Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis

Wook-Jin Chae[a], Thomas F. Gibson[a], Daniel Zelterman[b], Liming Hao[c], Octavian Henegariu[a], and Alfred L. M. Bothwell[a,1]

Departments of [a]Immunobiology, [b]Epidemiology and Public Health, and [c]Pathology, Yale University School of Medicine, New Haven, CT 06520

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The intrinsic role of endogenous IL-17A in spontaneous intestinal tumorigenesis has not been addressed previously to our knowledge. Ablation of IL-17A significantly reduced tumor development in mice bearing a heterozygote mutation in the adenomatous polyposis coli (APC) gene ($Apc^{Min/+}$ mice). There was also a decrease in inflammatory cytokines and proinflammatory mediators, reduced infiltration of lymphocytes including T cells, and preservation of intestinal architecture and the presence of APC protein in intestinal epithelial cells. Interestingly, IL-17A ablation also corrected immunological abnormalities such as splenomegaly and thymic atrophy in $Apc^{Min/+}$ mice. CD4 T cells from $Apc^{Min/+}$ mice showed hyperproliferative potential in vitro and in vivo and increased levels of IL-17A and IL-10. The effector CD4 T cells from $Apc^{Min/+}$ mice were more resistant to regulatory T cell–mediated suppression. Finally, these CD4 T cells induced colitis in immunodeficient mice upon adoptive transfer, whereas the ablation of IL-17A in CD4 T cells in $Apc^{Min/+}$ mice completely abolished this pathogenic potential in vivo. Taken together, our results show that CD4 T cell–derived IL-17A promotes spontaneous intestinal tumorigenesis with altered functions of CD4 T cells in $Apc^{Min/+}$ mice.

inflammation | T cells | colon cancer

The intestinal tract is subject to constant stimulation of immune cells and intestinal epithelial cells either by noninfectious antigens or infection. Recent studies identified the role of IL-17A in chronic inflammation in intestinal epithelium (1). Th17 cells (IL-17A–secreting T cells) are known to be involved in many autoimmune diseases, such as inflammatory bowel disease (IBD), experimental autoimmune encephalomyelitis, and rheumatoid arthritis (2, 3). It has been known that the level of IL-17A expression in the gut increases spontaneously over time in the presence of ATP (4). IL-6 is a critical inducer of Th17 cell development via STAT3 and retinoid-related orphan receptor (ROR)–γt in conjunction with TGF-β (5, 6), whereas IL-23 is critical to terminally differentiate Th17 (IL-17–secreting) cells (3). Recent studies using a colitis model showed complex roles for IL-17A in the gut (7, 8), suggesting that IL-17A can be protective or proinflammatory in the intestinal environment.

The most extensively studied model of intestinal dysplasia in either humans or mice is familial adenomatous polyposis, which harbors a mutation in the gene encoding adenomatous polyposis coli (APC). Mutation in this gene is one of the most frequently detected mutations in intestinal dysplasia in human and animal models as well as other types of cancers (9). The $Apc^{Min/+}$ mouse strain was generated by ethyl nitrosourea mutagenesis, resulting in a truncated form of APC (850 amino acids of 2,483) (10). WT APC plays a central role in normal colon to negatively regulate Wnt signaling, facilitating the proteosome-mediated degradation of β-catenin (11). The intestinal dysplasia actively develops from approximately week 11, with the complete loss of heterozygosity (LOH) of the APC gene, subsequently facilitating nuclear localization of phosphorylated β-catenin in colonic epithelial cells (12–14). These initial findings on the role of APC as a tumor suppressor gene in the Wnt–β-catenin pathway contributed to the understanding of intestinal tumorigenesis in colonic epithelial cells, but the role of the immune system to detect and eliminate these aberrant oncogenic cells has been in question based on its failure to prevent polyp growth. It has been suggested that the transfer of $CD4^+CD45RB^{hi}$ cells can increase tumor numbers whereas the transfer of $CD4^+CD45RB^{low}$ cells can inhibit tumor growth in $Apc^{Min/+}$ mice, suggesting that CD4 T cells may be an important cell subset to control intestinal tumorigenesis (15, 16). In addition, $Apc^{Min/+}$ mice have been shown to undergo thymic atrophy and splenomegaly, suggesting abnormalities in the immune system of these mice (17).

