

Regulation of T_H17 cells in the mucosal surfaces

Daniel Mucida, PhD,^a and Shahram Salek-Ardakani, PhD^b La Jolla, Calif

The mucosal surfaces represent the main intersection between jawed vertebrates and the environment. The mucosal surface of the intestine alone forms the largest surface that is exposed to exogenous antigens as well as the largest collection of lymphoid tissue in the body. Therefore, a protective immune activity must coexist with efficient regulatory mechanisms to maintain a health status of these organisms. The discovery of a new lineage of T_H cells that produce IL-17 has provided valuable new insight into host defense and the pathogenesis of inflammatory diseases at the mucosal surfaces. Of particular interest for these surfaces, it has been reported that peripherally-induced regulatory T cells and T_H17 effector cells arise in a mutually exclusive fashion, depending on whether they are activated in the presence of TGF- β or TGF- β plus inflammatory cytokines such as IL-6. This review addresses the protective and pathogenic roles of T_H17 cells in the mucosal surfaces and potential regulatory mechanisms that control their development. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Intestine, lung, mucosal, T_H17, regulatory T cells

The main intersection sites between the environment and our organism are the mucosal surfaces, represented by the gastrointestinal, respiratory, and genital tracts. The majority of contacts established at the mucosal surfaces are with nonpathogenic microbial antigens, dietary antigens, and airborne antigens. The mucosal surfaces also contain a diverse and large immune system. For example, the gut-associated lymphoid tissue (GALT) harbors more lymphocytes than all remaining lymphoid tissues together.¹ In contrast with the peripheral lymphoid tissue, the GALT consists of 70% constitutively activated T cells bearing an antigen-experienced phenotype (CD45RB^{lo}CD44^{hi}CD69^{hi}CD62L^{lo}).¹ A large proportion of these T cells can be classified as memory T cells on the basis of their phenotype and functional capacity to display immediate cytotoxicity and the prompt ability to secrete cytokines such as IFN- γ , IL-4, and TNF- α .¹ In the past few years, it has become clear that the GALT also naturally contains a population of T cells that constitutively produce proinflammatory cytokines such as IL-17A, IL-17F, TNF- α , and IL-22.

Abbreviations used

DC: Dendritic cell
Foxp3: Forkhead box protein 3-positive
GALT: Gut-associated lymphoid tissue
iTreg: inducible T regulatory cells
IEC: Intestinal epithelial cell
NK: Natural killer
RA: Retinoic acid
ROR: Retinoic acid-related orphan receptor
STAT: Signal transducer and activator of transcription
TLR: Toll-like receptor
Treg: Regulatory T
TSLP: Thymic stromal lymphopoietin

INTRODUCTION TO T_H17 CELLS

T_H17 cells were recently defined as a distinct lineage that does not share developmental pathways with either T_H1 or T_H2 cells.² In fact, both IFN- γ and IL-4 are able to inhibit the differentiation of IL-17-producing T cells while blocking antibodies to IFN- γ and IL-4 facilitate.^{3,4} Moreover, overexpression of either T-box expressed in T cells (T-bet) (main T_H1 transcription factor) or c-musculoaponeurotic fibrosarcoma (c-Maf) (transcription factor important for IL-4 expression) reduced IL-17 production.^{4,5} Another cytokine involved in both T_H1/T_H2 and regulatory T (Treg) cell development that negatively regulates T_H17 differentiation is IL-2. IL-2 is essential for the TGF- β -mediated inducible T regulatory cells (iTreg) induction, but its signaling via signal transducer and activator of transcription (Stat)-5 constrains T_H17 generation, whereas IL-2 deficiency was also shown to be associated with increased T_H17 generation *in vivo*.⁶ Finally, the cytokine IL-27, another member of the IL-12 cytokine family, is composed of EBV-induced gene 3 and p28 chains and also suppresses T_H17 development.² Although IL-27 signal activates several different pathways, including IL-6-related STAT3, the suppression of T_H17 development is dependent on Stat1.^{7,8}

In contrast with the T_H1/T_H2 subsets, which are inhibited by TGF- β , T_H17 cells rather require TGF- β , along with the proinflammatory cytokines IL-6 or IL-21, for their differentiation from naive precursors.^{2,9-11} Data originated from Cua and Littman's laboratory pointed to the retinoic acid-related orphan receptor (ROR) γ t as the key transcription factor for generation of T_H17 cells.² Accordingly, ROR γ t-deficient naive CD4 T cells have reduced ability to differentiate into IL-17-producing cells, whereas forced expression of ROR γ t in naive CD4 T cells is sufficient to induce expression of IL-17, IL-17F, and IL-22.¹² Current dogma proposes that initial steps are driven by TGF- β and IL-6 and/or IL-21, whereas IL-23 plays a fundamental role in stabilizing the phenotypic features of the T_H17 lineage.¹³

From the Divisions of ^aDevelopmental Immunology and ^bMolecular Immunology, La Jolla Institute for Allergy and Immunology.

Supported by a Career Development Award from the Crohn's and Colitis Foundation of America to D.M., and National Institutes of Health grant AI77079 to S.S.A. This is publication no. 1101 from the La Jolla Institute for Allergy and Immunology.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication January 9, 2009; revised February 27, 2009; accepted for publication March 9, 2009.

Reprint requests: Daniel Mucida, PhD, or Shahram Salek-Ardakani, PhD, La Jolla Institute for Allergy and Immunology, 9420 Athena Circle, La Jolla, CA 92037.

E-mail: mucida@liai.org or ssalek@liai.org.

