluted 1/100, and a 2-fold serial dilution was made. Samples were incubated at room temperature for 2 h: 0.25 µg/ml of biotinylated anti-IgA (KPL) was added for 1 h, followed by HRP-conjugated streptavidin (KPL) for 1 h. Plates were developed using a two-component TMB substrate (KPL) according to the manufacturer’s instructions, and the plate was analyzed at 450 nm. Results were quantified by normalizing to standard concentrations of IgA (Southern Biotechnology Associates).

**Quantitative real-time PCR**
RNA was extracted with TRIzol (Invitrogen) and followed by cDNA synthesis with Revertaid reverse transcriptase (Fermentas). Quantitative PCR was performed using TaqMan Gene Expression Assays. Predesigned primers and probes for PIGR and GAPDH were ordered from Applied Biosystems, and data were normalized to GAPDH mRNA expression.

**Dextran sulfate sodium induction of colitis**
As described previously (23), DSS (MP Biomedicals) was dissolved into drinking water and administered to mice ad libitum. For acute colitis, 2.5% w/v DSS was administered over 7 d, followed by 3 d of fresh water. For chronic colitis, 1.75% DSS was administered for 7 d, followed by 3 d of fresh water and repeated over 60 d.

**Histopathologic assessment**
At necropsy, the small intestine, cecum, and colon were separated and Swiss rolls of each were prepared. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections (5 µm) were sliced, stained with H&E, and blindly scored by an experienced pathologist. Histologic scoring was performed using a modification of a scoring system reported previously (24). In brief, longitudinal sections were examined for crypt epithelial hyperplasia, degeneration, and loss; goblet cell loss; crypt exudate; LP and submucosal inflammatory cell accumulation; submucosal edema; mucosal ulceration; and transmural inflammation. Each lesion component was scored as 1, 2, or 3 for mild, moderate, or severe, respectively (intensity), and 0 for absent, or 1, 2, 3, or 4 for 25%, 50%, 75%, or 100% of the tissue affected, respectively (extent). The total lesion severity score was calculated by summation of the products of extent and intensity scores for each individual lesion component.

**TGF-β bioassay**
As described previously (25), MFB-F11 cells are embryonic fibroblasts from Tgfb1−/− mice that are stably transfected with a reporter plasmid consisting of TGF-β-responsive Smad-binding elements coupled to a secreted alkaline phosphatase reporter gene. Secreted alkaline phosphatase activity shown as chemiluminescence units was measured using Great EscApe SEAP Chemiluminescence kit 2.0 (Clontech), following the manufacturer’s instructions and represents biologically active TGF-β activity.

**Bacterial enumeration**
Mesenteric lymph nodes were isolated and homogenized in 500 µL PBS; 10 µL was spotted onto blood agar plates (BD Biosciences) in serial dilution and incubated at 37°C under aerobic and anaerobic conditions. Anaerobic cultures were placed in a sealed jar with a lit candle to induce a microaerophilic environment.

**Statistical analysis**
For comparisons between samples, levels of significance were determined with Student t test in Prism 5.0 (GraphPad). Where appropriate, mean ± SEM is represented on graphs.

**Results**

**Low levels of intestinal IgA and plgR in IL-17R-deficient mice**
Analysis of fecal content in mice deficient in IL-17R (IL-17R−/−) revealed that the level of IgA was significantly decreased in the absence of IL-17 signaling compared with wild type mice (Fig. 1A). It has been shown that the plgR mediates the translocation of IgA into intestinal lumen, and a portion of the plgR is secreted with IgA to improve stability (16). Further analysis of fecal content revealed that the level of the plgR was also significantly reduced to a similar level as IgA in IL-17R−/− mice (Fig. 1B), indicating that the deficiency in intestinal IgA is partially due to a decrease in secretion. Pigr mRNA was also decreased in both the small intestines and large intestines of IL-17R−/− mice (Fig. 1C), indicating that the reduction in fecal plgR levels was not from variable levels of protein degradation. Although TLR signaling on epithelial cells can regulate plgR expression (26, 27), the large intestines contain significantly greater numbers of microflora than the small intestines. These data indicate that IL-17 signaling regulates plgR expression independent of microbiota.
Th17 cells to induce plgR from the bronchial epithelium (19). T regulatory cells (Tregs) have been shown to promote intestinal IgA production through production of TGF-β (20). Because Th17 cells are not stable and are able to convert into Tregs (29, 30), we measured TGF-β production in the intestines of TCR-βδ−/− mice that received CBir1 Th17 cells or PBS to determine whether TGF-β was involved in Th17 cell promotion of intestinal IgA. The intestines from both groups of mice produced TGF-β at a comparable level (Fig. 2E). Neutralization of IL-17A significantly decreased the amount of IgA present in the fecal content (Fig. 2F). Adoptive transfer of CBir1 Th1 cells slightly increased total IgA and Ag-specific IgA, but not to the extent seen in the transfer of CBir1 Th17 cells. Furthermore, neutralization of IL-17A decreased fecal IgA levels comparable to the transfer of CBir1 Th1 cells, signifying that the increases in intestinal IgA as a result of Th17 cell transfer is not solely due to the presence of T cell-mediated help, but that IL-17A contributes to IgA secretion as well. Collectively, our data indicate that Th17 cells increases plgR expression and IgA secretion in vivo.