To address the role of IL-17A in intestinal tumorigenesis, we ablated IL-17A in $Apc^{Min/+}$ mice. We observed a significant decrease in intestinal tumorigenesis and the recovery of thymic and splenic cellularity. We also showed that CD4 T cells from $Apc^{Min/+}$ mice are significantly altered to favor intestinal tumorigenesis and can induce colitis.

Results

IL-17A Ablation Significantly Decreases Intestinal Tumorigenesis. To address the intrinsic role of IL-17A in intestinal tumorigenesis, we bred $Apc^{Min/+}$ mice to IL-17A–deficient mice (IL-17A KO-$Apc^{Min/+}$). We observed a significant decrease in tumor numbers in both small (88.0%) and large intestine (80.3%; Fig. 1*A*). This marked decrease was evident both in the number of large tumors (>3.0 mm) and small tumors (<3.0 mm; Fig. 1*B*). The decrease in tumor numbers was consistently observed in each part of small intestine, whereas most tumors were found in the ileum in $Apc^{Min/+}$ mice (Fig. 1*C*). The weight loss that occurred in $Apc^{Min/+}$ mice is mostly a consequence of bowel obstruction, and the ablation of IL-17A largely restored mean body weight (Fig. S1). These results demonstrate that the ablation of IL-17A significantly decreased tumorigenesis in both the small and large intestinal tract.

Lymphocyte Infiltration and Loss of APC Were Decreased by IL-17A Ablation. As intestinal inflammation accompanies lymphocyte infiltration, we examined whether lymphocytes infiltrated the small intestine. Infiltration of lymphocytes was more evident in $Apc^{Min/+}$ mice compared with WT C57BL/6 mice and IL-17A KO- $Apc^{Min/+}$ mice in H&E-stained sections (Fig. 2*A*). Also, CD3 T cells in IL-17A KO- $Apc^{Min/+}$ mice were significantly reduced compared with $Apc^{Min/+}$ mice (Fig. 2*B*). This increased infiltration of T cells included both CD4 and CD8 T cell populations in the lamina propria (Fig. S2). Furthermore, we showed that IL-17A+ CD4 T cells were increased whereas there was only a marginal increase of IL-6R expression (Fig. S3). To investigate whether the ablation of IL-17A can affect the canonical Wnt–β-catenin pathway with the loss of APC gene, we assessed the accumulation of APC protein

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[1]To whom correspondence should be addressed. E-mail: alfred.bothwell@yale.edu.

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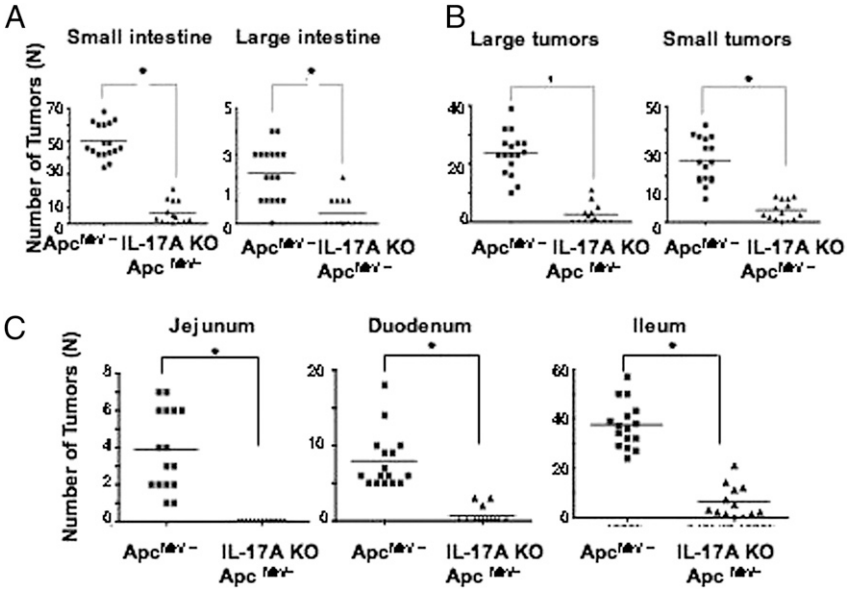