0091-6749/\$36.00

© 2009 American Academy of Allergy, Asthma & Immunology

doi:10.1016/j.jaci.2009.03.016

Similarly, a TGF- β -dependent pathway operates in human T_H17 cell differentiation from naive CD4 T cells.¹⁴

T_H17 cells have been linked to several autoimmune disorders (reviewed elsewhere^{2,13}) but are physiologically found in the lamina propria of the intestine. In the mucosal surfaces, the production of T_H17-related cytokines such as IL-22 and IL-17 itself is crucial for host protection against several extracellular pathogens, but is also related to the development of pathological inflammatory disorders. In this review, we focus on the role of these cells in the mucosal surfaces of the gut and the lung and discuss the potential mechanisms that govern their efficient generation and regulation at these sites.

ROLE OF T_H17 CELLS IN THE GUT

IL-17-producing cells are present at high numbers at steady state in the mucosal surfaces, represented mostly by CD4⁺TCR $\alpha\beta$ and CD8 $\alpha\alpha$ TCR $\gamma\delta$ T cells in lamina propria of the small intestine.¹⁵ New studies have started to elucidate physiologic factors that influence this spontaneous production of IL-17 in the intestinal lamina propria. In a recent study, Ivanov et al¹⁵ reported that commensal bacteria are required for IL-17 production in the small intestine, because germ-free mice from 3 different strains contained virtually no T_H17 cells in the lamina propria. Surprisingly, neither TIR domain-containing adaptor inducing IFN- β (*Trif*) nor myeloid differentiation factor 88 (*Myd88*) was required for this “spontaneous” IL-17 production in the lamina propria, indicating that Toll-like receptor (TLR) signaling was not involved in this phenomenon. An explanation for these findings could be found in a recent report by Atarashi et al,¹⁶ who have shown that ATP derived from commensal bacteria can activate a subset of lamina propria antigen-presenting cells (F4/80⁺CD11b⁺CD70^{hi}CD11c^{lo} cells) that are able to produce IL-6, IL-23, and TGF- β , triggering the differentiation of T_H17 cells.

Curiously, a different regulation seems to occur in large intestine. In contrast with what was described for the small intestine,¹⁵ Zaph et al¹⁷ showed that large intestine from germ-free mice contains more IL-17-producing cells than mice reared in specific pathogen-free conditions. The authors found that the microbiota present in the large intestine is responsible for the production of IL-17 family cytokine IL-25 (IL-17E), which counterregulates IL-17 production through inhibition of IL-23 production by lamina propria macrophages. Small and large intestines are indeed very different sites regarding the immune system. First, there are more intra-epithelial lymphocytes (IELs) per epithelial cell and more lamina-propria lymphocytes (LPLs) in the small intestine, and the lymphocyte migration to these sites is differentially regulated.¹ Peyer patches and M cells are also mostly found in the small intestine, although the large intestine contains a similar structure, the cecal patch. The composition of lymphocytes is distinct between these 2 anatomical sites. For example, the so-called *unconventional* lymphocytes such as CD8 $\alpha\alpha$ TCR $\alpha\beta$ and CD8 $\alpha\alpha$ TCR $\gamma\delta$ IELs are more frequent in the small than in the large intestine.¹ In addition, the great majority of commensal bacteria are found in the large intestine, and possibly the composition of bacteria species is distinct from the small intestine. Finally, “spontaneously” producing T_H17 cells are present at a higher frequency in the small intestine.¹⁵

Therefore, different regulation of small and large intestine is plausible. Nonetheless, the findings by Atarashi et al¹⁶ differ from those by Zaph et al¹⁷ because the former found a dramatic

decrease whereas the latter found an increase in the frequency of IL-17-producing cells in the large intestine of germ-free mice. These discrepancies could be related to different genetic backgrounds, although consistent data with the conclusion reached in each of the 3 studies (either increased¹⁷ or decreased^{15,16} IL-17 production in the lamina propria of mice maintained under germ-free conditions) were reported when comparing various conventional and germ-free mouse strains. Considering that germ-free mice should not harbor any microbiota, 1 reason for the divergent results might be related to alterations in the microbiota of conventional mice maintained in different facilities. For instance, Ivanov et al¹⁵ described that mice originated from Taconic Farms (Hudson, NY) contain significantly higher population of IL-17-producing cells in the lamina propria than from mice Jackson Laboratory.

The IL-23/IL-17 axis plays multiple roles in the intestinal immune system, protective against certain extracellular pathogens and detrimental in different models of inflammatory bowel diseases.² Genome-wide association studies from patients with inflammatory bowel diseases have pointed to IL-23R as one of the main genes associated with risk of developing Crohn disease and ulcerative colitis.¹⁸ IL-23 has been shown to mediate T-cell-independent colitis and innate inflammatory responses induced by agonist CD40 antibodies or by *Helicobacter hepaticus* infection of recombination-activating gene (RAG)-deficient mice.^{19,20} Although T_H17 cells were not required for the development of the disease in these models, predominantly mediated by IL-23 production, IL-17 expression by innate cells was found significantly inhibited on IL-23p19 blockade.²⁰ In T-cell-dependent models of colitis, induced either by *H hepaticus* infection associated with anti-IL-10R treatment, or by naive T-cell transfer into RAG-deficient mice, IL-23 but not IL-12 was associated the intestinal pathology.^{20,21} The involvement of IL-23 in the inflammatory process appeared to have strictly innate immunity as well as a T_H17-mediated components.^{20,21} A recent study directly linked the T_H17 lineage differentiation with colitis development. By using the transfer model of colitis, Leppkes et al²² showed that naive CD4⁺ T cells from wild-type but not from ROR γ -deficient mice induce colitis on transfer into RAG-deficient mice. In addition, by using blocking antibodies to IL-17A and naive CD4⁺ T cells from IL-17F-deficient mice, the authors showed that IL-17A and IL-17F redundantly trigger intestinal inflammation.

