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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-βΔ⁻/⁻ mice. Repletion of TCR-βΔ⁻/⁻ 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⁻/⁻ 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 antimicroflora 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.

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Abbreviations used in this article: DSS, dextran sulfate sodium; IBD, inflammatory bowel disease; ILF, isolated lymphoid follicle; LP, lamina propria; pIgR, polymeric immunoglobulin receptor; SFB, segmented filamentous bacteria; sIgA, secretory IgA; Treg, T regulatory cell.

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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-βδ6−/− 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 Biotechnology. Anti-α was purchased from Jackson ImmunoResearch Laboratories. Abs against phosphorylated NF-κB-p65 and Actin were purchased from Santa Cruz Biotechnology. Anti-μ was purchased from Jackson ImmunoResearch Laboratories. Abs against phosphorylated NF-κB-p65 and total NF-κB-p65 were purchased from Cell Signaling. NF-κB inhibitor Bay11-7082, PI3K 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 IL-12, and human recombinant IL-17A, TNF-α Abs. Cells were counterstained with fluorescence-labeled Abs for CD4, CD1A, 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 plgR 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 stained, 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 Tgflh−/− 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. Figr 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.

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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.

Th17 cells induced B cell IgA production in vitro

To determine whether Th17 cells directly induce B cell IgA production in vivo, splenic IgD+ B cells were cultured with in vitro polarized CBir1 Tg Th17, Th1, and unpolarized T cells (Th0). B cells were cultured with anti-μ, CD40L, TGF-β, and retinoic acid to serve as a positive control (31). B cells were also cultured with in vitro–polarized OTII Th17, Th1, and Th0 cells, without the presence of OVA. Total IgA in the supernatant was measured at day 5. As shown in Fig. 3, CBir1 Th17 cells greatly promoted IgA production, whereas CBir1 Th1 and Th0 cells only slightly enhanced IgA production. However, OTII T cells did not promote IgA production in the absence of their cognate Ag, indicating that the T cell activation and production of effector cytokines are required for Th17 cell–mediated induction of IgA. Th17 cells were more adept at promoting IgA secretion in an Ag-specific manner, both by directly inducing IgA production and by plgR expression.

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 resulted in an increase of PIGR mRNA, in a time- and dose-dependent manner, appearing as soon as 2 h after IL-17A treatment (Fig. 4A). This induction of PIGR mRNA also mirrors the induction by TNF-α, which is also produced by Th17 cells (32) and is known to be a potent stimulator of plgR (26). Most notably, the combination of human IL-17A and TNF-α resulted in strong induction of PIGR at all time points (Fig. 4A, 4B). 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, and 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-17 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 that would not inflict significant injury in wild type mice, but still injure the IL-17R−/− mice. Fecal pellets were collected, and IgA and plgR 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 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 plgR 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 plgR (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 TCR-β−/− 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.

FIGURE 5. IL-17R−/− mice suffer worsened colitis as a result of decreased pIgR and IgA secretion. (A) Age-matched wild type and IL-17R−/− mice, which had been cohoused from 3 wk old, were administered 1.75% DSS in drinking water. Weight was measured every 2 d. After 7 d of DSS, drinking water was replaced with fresh water for 3 d, and the cycle was repeated over 60 d. Weights are shown as a percentage of individual weight on day 0. Significant differences are compared between strains on DSS. *p < 0.05 compared with wild type mice; n = 4 mice per group. (B) Colonic histopathology of DSS-treated mice after 60 d of DSS administration. H&E, original magnification ×10. (C) IgA and pIgR in fecal pellets were quantified from mice by ELISA before DSS administration and plotted against their individual body weight after 54 d.

FIGURE 6. Blockade of IL-17 induces more severe colitis from DSS administration, and bacterial translocation is increased in IL-17R−/− mice. (A) C57BL/6 mice were injected i.p. with a neutralizing Ab to IL-17A, or isotype control, and administered DSS for 7 d. Weights are shown as a percentage of body weight on day 0. (B) Pathologic score of colitis was examined by blind histologic observation 10 d after DSS administration. **p < 0.01 compared with the mice treated with control mAb; n = 4 mice per group. (C) Mesenteric lymph nodes were harvested from wild type or IL-17R−/− mice under aseptic conditions. MLN homogenates were cultured onto blood agar plates and incubated in aerobic and anaerobic conditions at 37˚C. Aerobic cultures were incubated overnight; anaerobic cultures were incubated for 3 d. *p < 0.05 compared with wild type mice; n = 3 mice. (E) Serum IgG against commensal bacterial lysate were quantified from wild type or IL-17R−/− mice by ELISA. Wild type mice were injected i.v. with 200 μg A4 bacteria to indicate relative amount of serum IgG. *p < 0.05 compared with wild type mice; n = 4 mice per group.
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 lpIgA production (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 PIGR transcription; only blockade of both pathways resulted in significant downregulation of PIGR 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 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 conflicts of interest.

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