**FIGURE 1.** Intestinal IgA secretion and plgR expression is decreased in IL-17R−/− mice. (A and B) Fecal pellets were collected from age-matched 8-wk-old wild type or IL-17R−/− mice that were cohoused from 3 wk old. IgA (A) and plgR (B) levels were quantified through ELISA and normalized to total protein. *p < 0.05. (C) Pigr mRNA was analyzed from intestinal tissue from wild type or IL-17R−/− mice by RT-PCR. Pigr expression values were normalized to Gapdh expression. Significant differences are compared between respective tissues. *p < 0.05 compared with wild type mice. LB, Large bowel; SB, small bowel.

**Transfer of Th17 cells results in increased plgR and IgA in TCR-βδ−/− mice**

Although both T cell-dependent and -independent pathways are involved in regulation of IgA production, CD4+ T cells have a significant role in the induction of the plgR and secretion of IgA into the intestine, because TCR-βδ−/− mice have significantly lower amounts of fecal IgA (20) (Fig. 2A) as well as plgR (Fig. 2B). Because IL-17 is predominantly produced by Th17 cells that are enriched in the intestine, we asked whether the presence of Th17 cells could influence plgR expression and intestinal IgA secretion. We generated Th17 cells by polarizing CD4+ T cells from CBir1 Tg mice, which are specific for an immunodominant microbiota Ag (20, 28), under standard Th17 conditions with TGF-β and IL-6, and transferred them into TCR-βδ−/− mice. Th17 cells were also generated from OTII transgenic mice, which are specific for the model Ag OVA that is not present in intestinal lumen, and transferred into TCR-βδ−/− mice to serve as a control for Ag-specific stimulation in the intestines. The mice were sacrificed, and Pigr mRNA expression was measured in intestinal tissue 30 d later. Intestines displayed significant increases in Pigr mRNA after transfer of CBir1 Th17 cells, as compared with native TCR-βδ−/− mice receiving only PBS or OVA-specific OTII Th17 cells (Fig. 2C). Increases in fecal IgA and plgR were apparent after ~1 wk and continued to increase for the duration of the experiment (Fig. 2D). This finding is consistent with a recent report revealing that specific Ag-stimulation was required for T cell-dependent and -independent pathways.

**IL-17 directly induces plgR expression from epithelial cells through NF-κB and PI3K**

To further elucidate the role of IL-17 on the induction of plgR, we asked if IL-17 signaled directly upon intestinal epithelial cells to produce plgR, or whether there was another intermediate. Treatment of HT-29 human colon epithelial cells with human IL-17A appeared to last beyond 24 h, as indicated by NF-κB (2, 33) and PI3 kinase activation (33). This increase in plgR expression was greater than expected from the two cytokines alone and suggests a strong synergism between IL-17A and TNF-α. The effect of IL-17A and synergism of IL-17A and TNF-α appeared to last beyond 24 h, as Pigr mRNA steadily increased, whereas the effect of TNF-α began to decline at 24 h (Fig. 4A). Previous reports have detailed that IL-17 can stimulate a number of cytokines and antimicrobial peptides, and that this upregulation occurs through NF-κB (2, 33) and PI3 kinase activation (33). In order to ascertain the mechanisms of IL-17A–mediated Pigr mRNA induction, we examined the effect of IL-17A and the synergism of IL-17A and TNF-α on NF-κB activation. IL-17A was able to rapidly induce phosphorylation of p65, which is indicative of activated NF-κB signaling (Fig. 4C, 4D).
Next we questioned whether IL-17–induced pIgR was mediated through the NF-κB and PI3K pathways. We included inhibitors specific for NF-κB (Bay11-7082, 10 μM) and PI3K (LY294002, 10 μM) pathways to HT-29 cells cultured with IL-17A, TNF-α and PIGR mRNA was measured 4 h later. Blocking NF-κB activity greatly reduced levels of PIGR mRNA induced by IL-17A, TNF-α, or the combination of both IL-17A and TNF-α (Fig. 4E). However, inhibition of either pathway alone does not result in significant abrogation of PIGR transcription, which could be due to the short treatment time because it has been demonstrated that PIGR mRNA response to TNF-α stimulation in HT-29 cells peaks at 24 h (34, 35). Blocking both pathways at once resulted in significant downregulation of PIGR mRNA under all treatments, but did not completely shut down PIGR transcription, thereby signifying that although NF-κB and PI3K signaling may be identified as the major pathways involved, they do not appear to be the only pathways activated.

