

Review

Manipulation of T_H17 responses in pulmonary immunity and disease through vaccination

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Since the discovery of a novel subset of CD4⁺ T helper cells, T_H17 cells have been implicated in a wide range of human diseases, including autoimmunity, allergic reactions and auto-inflammatory diseases. Conversely, it has also been determined that T_H17 cells are required to mount a protective immune response to a number of pathogens. It has therefore been of great interest to manipulate T_H17 responses to either prevent disease or promote immunity. Vaccination is a particularly attractive approach for this manipulation. With a focus on the pulmonary mucosal environment, we herein review T_H17 responses and potential methods for manipulation.

Introduction

Over eight years ago, it was proposed that an alternative subset of IL-17A producing CD4⁺ T helper cells existed,¹ which were later termed T helper 17 cells (T_H17 cells).²⁻⁴ Since then, a substantial amount of research has followed investigating the importance of this new lineage. It has been discovered that T_H17 cells drive both pathogenic and protective host immune responses. In particular, this subset appears to play an important role at mucosal surfaces, such as the gut and lungs. Within the lung, it is necessary to maintain a balance of tolerance to non-threatening environmental antigens and readiness to mount an effective immune response to invasive ones. T_H17 cells are critical in maintaining this balance, as the lack of a T_H17 response can lead to pathogen-mediated disease, and an unnecessary T_H17 response can lead to immune-mediated disease.

Significant importance has also been placed on understanding the induction, maintenance, regulation and effector function of this subset. Understanding the complete T_H17 response is necessary for effective manipulation and development of therapies for pulmonary diseases. It is also important to investigate different

methods for manipulation. One attractive method of manipulation is vaccination to either promote or inhibit T_H17 responses. Several clinical trials aimed at suppressing T_H17-mediated disease have already been implemented, resulting in varying levels of success.

Here we review the vast knowledge of the induction, maintenance, regulation and function of T_H17 responses, in addition to the current data demonstrating a role for T_H17 cells in pulmonary immunity and disease. Comparisons will also be made between what is known in humans and mice. We propose that these pathways present excellent targets for therapeutic intervention and review potential methods for this manipulation, with a focus on vaccination and vaccination strategies.

T_H17 Cells

Adding to the dichotomy originally proposed by Mossman and Coffman of T_H1 and T_H2 cells,^{5,6} T_H17 cells are another lineage of CD4⁺ αβTCR expressing effector T-cells. Their name is derived from the fact that IL-17A is the major cytokine produced by this subset. A substantial amount of research has been performed to define the differentiation, maintenance, regulation and effector function of this subset. We will review these areas and focus on potential targets for the manipulation of the T_H17 responses.

T_H17 Cell Differentiation and Maintenance

In mice, several groups made the ground breaking finding that naïve T-cells can differentiate into T_H17 cells when stimulated in the presence of the proinflammatory cytokine IL-6, and TGFβ (Fig. 1).⁷⁻⁹ Stimulation with these cytokines led to induction of the lineage specific transcription factor RORγt,¹⁰ and later found RORα,¹¹ to promote both differentiation and function of the T_H17 lineage. Other important transcription factors include STAT3, which is critical for signaling of vital T_H17 cytokines IL-6, IL-21 and IL-23.¹²⁻¹⁴ Additionally, it has been shown that association with IRF-4 and Runx1 can act as a positive regulator of T_H17 differentiation.^{15,16} A more recent paper demonstrated that optimal T_H17 differentiation also requires the presence of aryl hydrocarbon receptor (AhR) agonists.¹⁷

Upon initial differentiation, murine T_H17 cells gain the ability to produce IL-21, which is able to act in an autocrine manner to promote and stabilize the T_H17 lineage. It has even been found

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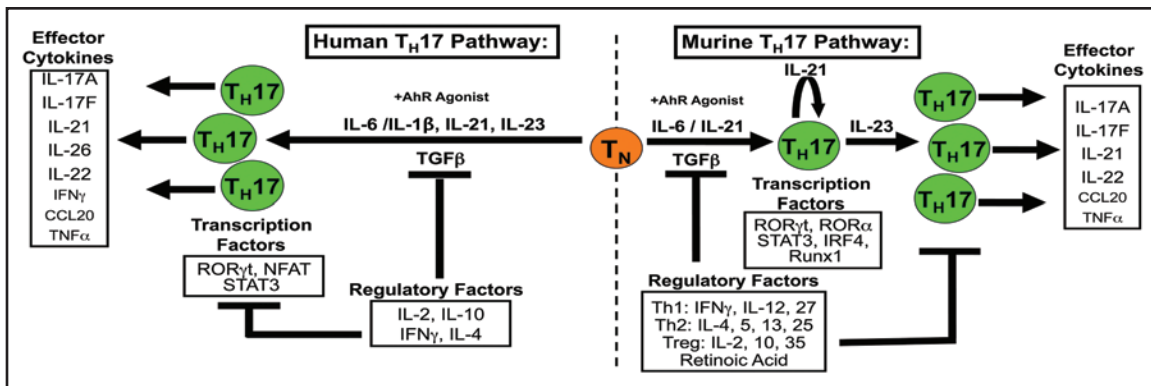