The above data underscore the importance of the IL-23/IL-17 axis in mediating detrimental intestinal inflammation, but in many cases inflammatory processes triggered by these cytokines are crucial for host protection. Mangan et al¹¹ have shown that although IL-23p19-deficient mice are able to mount a vigorous T_H17 response after *Citrobacter rodentium* infection, this response is not efficient enough, because p19^{-/-} but not wild-type mice die shortly after infection with uncontrolled bacterial growth and dissemination, suggesting that IL-23 is important for a full differentiation and for an efficient T_H17 response against this intestinal pathogen. The production of IL-23 has also been linked recently with the induction of IL-22-producing mucosal natural killer (NK) cells in mice and human beings.^{23,24} This population of CD56^{hi}NK cells seems to constitute another important branch of the immune response against intestinal pathogens and also requires stimulus from the microbiota and ROR γ t expression for their development.^{23,25,26} Furthermore, although in some inflammatory contexts IL-22 production by T_H17 cells can exert proinflammatory effects, IL-22 produced either by T_H17

cells or by IL-22–producing mucosal NK cells was shown to protect mice from inflammatory bowel disease (IBD), probably exerting protective effects on responding intestinal epithelial cells (IECs) in the colon.²⁵

A recent report extended the protective role of T_H17 cells to oropharyngeal fungus (*Candida albicans*) infection. Conti et al²⁷ showed that IL23p19 and IL-17RA–deficient mice but not IL-22 or IL-12p35–deficient mice are susceptible to oral candidiasis. The role played by IL-17–producing T cells in controlling invading pathogens, including *Candida* infection, is of special interest in infections that cause immune deficiency. Both simian immunodeficiency virus and HIV were shown to deplete intestinal memory CD4⁺ T cells massively at all stages of infection.²⁸ Raffatellu et al²⁹ extended these observations by demonstrating that simian immunodeficiency virus infection of rhesus macaques resulted in a severe depletion of T_H17 cells in the ileal mucosa. As a consequence of deprived T_H17 responses, the macaques displayed blunted responses against *Salmonella typhimurium*, resulting in bacterial dissemination.

ROLE OF T_H17 CELLS IN THE LUNGS

In addition to their role in the intestinal immune system, IL-17–producing CD4⁺ T cells also constitute an important arm of adaptive immune responses against airborne pathogens.³⁰ For instance, IL-17–producing cells play an important role in the establishment of effective immune responses to *Mycobacterium tuberculosis*, mainly through the recruitment of protective IFN- γ –producing CD4⁺ T cells.³¹ Increasing evidence suggests that IL-17, acting either directly or indirectly, significantly stimulates neutrophil maturation, migration, and function in the lung tissue and airways. IL-17 induction of neutrophil activation and migration is important in defense against a variety of organisms that infect the lung.^{32,33} It was reported that *Klebsiella pneumoniae*–pulsed dendritic cells (DCs) led to IL-17 production in an IL-23–dependent manner.³⁴ Accordingly, IL-23 p19–deficient and IL-17R–deficient mice were shown to be more susceptible to lung infection with *K pneumoniae*.^{34,35} The increased bacteremia and mortality observed in these mice were associated with reduced levels of chemokine (C-X-C motif) ligand 1 (CXCL1), CXCL2, granulocyte-colony stimulating factor (G-CSF), and subsequent neutrophilic influx in the lung on challenge with *K pneumoniae*. Likewise, in mice infected with *Mycoplasma pneumoniae*, infiltration of the lungs by neutrophils is dependent on IL-23–induced upregulation of IL-17.³⁶ Another important respiratory pathogen, *Bordetella pertussis*, the cause of whooping cough, uses multiple pathways to elicit IL-17 response. Protection of mice by pertussis vaccine requires production of IL-17, IL-23, and IL-1.³⁷ In addition to regulation of chemokines and cytokines, IL-17 regulates antimicrobial peptide production of β -defensins in human bronchial epithelial cells.^{32,37} Taken together, these data support the idea that T_H17 cells are critical for normal immunity against bacterial infections in the airways.

In some circumstances, however, IL-23 and/or IL-17 may also be associated with an unfavorable outcome to infection. A recent study by Zelante et al³⁸ demonstrated that the T_H17 pathway acted as a negative regulator of the T_H1-mediated immune resistance to both *C albicans* and *Aspergillus fumigatus*, and played an inflammatory role previously attributed to uncontrolled T_H1 responses. IL-23 acted as a molecular connection between uncontrolled fungal growth and inflammation, being produced by

DCs in response to high fungal burden and counterregulating IL-12p70 production. Both IL-23 and IL-17 impaired antifungal effector activities of neutrophils even in the presence of IFN- γ . In addition, both cytokines activated the inflammatory program of neutrophils by inducing the release of matrix metalloproteinase (MMP9) and myeloperoxidase (MPO), which likely contributes to the high inflammatory pathology and tissue destruction associated with T_H17-cell activation. Together, these results imply that the relative contribution of IL-17 to host defense against respiratory pathogens might vary depending on the infectious setting.