Fig. 1. IL-17A ablation significantly reduces intestinal tumorigenesis. (*A*) Tumor numbers of small and large intestine were counted in 20-week-old $Apc^{Min/+}$ mice ($n = 16$) and IL-17A KO- $Apc^{Min/+}$ mice ($n = 13$). (*B*) Tumors smaller than 3 mm and larger than 3 mm were counted separately. (*C*) Tumor numbers from each part of small intestine (duodenum, jejunum, and ileum). For both comparisons, $^*P < 0.002$ using two-tailed *t* tests.

in the small intestine of $Apc^{Min/+}$ and IL-17A KO- $Apc^{Min/+}$ mice. The level of APC protein was decreased in $Apc^{Min/+}$ compared with WT or IL-17A KO- $Apc^{Min/+}$ mice (Fig. 2*C*). Taken together, our results show that lymphocyte infiltration including CD4 and CD8 T cells does occur and this was inhibited by ablation of IL-17A in $Apc^{Min/+}$ mice.

Proinflammatory Cytokines and Mediators Are Significantly Decreased in the Tumors of IL-17A KO- $Apc^{Min/+}$ Mice. To investigate whether the ablation of IL-17A can down-regulate inflammation in the intestinal tract, we isolated tumors (3.0 mm in size) from small intestines of $Apc^{Min/+}$ and IL-17A KO- $Apc^{Min/+}$ mice and quantitated the mRNA expression of proinflammatory cytokines and mediators. There was a marked decrease in IL-6 and IL-23 (p19), as well as a decrease in IL-1β (Fig. 3), suggesting that the inflammatory microenvironment is significantly changed by ablating IL-17A. We also found a significant decrease in CD44 and proinflammatory mediators such as keratinocyte chemoattractant and Cox-2 (Fig. 3). This suggests that the deletion of IL-17A abrogated the initiation and stabilization of an IL-17A–mediated inflammatory environment.

IL-17A Ablation Corrected Immune Abnormalities. It has been demonstrated that $Apc^{Min/+}$ mice undergo thymic atrophy and splenomegaly with lymphodepletion as well as loss of Peyer's patches accompanying intestinal tumorigenesis (17, 18). It has also been reported that the rapid onset of tumors in the intestine coincides with the loss of thymus (15). Interestingly, thymic atrophy and splenomegaly were largely reversed in IL-17A KO- $Apc^{Min/+}$ mice (Fig. 4). We further observed that the loss of CD4 and CD8 T cells as well as B cells from spleen in $Apc^{Min/+}$ mice was also restored while maintaining the normal CD4/CD8 ratios by ablating IL-17A (Fig. 4*C*). However, the ratio of CD4 and CD8 thymocytes did not change in $Apc^{Min/+}$ mice and it remained unchanged in IL-17A KO- $Apc^{Min/+}$ mice (Fig. 4*C*). Of note, we did not observe significant lymphodepletion in mesenteric lymph nodes. Treatment with anti–IL-17A mAb substantially reduced

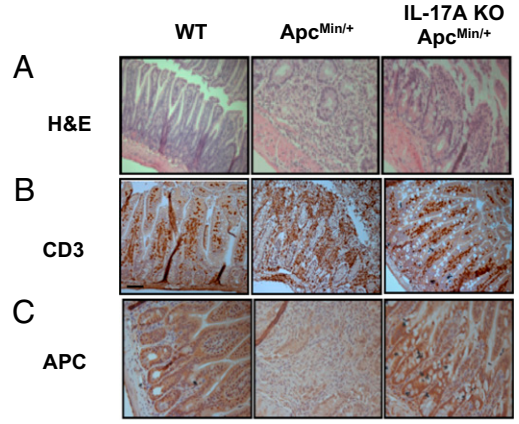

Fig. 2. Ablation of IL-17A preserved small intestinal architecture in $Apc^{Min/+}$ mice and reduces immune cell infiltration. Small intestines (ileum) of 20-week-old C57BL/6 ($n = 5$), $Apc^{Min/+}$ ($n = 4$), and IL-17A KO- $Apc^{Min/+}$ ($n = 4$) mice were harvested and stained with H&E (*A*) and murine anti-CD3 monoclonal antibody (*B*). Ileums of same group of mice were stained with anti-APC monoclonal antibody (*C*) with paraffin-embedded section.