Figure 1. Developmental, maintenance, regulation and effector pathways of human and murine T_H17 responses. Murine and human T_H17 responses develop and are regulated by similar mechanisms. The murine T_H17 pathway has been much more defined, however, many of the findings are holding to be true within humans.

that exogenous IL-21 can substitute for the absence of IL-6 in initial differentiation stages.¹⁸⁻²⁰ At this point there is also an upregulation of the IL-23R.^{10,20} It is clear that IL-23 signaling is critical, as its absence can abrogate either pathogenic or protective properties of the T_H17 pathway. Recent work by Daniel Cua and colleagues has demonstrated that in the selective loss of the IL-23 receptor, there is an inability to downregulate IL-2, decreased proliferation and unsustainable effector cytokine production, thus suggesting that IL-23 is essential for the terminal differentiation of T_H17 cells.²¹

Differentiation of human T_H17 cells share many similarities to what was found in the mouse. TGFβ was initially found to inhibit IL-17A production in T-cells isolated from the peripheral blood of humans.^{22,23} However it was found that this inhibition was acting on memory CD4⁺ T-cells and not naïve CD4⁺ T-cells. Recent reports have demonstrated that human naïve CD4⁺ T-cells require TGFβ, IL-21, IL-23 and the inflammatory cytokines IL-1β or IL-6 for optimal T_H17 cell differentiation.²⁴⁻²⁶ Human T_H17 cells also upregulate the lineage specific transcription factor RORγt upon differentiation and also rely on STAT3 signaling.²² Additionally, the human *iL17A* gene has several NFAT transcriptional binding sites, suggesting a critical role for NFAT in IL-17A cytokine regulation.²⁷ Other transcription factors are also believed to be important, however, direct evidence has not yet been found.

T_H17 Cell Regulation

A number of regulatory mechanisms in murine T_H17 cells have been discovered. Similar to other T helper cell subsets, lineage specific cytokines of alternative subsets can inhibit differentiation of T_H17 cells, including IL-12, IL-27 and IFNγ driving the T_H1 subset, and, IL-4, IL-5, IL-13 and IL-25 driving the T_H2 subset.²⁸⁻³⁰ A number of cytokines involved in the regulatory T-cell lineage (T_{reg}) have also been found to inhibit T_H17 responses, including IL-2, IL-10 and IL-35.³¹⁻³³ Many of these cytokines have also been found to be inhibitory in human T_H17 differentiation.^{23,34}

Interestingly, Daniel Cua and colleagues found a mechanism of self-regulation in murine T_H17 cells. This self-regulation occurs

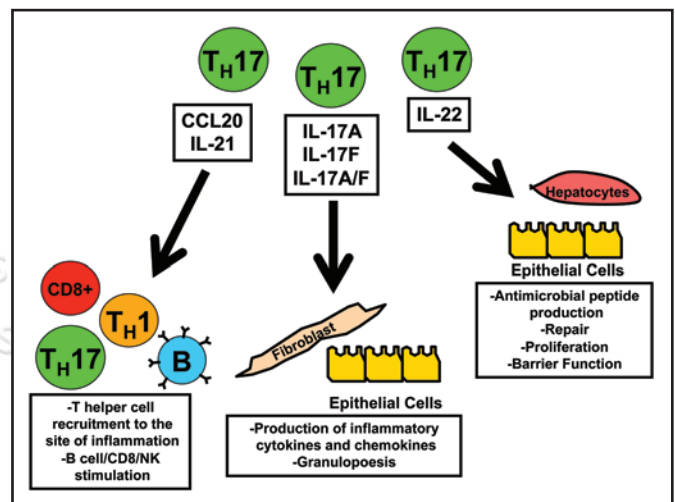


Figure 2. Cytokines produced by T_H17 cells and their effector function. Cytokines produced by T_H17 cells do not only act on other immune cells, but many also have the ability to act on cells of a non-hematopoietic origin. This allows for a powerful crosstalk between immune and non-immune cells to provide protection and promote inflammation.

when T_H17 effectors are restimulated with the differentiation cytokines IL-6 and TGFβ in the absence of IL-23, leading to coexpression of the regulatory cytokine IL-10 with IL-17A. These self-regulating cells are able to provide protection in a T_H17 mediated model of autoimmunity.³⁵

T_H17 Cell Effector Cytokines

In addition to IL-17A, other major cytokines produced by T_H17 cells include IL-17F, IL-21 and IL-22, in addition to CCL20 and TNFα to a lesser extent (Fig. 1). These cytokines play many diverse roles in the ability of T_H17 cells to drive both immunity and disease (Fig. 2).