More severe colitis in IL-17R−/− mice with chronic DSS

Previous reports have presented conflicting results on the role of IL-17 in IBD. Some reports have suggested a pathogenic role for IL-17 in the development of colitis (4, 8), whereas other work details that IL-17 may alleviate disease (7). Next, we wanted to assess whether there was a functional deficiency in epithelial protection in the absence of IL-17R signaling. We subjected IL-17R−/− mice to intestinal injury through DSS administration to determine whether the decrease in intestinal IgA played a significant role in protecting the epithelium. We decided on a suboptimal dose of DSS (2% w/v) for 7 d to induce severe colitis. In contrast to what has been demonstrated in IL-17−/− mice (16), IL-17R−/− mice displayed a more severe DSS-induced colitis compared with wild-type mice (Fig. 5).

More severe colitis was associated with lower levels of IL-17 that was able to translocate across the gut barrier (Fig. 5C). This was consistent with the marked decrease in the expression of IL-17R on Paneth cells and goblet cells in IL-17R−/− mice, indicating that epithelial cells do not express IL-17R in IL-17R−/− mice. We also observed that IL-17−/− mice had larger crypt areas and less Paneth cell expression of IL-17R (Fig. 5B). IL-17R−/− mice also displayed lower levels of IL-17A, LTB4, and TNF-α, consistent with previous reports (16). The decrease in Th17 cell activity was also associated with lower numbers of Foxp3+ Tregs in the colon of IL-17R−/− mice (16). We also found that IL-17−/− mice displayed lower levels of IL-17R expression on Paneth cells and goblet cells compared with wild-type mice (Fig. 5B). These findings were consistent with previous reports (16). The decrease in IL-17R expression on Paneth cells and goblet cells was also associated with lower levels of IL-17A, LTB4, and TNF-α (Fig. 5C). We also found that IL-17−/− mice had lower levels of IL-17R expression on Paneth cells and goblet cells compared with wild-type mice (Fig. 5B). These findings were consistent with previous reports (16). The decrease in IL-17R expression on Paneth cells and goblet cells was also associated with lower levels of IL-17A, LTB4, and TNF-α (Fig. 5C).
DSS that would not inflict significant injury in wild type mice, but still injure the IL-17R−/− mice. Fecal pellets were collected, and IgA and pIgR levels were quantified before colitis induction. Administration of 1.75% DSS induced colitis after 5 d in the IL-17R−/− and control mice, and continued over six cycles of 7 d of DSS administration, followed by 3 d of fresh water. Disease progression was characterized by weight loss and visual examination of loose or bloody stool every 48 h. As shown in Fig. 5A, the IL-17R−/− mice displayed more significant disease as witnessed by increased weight loss and loose, mucoid, and bloody stool. Weight loss and recovery in the control mice were responsive shortly after the switch from DSS to water. IL-17R−/− mice showed a delayed recovery in weight at the end of the first cycle and continued to display irregular responses to the treatment cycles. As a whole, IL-17R−/− mice showed a delayed recovery in weight at the end of the first cycle and continued to display irregular responses to the treatment cycles. As a whole, IL-17R−/− mice suffered from a more severe colitis than the control mice (Fig. 5B), detailing that IL-17 provides significant protection in chronic DSS colitis. Although the control mice recovered their weight after the initial cycle of DSS, the IL-17R−/− mice repeatedly lost more than 10% of their body weight with each cycle. Interestingly, mice that expressed the lowest levels of fecal IgA and pIgR under healthy conditions before DSS administration went on to exhibit a more severe disease and more severe weight loss than mice that expressed higher levels of IgA and the pIgR (Fig. 5C).

Blockade of IL-17 increases severity of acute colitis in response to DSS

To further address the nature of IL-17 in the context of IBD, we injected a neutralizing Ab to IL-17A into C57BL/6 mice, followed by DSS administration. As shown in Fig. 6A–C, mice that received neutralizing Ab to IL-17A demonstrated more severe colitis than did mice receiving a control Ab after 10 d, as measured by weight loss and histologic examination. The differences were seen in weight loss after 6 d of DSS administration—although it did not reach statistical significance (Fig. 6A)—and the histopathologic scores (Fig. 6B–C), thus confirming a protective role of IL-17 in DSS-induced intestinal inflammation.