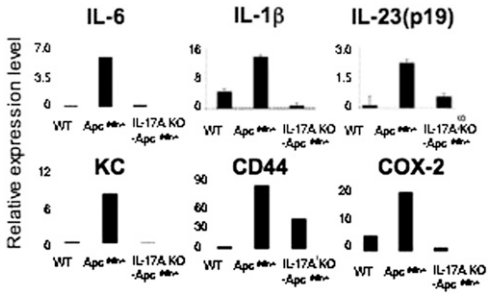

Fig. 3. IL-17A ablation reduced proinflammatory cytokines and mediators in tumors. Tumors (3 mm; one or two from each mouse) were dissected from 20-week-old $Apc^{Min/+}$ mice ($n = 5$) and IL-17A KO- $Apc^{Min/+}$ mice ($n = 4$). mRNA level was normalized against HPRT. For WT C57BL/6 mice, 5 mm of ileum was used. Values are means ± SD of all experiments.

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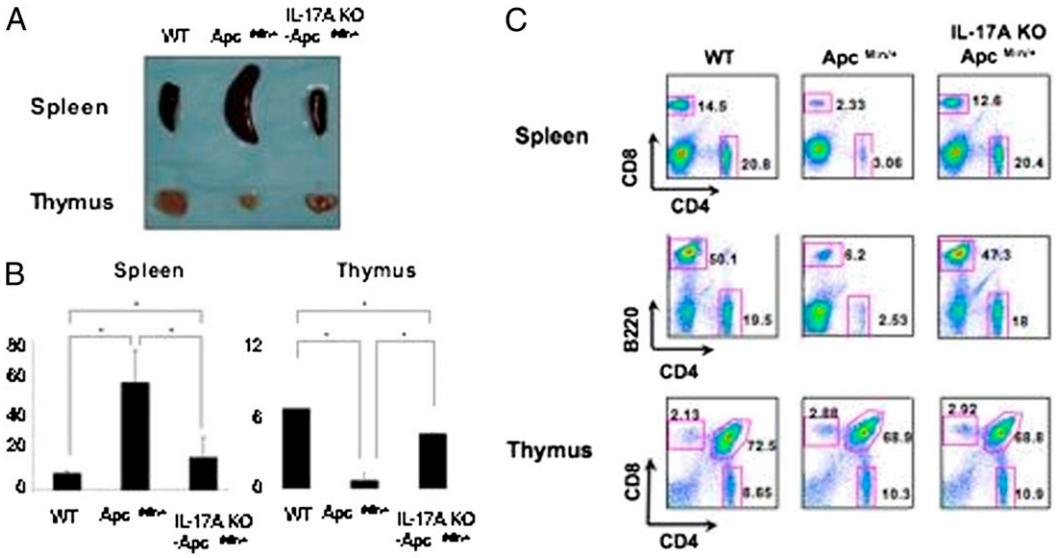

Fig. 4. Splenomegaly and thymic atrophy of $Apc^{Min/+}$ mice are corrected by IL-17A ablation. Spleens and thymuses from 20-week-old $Apc^{Min/+}$ and IL-17A KO $Apc^{Min/+}$ mice and littermate controls of $Apc^{Min/+}$ were dissected (*A*) and counted for cell numbers (*B*), and subsequently analyzed by flow cytometry (*C*). Flow cytometry is a representative of five mice per group. $*P \leq 0.002$; one-way analysis was used with Tukey correction at an α of 0.05.

the number of tumors whereas treatment with anti–IFN-γ had no significant effect (Fig. S4). Similar findings were made regarding reversal of thymic atrophy and splenomegaly (Fig. S4). Collectively, our data suggest that the thymus and spleen are the primary immune organs affected by $Apc^{Min/+}$ in the presence of endogenous IL-17A in addition to intestinal tumorigenesis.