There is growing evidence from clinical studies that exaggerated recruitment and activation of neutrophils in the airways is linked to the clinical course of several inflammatory diseases in the airways and lungs, such as asthma, nonspecific bronchial hyperreactivity, chronic bronchitis, chronic obstructive pulmonary disease, cystic fibrosis, and acute respiratory distress syndrome.^{32,39} The precise role of T_H17 cells in lung diseases is still largely unclear, but there is growing clinical and experimental evidence to suggest that they may be important for neutrophilic influx in acute and chronic airway inflammation. In patients with asthma, IL-17 expression was increased in the lungs, sputum, bronchoalveolar lavage fluid, or sera, and severity of airway hypersensitivity in patients correlated with the level of IL-17 expression.⁴⁰ Human bronchial fibroblasts cells, airway smooth muscle cells, and lung epithelial cells respond to stimulation with IL-17 *in vitro* by producing IL-6, IL-8, and growth-regulated oncogene (GRO)- α . IL-8 and GRO- α are known chemoattractants for neutrophils, and IL-6 is a neutrophil-activating cytokine.⁴¹ Thus, the increased expression of IL-17 in the lung during allergic responses may explain the increased accumulation and activation of lung neutrophils. Consistent with this, Hellings et al⁴² in a mouse model of allergic asthma showed that acute allergen provocation is followed by a rapid increase in IL-17 mRNA in mouse lungs, which correlated with an increase in neutrophilic influx in the airways. Neutralizing anti-IL-17 mAb treatment ablated bronchial neutrophilia in parallel with reduction of bone marrow and blood neutrophilia. Moreover, IL-23 and T_H17 cells not only induce T_H17-cell-mediated neutrophil recruitment but can also upregulate T_H2-mediated eosinophilic airway inflammation and hyperresponsiveness.^{43,44} Recently, by using a well established murine model of hypersensitivity pneumonitis and pulmonary fibrosis, Simonian et al⁴⁵ repeatedly exposed C57BL/6 mice to *Saccharopolyspora rectivirgula* and demonstrated that CD4 T cells expressing IL-17 and IL-22 are recruited into the lung. Importantly, after exposure of IL-17R $\alpha^{-/-}$ mice to *S rectivirgula*, a significantly decreased T-cell alveolitis was seen, with an associated decrease in collagen deposition. These data highlight a role for T_H17 cells in the immune response directed against bacterial pathogens and for the subsequent development of lung fibrosis. Thus, in contrast with T_H1 and T_H2 cells, T_H17 cells may be involved in the pathogenesis of various inflammatory and allergic responses in the lung in part by supporting neutrophil recruitment and survival and inducing proinflammatory cytokines by structural cells.

Similar to the intestine, IL-17 producing cells are present at high numbers at steady-state in the airways, mostly represented by invariant NK T cells, TCR $\gamma\delta$ T cells, and non-T cells.¹⁵ Although many studies have characterized pathways that induce and regulate T_H17-related cytokines in the intestine, very few

studies have attempted to describe how these pathways operate in the airways. For example, are there microbial or endogenous stimuli required for the spontaneous production of IL-17 in the lungs? Because intestinal and lung-associated lymphoid tissue share characteristics typically found at mucosal sites, such as predominant induction of tolerance at steady-state, it is plausible that parallel regulatory mechanisms to the intestine can be found in the airways (discussed below).

REGULATION OF T_H17 CELLS IN THE MUCOSA: IL-25 AND THYMIC STROMAL LYMPHOPOIETIN

The cytokine IL-25 is expressed by lung epithelial cells and activated eosinophils and basophils. It mediates early T-cell differentiation toward a T_H2 phenotype and development of airway hyperactivity and allergic diseases. This characteristic, associated with the ability to suppress T_H17 differentiation, suggested that IL-25 could be an important regulatory mechanism to suppress the development of different inflammatory diseases. In addition, IL-25 promotes T_H2-cell differentiation on thymic stromal lymphopoietin (TSLP)-activated DC stimulation.⁴⁶ This IL-25/TSLP pathway seems to operate in the intestinal mucosa as well. Mice with an IEC-specific deletion of the nuclear factor- κ B-related kinase, IKK β , show a reduced expression of TSLP in the intestine and fail to develop a protective T_H2 response against the intestinal parasite *Trichuris*. Moreover, these mice develop exaggerated IFN- γ and IL-17 responses.⁴⁷ These results underscore the importance of mechanisms that regulate T_H17 responses under pathogen-induced or autoimmune and allergic diseases-induced inflammation in the mucosal surfaces.

REGULATION OF T_H17 CELLS IN THE MUCOSA: RECIPROCAL DEVELOPMENT OF T_H17 AND Treg CELLS

In addition to its effects on T_H17-differentiation, TGF- β has multiple roles in both systemic and mucosal immune regulation. Among them is the ability to convert naive CD4 cells into forkhead box protein 3-positive (Foxp3)-expressing regulatory T cells (iTreg).⁴⁸ This contrasting deviation underscores a central role of TGF- β in orchestrating the proinflammatory and anti-inflammatory nature of adaptive immunity. This pathway has particular relevance at mucosal surfaces such as the intestine, where both intense microbial load and production of TGF- β are constant under physiological conditions. How is the balance of proinflammatory and anti-inflammatory responses achieved in the highly stimulated surface of the intestinal mucosa?

It was recently found that Foxp3 can directly inhibit T_H17 differentiation by direct interaction with ROR γ t.⁴⁹⁻⁵¹ Actually, ROR γ t and Foxp3 may coexist in the same cell.^{50,52} Lochner et al⁵² reported that ROR γ t-expressing T cells contain a sizeable population of cells with regulatory properties, including IL-10-producing Foxp3⁺ Treg cells *in vivo*. The balance between IL-17-producing ROR γ t⁺ T cells and IL-10-producing Foxp3⁺ cells depends on factors such as inflammatory cytokines and the expression of Foxp3 itself. IL-6-deficient mice show decreased IL-17-producing ROR γ t cells in various tissues, including the airways and mesenteric lymph nodes. Remarkably, the relative frequency of IL-17-producing ROR γ t⁺ or IL-10-producing Foxp3⁺

ROR γ t⁺ T cells remained constant during chronic intestinal inflammation as well as after lung infection with influenza A virus.⁵² The authors proposed the existence of a robust mechanism maintaining the equilibrium between T_H17 and Treg cells within ROR γ t cells during infection.⁵² Keeping the balance of IL-17 versus IL-10 production would promote inflammation while limiting collateral damage, a necessary compromise between maintaining effective immunity and tissue integrity.