Increased commensal bacterial stimulation in IL-17R-deficient mice

Our data indicate a role of IL-17 in maintenance of intestinal homeostasis. We then questioned whether the lack of IL-17 signaling would result in more commensal bacterial translocation with increased systemic response to commensal bacterial activities. There were more bacteria in the MLN of IL-17R−/− mice compared with that in wild type mice (Fig. 6D). Consistent with our previous observations (36), there was no serum IgG against commensal bacterial Ags in wild type mice, but significant serum IgG against the bacterial Ags was observed in wild type mice immunized i.v. with commensal A4 bacteria (37). In contrast, analysis of serum Ab titers revealed detectable levels of IgG specifically directed against commensal bacterial Ags in IL-17R−/− mice (Fig. 6E). This finding signifies an important role for IL-17 signaling in the prevention of bacterial translocation across the epithelium, thereby limiting the activation of inflammatory responses against innocuous commensal Ags, both in the intestinal tract as well as systemically (Fig. 7).
Discussion

Despite enormous bacterial challenge, the host intestine establishes a mutualistic relationship with the microbiota. Multiple mechanisms have evolved to regulate this relationship. The intestinal tract has been shown as a natural site for Th17 cell development, which is stimulated by specific species of microbiota (14), with SFB being recently identified as one such stimulator (13). Although both proinflammatory and anti-inflammatory functions of Th17 have been demonstrated in different experimental systems (4–8), the enrichment of Th17 cells in the intestine suggests a role for these cells in mucosal homeostasis and more specifically in the containment of the vast local microbiota. In consistency with this argument, our data demonstrated that Th17 cells are able to promote intestinal IgA secretion via induction of epithelial cell pIgR expression, thereby contributing to the maintenance of host immune homeostasis to microbiota.

One of the most important strategies to generate immune protection and maintain intestinal homeostasis is the production of IgA (9), which is the primary Ab in the gut. IgA regulates the microbiota, and bacteria in turn adapt to IgA by altering their gene expression patterns (38). Although IgA also plays a role in host resistance to infection, it has been argued that the major role of IgA in the intestine is in maintaining the balance between the host and its microbiota (39). In the absence of pathogen exposure, specific pathogen–free mice have abundant levels of IgA, whereas germ-free mice have very low levels of IgA (9). B cell IgA production can be stimulated by dendritic cell–B cell or epithelial cell–B cell interactions via BAFF, APRIL, inducible NO synthase, and TLR ligands, or utilizing T cell help and a number of cytokines including TGF-β, IL-4, IL-6, and IL-10 (10). Although the relative contribution of T cell-dependent and -independent regulation to intestinal IgA production is still not completely understood, decreased levels of intestinal IgA in T cell-deficient TCRβ/−/− mice compared with wild type mice indicates a predominant role of the T cell-dependent pathway (20, 39). However, it is still unclear which T cells provide help and which sources of cytokines are needed for intestinal IgA production in the mucosa.
Although TGF-β has been shown as a crucial cytokine in promoting IgA class switching (10), and Treg production of TGF-β greatly contributes to intestinal IgA production (20), it cannot completely explain why high levels of IgA are present only in the intestine, but not other lymphoid tissues even though TGF-β are also present in those sites. Our data indicated that repletion of Th17 cells promoted intestinal IgA secretion in the TCR-βδ−/− mice. Blockade of Th17 cytokine IL-17 decreased intestinal IgA (Fig. 2). In addition, IL-17R deficiency resulted in lower intestinal IgA secretion compared with wild type mice (Fig. 1), indicating that Th17 cells and their signature cytokine IL-17 greatly contribute to intestinal IgA secretion. Promotion of IgA secretion is not due to Tregs that were converted from Th17 cells, because the intestinal tissues produced TGF-β at a similar level. Several types of innate cells have been identified recently in the intestines that could also provide sources of IL-17 to promote intestinal IgA production (40–42). Indeed, a previous report showed that RORγt+ LTi cells but not RORγt+ CD4+ T cells induced T cell-independent LP IgA production in the absence of Peyer patches (43). In RORγt-deficient mice, transfer of RORγt+ LTi cells induced isolated lymphoid follicle (ILF) formation as well as LP IgA. However, transfer of RORγt+ CD4+ T cells did not induce ILF or PP formation, nor intestinal IgA, indicating that in the absence of PP and ILF, Th17 cells would not be activated and thus would not produce cytokines required for induction of intestinal IgA. Several recent studies demonstrated that commensal microbiota greatly affect intestinal Treg, Th17 cell, and IgA responses. SFB preferably induces intestinal Th17 cells (13) and IgA (12, 13), whereas colonization with Clostridium species and Schaedler flora, which contain eight known commensal bacteria including Clostridium, induces Tregs (44, 45). Interestingly, failure to activate Tregs results in the induction of Th17 cells; therefore, commensal bacteria regulate the balance between Tregs and Th17 cells. As Tregs have been shown to promote intestinal IgA response (20), and we now show that Th17 cells are also able to upregulate intestinal IgA, the microbiota greatly influence intestinal IgA responses at least partially through regulation of Tregs and Th17 cells.