CD4 T Cells from $Apc^{Min/+}$ Mice Have Extensively Altered Functions. It has been demonstrated that CD4 T cells are a primary source of IL-17A. Based on our phenotype in $Apc^{Min/+}$ mice and IL-17A KO $Apc^{Min/+}$ mice, we questioned whether CD4 T cells from $Apc^{Min/+}$ mice were significantly altered. First, we tested whether CD4 T cells from $Apc^{Min/+}$ mice are more proliferative based on its role as a tumor suppressor gene, thereby regulating cell cycle. We observed that CD4 T cells from $Apc^{Min/+}$ mice were hyperproliferative compared with their WT littermate controls upon T cell receptor stimulation (Fig. 5*A*). This hyperproliferation was more obvious following stronger stimulation through the T cell receptor. Interestingly, the activation status of naive T cells from $Apc^{Min/+}$ mice and IL-17A KO $Apc^{Min/+}$ mice did not show significant differences (Fig. S5). Next, we tested whether the effector CD4 T cells are more refractory to regulatory T cell (Treg)–mediated suppression. Upon coculture with WT Treg cells, $Apc^{Min/+}$ CD4 effector T cells were more resistant to Treg-mediated suppression, especially at a high ratio of effectors to Treg, suggesting that the suppression on $Apc^{Min/+}$ CD4 effector T cells are not as efficient as for WT CD4 effector T cells (Fig. 5*B*). We also tested Tregs from $Apc^{Min/+}$ and littermate controls using an in vitro suppressor assay, but no significant differences were observed (Fig. S6). However, this suppression was not maintained in vivo (Fig. S7) in an IBD model. We further tested whether IL-17A acts on $Apc^{Min/+}$ CD4 T cells in an autocrine manner by treatment with IL-17A, but it did not enhance proliferation (Fig. S8). Finally, we tested whether CD4 T cells are altered in their cytokine secretion. $Apc^{Min/+}$ CD4 T cells indeed secreted significantly more IL-17A and IL-10 than WT CD4 T cells, whereas they produced less IFN-γ upon stimulation (Fig. 5*C*). We also observed preferential expression of IL-17A and IL-10 in Peyer's patch CD4 T cells in $Apc^{Min/+}$ mice (Fig. S9).

Ablation of IL-17A $Apc^{Min/+}$ CD4 T Cells Corrects Their Aberrant Phenotype in Vivo. Based on our results in vitro, we questioned whether $Apc^{Min/+}$ CD4 T cells can be more proliferative and induce inflammation in the intestinal tract. To this end, we used adoptive transfer of CD4 T cells into Rag2-deficient mice. We measured CD4 and Foxp3 expression before transfer the transfer of CD4 T cells, and it showed no significant changes for each group of mice. First, we measured cell numbers in spleen of Rag2-deficient mice after 4 weeks of adoptive transfer (Fig. 6 *A* and *B*), and it showed that $Apc^{Min/+}$ CD4 T cells proliferated more, whereas IL-17A KO $Apc^{Min/+}$ CD4 T cell transfer did not show this hyperproliferation. This suggests that the ablation of IL-17A corrected the hyperproliferative potential of $Apc^{Min/+}$ CD4 T cells. Next, we observed that $Apc^{Min/+}$ CD4 T cells can cause more severe colitis than WT CD4 T cells whereas IL-17A $Apc^{Min/+}$ CD4 T cells did not induce colitis (Fig. 6 *C* and *D*).

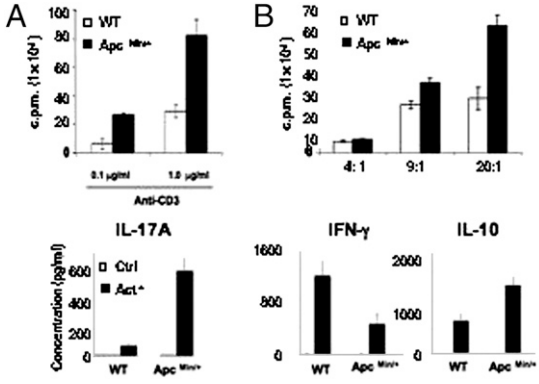

Fig. 5. CD4 T cells from $Apc^{Min/+}$ mice are functionally altered. (*A*) CD4 T cells from $Apc^{Min/+}$ mice were isolated and activated with given amount of anti-CD3 and T cell–depleted irradiated splenocytes for 72 h. (*B*) Foxp3+ regulatory T cells were isolated from $Apc^{Min/+}$ Foxp3-IRES-RFP mice and cocultured with Foxp3- CD4 effector T cells from either $Apc^{Min/+}$ mice or their littermate control for 72 h for given ratios. (*C*) CD4 T cells from $Apc^{Min/+}$ mice were isolated and activated for 96 h, and cytokines were measured by cytokine bead array. Data indicate means ± SD of three separate experiments as triplicates.