The constant exposure to luminal antigens creates a so-called *physiological chronic inflammation* in the gut, which could easily favor the TGF- β -driven effector differentiation at the expense of iTreg cells. The high amounts of IL-17-producing T cells at steady-state correlate with the fact that inflammatory cytokines are produced physiologically in the intestine.^{12,15,52} At the same time, the intestine is highly regulated, containing several different mechanisms to control pathological inflammation. In normal circumstances, previous exposure to antigens via oral or nasal routes efficiently inhibits the development of immune responses to subsequent challenges with the same antigen.^{53,54} The intestinal and airway mucosa are highly effective at inducing iTreg cells.^{48,54} Mucida et al⁴⁸ and Curotto et al⁵⁴ have shown that Treg cells are induced after oral or nasal exposure to the cognate antigen and these iTreg cells are sufficient and required for mucosal tolerance in this system. Coombes et al⁵⁵ and Sun et al⁵⁶ confirmed and extended these findings by demonstrating that iTreg cells were preferentially induced in mesenteric lymph nodes and lamina propria by a subpopulation of DCs, rather than in spleen or peripheral lymph nodes, reinforcing that the intestine is a privileged site for Treg cell induction. Importantly, these intestinal DC subpopulations specifically produce a "cofactor" for Treg cell development, the vitamin A metabolite retinoic acid (RA).⁵⁵⁻⁵⁸

REGULATION OF T_H17 CELLS IN THE MUCOSA: RA

Mucosal antigen-presenting cells, especially CD11b⁺CD11c⁺ or CD103⁺CD11c⁺ DCs, are able to help the development of the T_H17 cells in the lamina propria of the intestine (Fig 1).^{55,58} On the other hand, important regulatory functions can also be ascribed to DCs present in the mucosal sites. For example, antigen presentation by mucosal DCs in the MLN plays crucial roles in the development of oral tolerance.⁵⁹ Also, mucosal plasmacytoid CD8 α ⁺ DCs are inefficient at inducing CD4 T_H proliferation but instead can induce their differentiation to IL-10-producing CD4 cells.⁶⁰ Recently, the CD103⁺ DC population was shown to be the main population involved in the production of RA. Importantly, the production of RA by mucosal antigen-presenting cells, including lamina propria macrophages, was associated with their high efficiency in converting naive CD4⁺ T cells into FoxP3⁺ T cells in a TGF- β -dependent fashion.⁵⁵⁻⁵⁸ In contrast with the effect on Treg cells, RA potently constrains T_H17 conversion.⁵⁷ Different groups have shown that RA signaling through RAR receptors in the T cells is able to block the inhibitory effects of inflammatory cytokines, such as IL-6, on the TGF- β -mediated Foxp3 induction, consequently suppressing the development of IL-17-producing CD4 and CD8 T cells.^{57,61} In addition, RA directly inhibits TGF- β and IL-6-induced ROR γ t on naive CD4⁺ T cells,^{57,62} possibly through the inhibition of IL-6R α and IL-23R expression.⁶³

The dose of RA associated with TLR agonists was also suggested to play a role on the suppression of T_H17 differentiation. Although previous studies have described that RA has suppressive effects on T_H17 development in all doses examined,