IgA translocation across the intestinal epithelium is mediated by the plgR (9). IgA function in the intestinal lumen is dependent on plgR expression, and reduction in plgR expression has been shown to lead to decreased IgA-mediated protection against luminal Ags (15). Intestinal plgR expression was lower in TCR-βδ−/− mice compared with wild type mice, indicating a role for T cells in the induction of plgR (Fig. 2). Consistent with a previous report describing IL-17–mediated plgR expression in airway epithelial cells (19), repletion of Th17 cells restored intestinal plgR expression in TCR-βδ−/− mice, and IL-17R deficiency resulted in lower expression of intestinal plgR, demonstrating that Th17 and IL-17 signaling regulate intestinal epithelial plgR expression. Indeed, treatment with IL-17 greatly increased HT-29 epithelial cell expression of plgR, alone or synergistically with TNF-α. IL-17 was able to activate NF-κB p65 signaling in intestinal epithelial cells (Fig. 4). Blockade of NF-κB signaling and PI3 kinase activity with selective chemical inhibitors inhibited IL-17 induction of plgR. Interestingly, both pathways work independently in IL-17 signaling as the inhibition of either pathway did not result in strong abrogation of PlgR transcription; only blockade of both pathways resulted in significant downregulation of PlgR mRNA. Intestinal Th17 cells require cognate luminal Ag stimulation to produce effector cytokines. Once cytokines are produced by the activated T cells, they regulate intestinal IgA production in an Ag-nonspecific manner.

Both intestinal plgR and IgA have been implicated in maintenance of intestinal immune homeostasis, because deficiency of either plgR or IgA results in greater commensal bacterial translocation across the intestinal epithelium and more severe intestinal inflammation in response to DSS (17, 18, 36). Thus, Th17 cell regulation of intestinal plgR and IgA could have a crucial role in protection against intestinal inflammation induced by mucosal breach by commensal flora. Indeed, there was higher level of systemic anti-commensal bacterial IgG in IL-17R−/− mice but not in wild type mice (Fig. 6E), which is indicative of the presence of commensal bacteria in the systemic immune system. This finding revealed that deficiency of IL-17 signaling resulted in more commensal bacterial translocation from lumen, and sequentially, to more severe intestinal inflammation in response to DSS (Fig. 5). Consistent with these observations, we also found higher numbers of bacteria in the mesenteric lymph nodes of IL-17R−/− mice (Fig. 6D). This finding is likely due to impaired intestinal plgR expression and IgA secretion, although the induction of a number of cytokines and antimicrobial peptides from epithelial cells by IL-17 could also contribute to IL-17–mediated protection against intestinal inflammation. However, we cannot exclude the possibility that wild type and IL-17R−/− mice may have differences in the composition of their respective gut microbiota, which could have contributed to our results.

In summary, our data demonstrate that enriched microbiota Ag-specific Th17 cells protect the host from chronic inflammation and contribute to intestinal immune homeostasis by regulating epithelial plgR expression, thereby promoting intestinal IgA. However, it certainly does not mean that this is the only function of Th17 cells that contributes to intestinal immune homeostasis, because Th17 cells and IL-17 have been shown to stimulate a number of cytokines and antimicrobial peptides that also contribute to the regulation of host immune responses to microbiota (33). Tregs have been shown to greatly promote intestinal IgA production via directly promoting B cell IgA class switching through production of TGF-β. We now show that Th17 cells promote IgA translocation across the intestinal epithelium via induction of plgR by IL-17. Thus, Tregs and Th17 cells coordinately regulate intestinal IgA production and secretion (Fig. 7). A deficiency in either pathway...
will result in decreased intestinal IgA and disruption of intestinal immune homeostasis.

Disclosures

The authors have no financial interests of conflict.

References