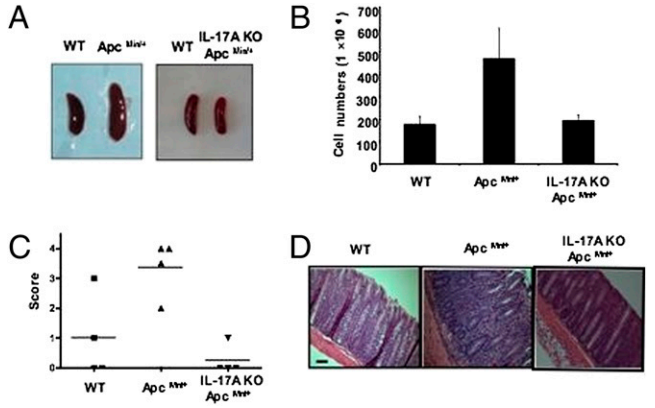

Fig. 6. Ablation of IL-17A in CD4 T cells corrects altered functions in CD4 T cells from $Apc^{Min/+}$ mice. (*A*) CD4 T cells from 11-week-old $Apc^{Min/+}$ and IL-17A KO $Apc^{Min/+}$ mice and littermate controls $Apc^{Min/+}$ mice were isolated and transferred into Rag 2 KO mice. After 4 weeks of transfer, spleens were harvested and cell numbers were counted, and the ratio between CD4 effector T cells and Foxp3+ CD4 T cells was measured by flow cytometry (*B*). Colons were harvested and scored for their chronicity (*C*) and stained with H&E (*D*). The experiments are the representative of two independent experiments.

This suggests that IL-17A ablation in $Apc^{Min/+}$ CD4 T cells largely restored their altered functions in vivo. We further showed that regulatory T cells from $Apc^{Min/+}$ mice could not prevent inflammatory bowel disease whereas those from IL-17A KO $Apc^{Min/+}$ mice could prevent colitis (Fig. S7). Collectively, our data thus suggest that the altered functions of CD4 T cells from $Apc^{Min/+}$ mice can be corrected by ablating IL-17A.

Discussion

Several studies demonstrated the role of IL-17A in autoimmune diseases as a proinflammatory cytokine. However, it has not been clearly addressed whether IL-17A does contribute clearly as a proinflammatory cytokine in spontaneous intestinal tumorigenesis. Controversial results regarding the role of IL-17A in different types of colitis models have been reported (7, 8). In addition, infection with enterotoxigenic bacteria caused intestinal tumorigenesis involving STAT3, perhaps through a Toll-like receptor (TLR)–mediated pathway by a specific bacterial pathogen (19, 20). Although these studies may imply that our intestinal tumorigenesis model is mainly contributed by a commensal TLR-mediated pathway, we cannot exclude the possibility there are other pathways that can contribute intestinal tumorigenesis significantly by Th17 cell generation. For instance, a previous report suggests that the complete ablation of TLR-mediated pathways in vivo by using MyD88-Trif DKO (i.e., double-KO) mice did not show any significant changes in the Th17 cell population in the gut. Our data suggest that the increase of $IL\text{-}17A^{+}CD4^{+}$ T cells drive intestinal tumorigenesis, but this may not be mediated by IL-6R. Indeed, IL-6–independent induction of Th17 cells has been reported (21). Furthermore, the use of antibiotics such as vancomycin and metronidazole to eliminate commensals reduced ATP level to generate Th17 cells in the gut, suggesting that the generation of Th17 cells and hence IL-17A secretion is not wholly dependent on bacterial pathogens through TLR-mediated pathways (4).