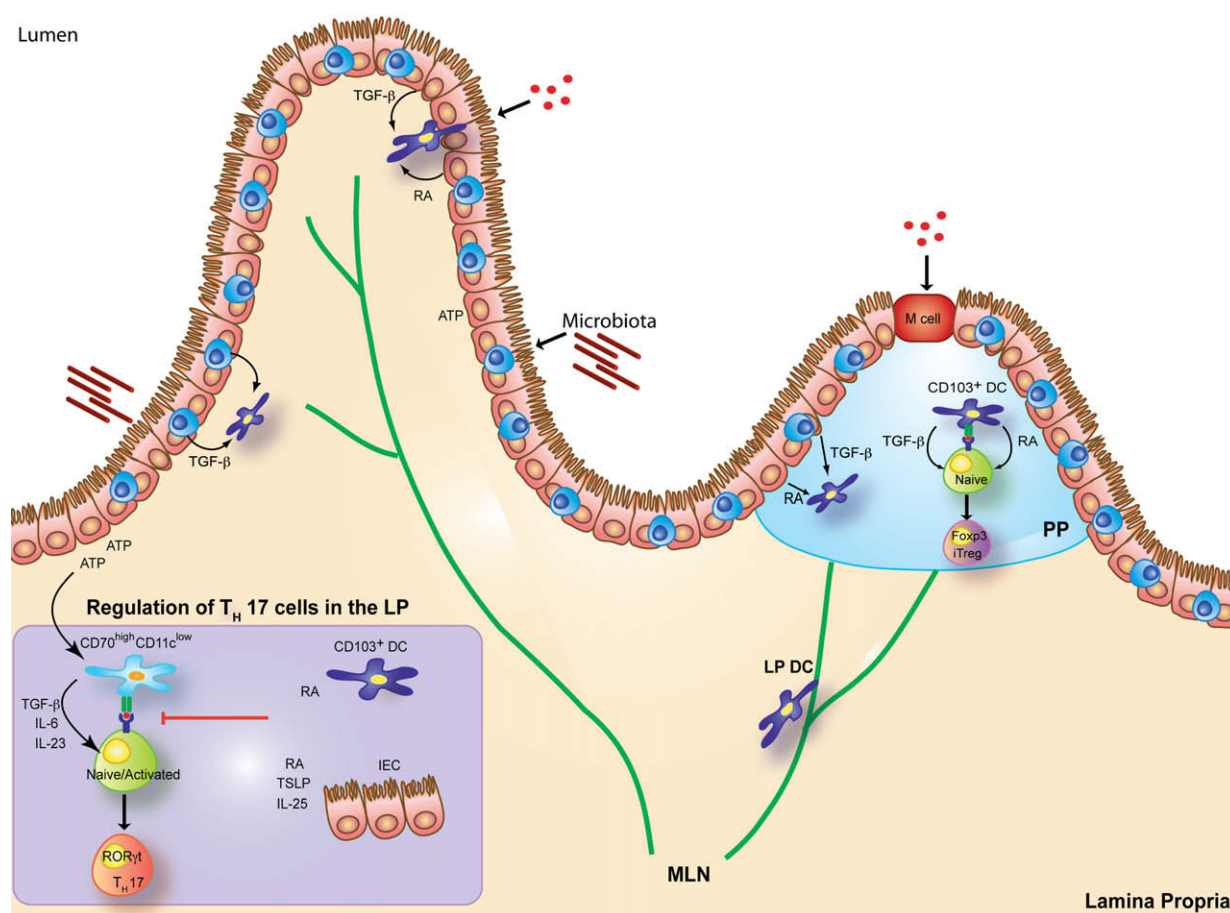


FIG 1. Generation and regulation of T_H17 responses in the intestine. Under the influence of microbial-derived stimuli and ATP, lamina propria (LP) $CD70^{high}$ and/or $CD103^+$ DCs induce T_H17 differentiation through the production of TGF- β , IL-6, and IL-23. Production of TGF- β by LP cells also promotes the anti-inflammatory Foxp3 $^+$ -induced T regulatory cells (iTreg). The balance between T_H17 and Treg cell development is favored toward Treg cells on the influence of RA produced by LP and Peyer patch $CD103^+$ DCs, LP macrophages, and IECs. Besides RA, TSLP expressed by IECs and the cytokine IL-25 were reported to suppress T_H17 development in the intestine.

including spontaneous production of RA by mucosal DCs in T/DC cocultures.^{57,62} Uematsu et al⁶⁴ have recently suggested that production of RA by $CD11c^{hi}CD11b^{hi}$ lamina propria DCs, when stimulated by TLR5-ligand flagellin, promoted a modest differentiation of antigen-specific T_H17 and T_H1 cells, suggesting that innate stimuli may induce contrasting effects of RA on either DCs or T cells.

Clinically, vitamin A derivatives have been used to treat certain types of cancer and skin-related diseases such as psoriasis.⁶⁵ Interestingly, the production of IL-6 and IL-23 and differentiation of IL-17 and IL-22-producing T_H17 cells have been implicated with the pathogenesis of autoimmune psoriasis.⁶⁶ Several clinical trials currently investigate potential application of the inhibition of T_H17 development—for example, using antibodies against IL-23—in the treatment of autoimmune diseases.⁶⁷ In fact, RA and other vitamin A derivatives were previously shown to suppress experimental models of autoimmune diseases recently linked to a T_H17 phenotype, such as experimental autoimmune encephalomyelitis.⁶⁸ It is possible that part of the mechanism of action of retinoids in the treatment of psoriasis and experimental autoimmune encephalomyelitis is related to suppression of T_H17 development. Therefore, it

is of utmost interest to dissect the mechanism of action of RA-mediated suppression of these inflammatory processes as well as to evaluate possible therapeutic applications for RA in T_H17 -related diseases.

CONCLUSION

The discovery of T_H17 cells represents a major step in our understanding of protective and pathological responses at the mucosal surfaces. However, there are still many unanswered questions, such as how endogenous and/or microbiota-derived stimuli trigger ROR γ t and consequently T_H17 development in the mucosa. In addition, it is not known which types/strains of commensal bacteria trigger natural production of T_H17 in the intestine. A great deal of work needs to be done on the precise regulatory mechanisms that govern the efficient generation, persistence, and reactivation of T_H17 cells at different mucosal sites. The relationship between Treg cells and T_H17 cells as well as the possible proinflammatory role of Treg cells that lose Foxp3 expression or coexpress Foxp3 and ROR γ t are also of great interest. Another largely unexplored topic is the role of IL-17 production by cells other than $CD4^+$ T cells. IL-17 can also be produced by $CD8^+$ T cells,

$\gamma\delta$ T cells, and in some cases even NK T cells, macrophages, neutrophils, and eosinophils. Last, a critical direction for future studies of T_H17 cells will be to determine what roles these cells play in human disease, and ultimately how useful or safe they will be as a therapeutic target.

We thank Hilde Cheroutre and Alexandre Basso for their valuable suggestions for this article.