Our results clearly show that the role of IL-17A in spontaneous intestinal tumorigenesis is proinflammatory and very potent in a relatively early stage of tumor development. Many in vitro experiments to differentiate Th17 cells use the combination of TGF-β and IL-6 (22). Interestingly, the magnitude of inhibition by IL-17A ablation is much more potent than IL-6 ablation in $Apc^{Min/+}$ mice (30% reduction in polyp numbers) (23). This suggests that IL-17A plays a central role in controlling intestinal tumorigenesis and is not solely dependent on the IL-6 and TGF-β–mediated Th17 cell pathway. Indeed the ablation of IL-17A did decrease the level of IL-6 significantly, but other proinflammatory mediators such as keratinocyte chemoattractant and Cox-2 were also reduced. The normal intestinal architecture of the small intestine in IL-17A KO $Apc^{Min/+}$ mice suggested that IL-17A amplifies $Apc^{Min/+}$–mediated intestinal tumorigenesis, and it is likely that it prevents LOH significantly based on our immunohistochemistry of Apc. Although a more thorough examination is required, it has been extensively demonstrated that LOH is an indicator of adenoma formation in our model (24). It has been suggested that the mutation of KRas can significantly increase adenocarcinoma formation when combined with $Apc^{Min/+}$ genotype (25). As more than 50% of patients with colon cancer have these two mutations together, the ablation or elimination of IL-17A may abrogate this sequential pathway of adenocarcinoma development at an early point. Polyps were mainly found in the ileum of the small intestine, and we showed that Peyer's patch CD4 T cells showed more IL-17A expression in $Apc^{Min/+}$ mice.

Alongside with intestinal tumorigenesis, $Apc^{Min/+}$ mice have splenomegaly and thymic atrophy at later times. Interestingly, the ablation of IL-17A has corrected these immune abnormalities that contribute to lymphodepletion, especially the complete reversion of thymic atrophy. It has been suggested that the polyp growth rate is accelerated when thymic atrophy appears, indicating that T cell–mediated responses may be important. In line with this, it has been suggested that effector CD4 T cells or regulatory T cells have the potential to control polyp numbers (15, 16), but the phenotype of CD4 T cells from $Apc^{Min/+}$ mice has never been characterized to our knowledge. We showed the hyperproliferation of CD4 T cells in vitro and in vivo and showed that CD4 T cells from $Apc^{Min/+}$ mice are altered in their control of proliferation. Interestingly, the ratio of effector T cells and regulatory T cells before and after transfer did not show significant changes, whereas the total numbers of CD4 T cells were increased in CD4 T cells from $Apc^{Min/+}$ mice. This may be a result of the altered function of regulatory T cells or the resistance of CD4 effector T cells over regulatory T cell–mediated suppression. Indeed, we showed that the resistance of $Apc^{Min/+}$ effector CD4 T cells was increased as the effector to Treg ratio is increased, which is closer to physiological conditions in vivo. Also, the inability of regulatory T cells from $Apc^{Min/+}$ mice to block effector CD4 T cell–mediated IBD further suggests altered functions of regulatory T cells in vivo. The altered cytokine secretion from $Apc^{Min/+}$ CD4 T cells indicates that there are qualitative alterations. Our result using IL-17A KO $Apc^{Min/+}$ mouse CD4 T cells in vivo clearly shows that ablation of IL-17A in $Apc^{Min/+}$ CD4 T cells abrogated hyperproliferation and colitogenic potential in immunodeficient mice, consistent with our observation of the phenotypic changes in intestinal tumorigenesis.

Taken together, our results suggest an important proinflammatory role of IL-17A and its correction of immune abnormalities in spontaneous intestinal tumorigenesis. We demonstrate altered CD4 T cell functions from $Apc^{Min/+}$ mice, which favors intestinal tumorigenesis, and the ablation of IL-17A can abolish these altered function in Apc^{Min+} mice, suggesting the importance of T cell mediated IL-17A in spontaneous intestinal tumorigenesis.

Materials and Methods

Mice. $Apc^{Min/+}$ mice (expressing a mutant gene encoding an adenomatous polyposis coli protein truncated at amino acid 850) were established on a C57BL/6 background. In all experiments, WT littermates of $Apc^{Min/+}$ mice on C57BL/6 background were used. Rag2-deficient mice were housed in specific pathogen-free conditions. IL-17A–deficient (i.e., KO) mice were used under an agreement with Yoichiro Iwakura (Tokyo, Japan). For isolation of Foxp3+ CD4 T cells, $Apc^{Min/+}$ mice were bred to Foxp3-IRES-RFP (FIR) mice on a C57BL/

6 background (a gift from Richard Flavell, New Haven, CT). For all experiments, C57BL/6 mice were obtained as littermate controls of $Apc^{Min/+}$ mice. For histopathology of C57BL/6 (littermate control of $Apc^{Min/+}$ mice), $Apc^{Min/+}$, and IL-17A KO- $Apc^{Min/+}$ mice, ileum was dissected, fixed in 10% formalin, washed in PBS solution, paraffin-embedded, sectioned at 5 μm, and stained. All mouse protocols were approved by the Yale University Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International.