REFERENCES

- Cheroutre H. Starting at the beginning: new perspectives on the biology of mucosal T cells. *Annu Rev Immunol* 2004;22:217-46.
- Kastelein RA, Hunter CA, Cua DJ. Discovery and biology of IL-23 and IL-27: related but functionally distinct regulators of inflammation. *Annu Rev Immunol* 2007;25:221-42.
- Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4⁺ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. *Nat Immunol* 2005;6:1123-32.
- Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005;6:1133-41.
- Ivanov II, Zhou L, Littman DR. Transcriptional regulation of Th17 cell differentiation. *Semin Immunol* 2007;19:409-17.
- Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, Yao Z, et al. Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. *Immunity* 2007;26:371-81.
- Batten M, Li J, Yi S, Kljavin NM, Danilenko DM, Lucas S, et al. Interleukin 27 limits autoimmune encephalomyelitis by suppressing the development of interleukin 17-producing T cells. *Nat Immunol* 2006;7:929-36.
- Stumhofer JS, Laurence A, Wilson EH, Huang E, Tato CM, Johnson LM, et al. Interleukin 27 negatively regulates the development of interleukin 17-producing T helper cells during chronic inflammation of the central nervous system. *Nat Immunol* 2006;7:937-45.
- Betelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006;441:235-8.
- Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGF β in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 2006;24:179-89.
- Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor- β induces development of the T(H)17 lineage. *Nature* 2006;441:231-4.
- Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelletier A, Lafaille JJ, et al. The orphan nuclear receptor ROR γ directs the differentiation program of proinflammatory IL-17⁺ T helper cells. *Cell* 2006;126:1121-33.
- Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol* 2008;8:337-48.
- Manel N, Unutmaz D, Littman DR. The differentiation of human T(H)-17 cells requires transforming growth factor- β and induction of the nuclear receptor ROR γ . *Nat Immunol* 2008;9:641-9.
- Ivanov II, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, et al. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008;4:337-49.
- Atarashi K, Nishimura J, Shima T, Umesaki Y, Yamamoto M, Onoue M, et al. ATP drives lamina propria T(H)17 cell differentiation. *Nature* 2008;455:808-12.
- Zaph C, Du Y, Saenz SA, Nair MG, Perrigoue JG, Taylor BC, et al. Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine. *J Exp Med* 2008;205:2191-8.
- Duerr RH, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, et al. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006;314:1461-3.
- Uhlir HH, McKenzie BS, Hue S, Thompson C, Joyce-Shaikh B, Stepankova R, et al. Differential activity of IL-12 and IL-23 in mucosal and systemic innate immune pathology. *Immunity* 2006;25:309-18.
- Hue S, Ahern P, Buonocore S, Kullberg MC, Cua DJ, McKenzie BS, et al. Interleukin-23 drives innate and T cell-mediated intestinal inflammation. *J Exp Med* 2006;203:2473-83.
- Kullberg MC, Jankovic D, Feng CG, Hue S, Gorelick PL, McKenzie BS, et al. IL-23 plays a key role in *Helicobacter hepaticus*-induced T cell-dependent colitis. *J Exp Med* 2006;203:2485-94.
- Leppkes M, Becker C, Ivanov II, Hirth S, Wirtz S, Neufert C, et al. ROR γ -expressing Th17 cells induce murine chronic intestinal inflammation via redundant effects of IL-17A and IL-17F. *Gastroenterology* 2009;136:257-67.
- Sanos SL, Bui VL, Mortha A, Oberle K, Heners C, Johnner C, et al. ROR γ and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46⁺ cells. *Nat Immunol* 2009;10:83-91.
- Cella M, Fuchs A, Vermi W, Facchetti F, Otero K, Lennerz JK, et al. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 2009;457:722-5.
- Zenewicz LA, Yancopoulos GD, Valenzuela DM, Murphy AJ, Stevens S, Flavell RA. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 2008;29:947-57.
- Satoh-Takayama N, Voshenrich CA, Lesjean-Pottier S, Sawa S, Lochner M, Rattis F, et al. Microbial flora drives interleukin 22 production in intestinal NKp46⁺ cells that provide innate mucosal immune defense. *Immunity* 2008;29:958-70.
- Conti HR, Shen F, Nayyar N, Stocum E, Sun JN, Lindemann MJ, et al. Th17 cells and IL-17 receptor signaling are essential for mucosal host defense against oral candidiasis. *J Exp Med* 2009;206:299-311.
- Johnson RP. How HIV guts the immune system. *N Engl J Med* 2008;358:2287-9.
- Raffatellu M, Santos RL, Verhoeven DE, George MD, Wilson RP, Winter SE, et al. Simian immunodeficiency virus-induced mucosal interleukin-17 deficiency promotes *Salmonella* dissemination from the gut. *Nat Med* 2008;14:421-8.
- Infante-Duarte C, Horton HF, Byrne MC, Kamradt T. Microbial lipopeptides induce the production of IL-17 in Th cells. *J Immunol* 2000;165:6107-15.
- Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4⁺ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. *Nat Immunol* 2007;8:369-77.
- Linden A, Laan M, Anderson GP. Neutrophils, interleukin-17A and lung disease. *Eur Respir J* 2005;25:159-72.
- Linden A, Adachi M. Neutrophilic airway inflammation and IL-17. *Allergy* 2002;57:769-75.
- Happel KI, Dubin PJ, Zheng M, Ghilardi N, Lockhart C, Quinton LJ, et al. Divergent roles of IL-23 and IL-12 in host defense against *Klebsiella pneumoniae*. *J Exp Med* 2005;202:761-9.
- Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, et al. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating factor expression, neutrophil recruitment, and host defense. *J Exp Med* 2001;194:519-27.
- Wu Q, Martin RJ, Rino JG, Breed R, Torres RM, Chu HW. IL-23-dependent IL-17 production is essential in neutrophil recruitment and activity in mouse lung defense against respiratory *Mycoplasma pneumoniae* infection. *Microbes Infect* 2007;9:78-86.
- Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev* 2008;223:87-113.
- Zelante T, De Luca A, Bonifazi P, Montagnoli C, Bozza S, Moretti S, et al. IL-23 and the Th17 pathway promote inflammation and impair antifungal immune resistance. *Eur J Immunol* 2007;37:2695-706.
- Dubin PJ, McAllister F, Kolls JK. Is cystic fibrosis a TH17 disease? *Inflamm Res* 2007;56:221-7.
- Oboki K, Ohno T, Saito H, Nakae S. Th17 and allergy. *Allergol Int* 2008;57:121-34.
- Aujla SJ, Dubin PJ, Kolls JK. Interleukin-17 in pulmonary host defense. *Exp Lung Res* 2007;33:507-18.
- Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, Overbergh L, et al. Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol* 2003;28:42-50.
- McKinley LE, Alcorn JF, Peterson A, Dupont RB, Kapadia S, Logar A, et al. TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 2008;181:4089-97.
- Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, Watanabe N, et al. IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 2008;178:1023-32.
- Simonian PL, Roark CL, Wehrmann F, Lanham AK, Diaz del Valle F, Born WK, et al. Th17-polarized immune response in a murine model of hypersensitivity pneumonitis and lung fibrosis. *J Immunol* 2009;182:657-65.
- Wang YH, Angkasekwinai P, Lu N, Voo KS, Arima K, Hanabuchi S, et al. IL-25 augments type 2 immune responses by enhancing the expansion and functions of TSLP-DC-activated Th2 memory cells. *J Exp Med* 2007;204:1837-47.
- Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, Du Y, et al. Epithelial-cell-intrinsic IKK- β expression regulates intestinal immune homeostasis. *Nature* 2007;446:552-6.
- Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. *J Clin Invest* 2005;115:1923-33.

49. Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, et al. Foxp3 inhibits RORgamma t-mediated IL-17A mRNA transcription through direct interaction with RORgamma t. *J Biol Chem* 2008;283:17003-8.
50. Zhou L, Lopes JE, Chong MM, Ivanov II, Min R, Victora GD, et al. TGF-beta-induced Foxp3 inhibits T(H)17 cell differentiation by antagonizing RORgamma t function. *Nature* 2008;453:236-40.
51. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, Chung Y, et al. T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* 2008;28:29-39.
52. Lochner M, Peduto L, Cherrier M, Sawa S, Langa F, Varona R, et al. In vivo equilibrium of proinflammatory IL-17+ and regulatory IL-10+ Foxp3+ RORgamma t+ T cells. *J Exp Med* 2008;205:1381-93.
53. Vaz NM, Maia LC, Hanson DG, Lynch JM. Inhibition of homocytotropic antibody responses in adult inbred mice by previous feeding of the specific antigen. *J Allergy Clin Immunol* 1977;60:110-5.
54. Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. *Immunity* 2008;29:114-26.
55. Coombes JL, Siddiqui KR, Arancibia-Carcamo CV, Hall J, Sun CM, Belkaid Y, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007;204:1757-64.
56. Sun CM, Hall JA, Blank RB, Bouladoux N, Oukka M, Mora JR, et al. Small intestine lamina propria dendritic cells promote de novo generation of Foxp3 T reg cells via retinoic acid. *J Exp Med* 2007;204:1775-85.
57. Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256-60.
58. Denning TL, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 2007;8:1086-94.
59. Worbs T, Bode U, Yan S, Hoffmann MW, Hintzen G, Bernhardt G, et al. Oral tolerance originates in the intestinal immune system and relies on antigen carriage by dendritic cells. *J Exp Med* 2006;203:519-27.
60. Bilsborough J, George TC, Norment A, Viney JL. Mucosal CD8alpha+ DC, with a plasmacytoid phenotype, induce differentiation and support function of T cells with regulatory properties. *Immunology* 2003;108:481-92.
61. Schambach F, Schupp M, Lazar MA, Reiner SL. Activation of retinoic acid receptor-alpha favours regulatory T cell induction at the expense of IL-17-secreting T helper cell differentiation. *Eur J Immunol* 2007;37:2396-9.
62. Elias KM, Laurence A, Davidson TS, Stephens G, Kanno Y, Shevach EM, et al. Retinoic acid inhibits Th17 polarization and enhances FoxP3 expression through a Stat-3/Stat-5 independent signaling pathway. *Blood* 2008;111:1013-20.
63. Xiao S, Jin H, Korn T, Liu SM, Oukka M, Lim B, et al. Retinoic acid increases Foxp3+ regulatory T cells and inhibits development of Th17 cells by enhancing TGF-beta-driven Smad3 signaling and inhibiting IL-6 and IL-23 receptor expression. *J Immunol* 2008;181:2277-84.
64. Uematsu S, Fujimoto K, Jang MH, Yang BG, Jung YJ, Nishiyama M, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. *Nat Immunol* 2008;9:769-76.
65. Albanesi C, Scarponi C, Pallotta S, Daniele R, Bosisio D, Madonna S, et al. Chemerin expression marks early psoriatic skin lesions and correlates with plasmacytoid dendritic cell recruitment. *J Exp Med* 2009;206:249-58.
66. Aujla SJ, Chan YR, Zheng M, Fei M, Askew DJ, Pociask DA, et al. IL-22 mediates mucosal host defense against gram-negative bacterial pneumonia. *Nat Med* 2008;14:275-81.
67. Boniface K, Blom B, Liu YJ, de Waal Malefyt R. From interleukin-23 to T-helper 17 cells: human T-helper cell differentiation revisited. *Immunol Rev* 2008;226:132-46.
68. Massaccesi L, Castigli E, Vergelli M, Olivetto J, Abbamondi AL, Sarlo F, et al. Immunosuppressive activity of 13-cis-retinoic acid and prevention of experimental autoimmune encephalomyelitis in rats. *J Clin Invest* 1991;88:1331-7.