Colitis Model. Splenic CD4 T cells (3×10^5) from 12-week-old $Apc^{Min/+}$ mice and their littermate controls were isolated using a $CD4^+$ T cell isolation kit (Miltenyi Biotech). The purity was higher than 97%. CD4 T cells were transferred into 7-week-old Rag2-deficient mice. Four weeks after transfer, spleens were harvested and cell numbers were counted. Splenocytes were stained with anti-CD4 (clone RM4-5) and anti-Foxp3 (clone FJK-16s) antibodies followed by CD16/CD32 (clone CD16/CD32) antibody. Colons were processed (four mice per group) and paraffin-embedded, and then stained with H&E. Colonic inflammation was scored for chronicity on a scale from 0 to 4 [0, normal mucosa; 1, minimally increased chronic inflammation; 2, mildly increased inflammation; 3, moderately increased inflammation (thickening of intestinal wall); and 4, severely increased inflammation (architecture distortion)].

Flow Cytometry. Spleens and thymuses of 20-week-old $Apc^{Min/+}$ mice and their littermate controls, and IL-17A KO- $Apc^{Min/+}$ mice were harvested. After RBC lysis by RBC lysis buffer, cells were stained with CD16/CD32 antibody and then subsequently stained with anti-CD4 (clone RM4-5), anti-CD8α (clone 53–6.7), and anti-B220 (clone RA3-6B2) antibodies. For the stimulation of Peyer patch cells, PMA (100 ng/mL) and ionomycin (1μM) were used. Brefeldin A (1 μg/mL) was added for the last 4 h of culture. Cells were stained with anti-CD4 (clone RM4-5), anti–IL-17A (eBio17B7), anti–IL-10 (JES5-16E3) and isotype control antibodies. For lamina propria preparation, 13-week-old $Apc^{Min/+}$ mice and their littermate control mice were killed, and the individual small intestines were processed as described (3). After Percoll gradient separation, lymphocytes were counted and stained with anti-CD4 (clone RM4-5) and anti-CD8α (clone 53–6.7) antibodies. FACSCalibur (BD Biosciences) was used for flow cytometry and data were analyzed by FlowJo software (Treestar).

Real-Time PCR. Tumors (3 mm) from $Apc^{Min/+}$ and IL-17A KO- $Apc^{Min/+}$ mice were isolated and homogenized by Lysing Matrix D beads and homogenizer (MPBio). TRIzol (Invitrogen) was used to isolate mRNA and genomic DNA was further cleaned by genomic DNA removal kit (Qiagen). cDNA was synthesized with BDsprint cDNA synthesis kit (Clontech) and used with SYBR Green (Molecular Probes). Real-time PCR was performed with SYBR MX3000 bioanalyzer (Stratagene). Primers were synthesized by the Keck Biotechnology facility. We calculated relative gene expression by the ΔC_T method. The mRNA expression was normalized against HPRT. Primer sequences are provided upon request.

In Vitro T Cell Activation and Cytokine Analysis. For proliferation assays, 2×10^5 CD4 T cells from $Apc^{Min/+}$ mice and their littermate controls were activated with 0.1 μg/mL or 1.0 μg/mL of murine anti-CD3 monoclonal antibody in the presence of T cell depleted irradiated splenocytes for 72 h. T cells were depleted with Thy1.1 microbeads (Miltenyi Biotec). Thymidine [H^3] was added for the last 10 h of incubation. For cytokine analysis, 2×10^5 CD4 T cells were activated with plate bound anti-CD3 (clone 145.2C-11) and anti-CD28 (clone 37.51) for 96 h. Supernatants were harvested and analyzed by Luminex (Bio-Rad).

Statistical Analysis. Significance tests were performed in SAS 9.1 software using either the Pearson χ^2 statistic or one-way analysis of variance at significance levels of at least 0.05. Multiple comparisons were performed using a one-way analysis of variance in SAS with the Tukey correction for multiple comparisons keeping a type I experiment-wise error rate at 0.05. All comparisons were statistically significant at an α of 0.05 or more extreme.

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