IL-17A and IL-17F are closely related members of the IL-17 cytokine family. They commonly exist as homodimers, but can also form IL-17A/F heterodimers.³⁶ They signal through a heterodimeric IL-17RA and IL-17RC receptor complex, which can be

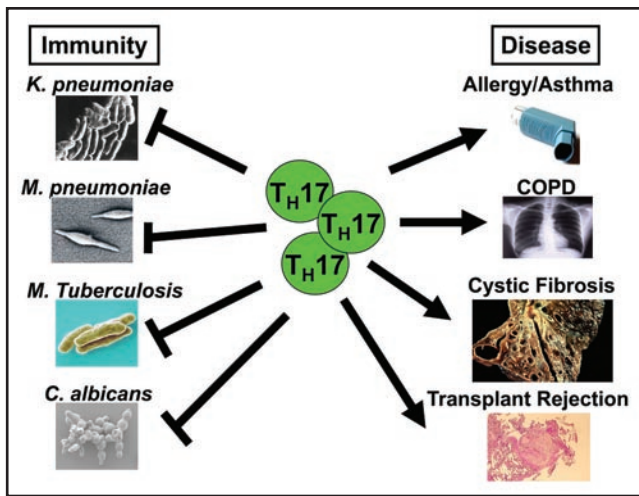


Figure 3. A T_H17 response can be both protective and pathogenic. T_H17 responses have been shown to be critical in providing pulmonary immunity to a range of pathogens. In addition, T_H17 responses can cause a range of pulmonary diseases.

found on cell types such as fibroblasts and epithelial cells,^{37,38} allowing for the T_H17 subset to cross-talk to non-hematopoietic cell types of the body. Stimulation of these cells with IL-17A or IL-17F leads to the production of inflammatory cytokines, chemokines, G-CSF and GM-CSF. This in turn supports an inflammatory state, extramedullary hematopoiesis and efficient neutrophil recruitment.³⁹⁻⁴² The differences between IL-17A and IL-17F are still under debate and investigation has been difficult. It is believed that IL-17A and IL-17F have dramatically redundant function, but that IL-17F may carry out those functions at a much lower degree of efficiency, perhaps due to its lower binding affinity to IL-17RA/RC.⁴³ However, recent work directly comparing IL-17A and IL-17F deficient mice has revealed some non-redundant functions for these two cytokines in infection and allergic responses.⁴⁴

As discussed above, IL-21 can help to maintain T_H17 lineage commitment. In addition, it has also been found that IL-21 can provide B-cell help and stimulate CD8⁺ T-cells, NK cells and myeloid cells.⁴⁵⁻⁴⁹ However, it has not yet been determined whether or not T_H17 derived IL-21 carries out these functions *in vivo*.

IL-22 is another effector cytokine produced by T_H17 cells that can act on non-hematopoietic cells including epithelial cells and hepatocytes.^{50,51} Under some circumstances IL-22 can act in synergy with IL-17A to promote the production of inflammatory mediators.⁵² In addition, IL-22 can promote the production of antimicrobial peptides to provide defense against a number of pathogens. IL-22 can also act to protect epithelium by promoting proliferation, repair mechanisms and barrier function.^{52,53} This cytokine is of particular interest because it appears to often provide protection in inflammatory states such as during inflammatory bowel disease (IBD),⁵⁴ colitis⁵⁵ and hepatitis.⁵⁶

Stimulation of purified naïve T-cells with IL-6 alone is able to induce expression of IL-22, but not IL-17A. However, further stimulation with increasing concentrations of TGFβ induces

expression of IL-17A, but dose dependently suppresses IL-22 expression.⁵⁷ Additionally, regulation with either IFNγ or IL-4 has a much more potent suppressive effect on IL-17A production than IL-22.⁵⁷ This information thereby provides potential targets for the suppression of a single effector molecule of T_H17 cells. As it appears that in some circumstances IL-17A promotes inflammation an IL-22 can provide protection, it may be useful to target this differential regulation and tip the scales towards protection in inflammatory disorders.

Much of the above work was determined using mouse models, however, many of the findings hold true using human cell lines and other *in vitro* studies. In addition to these cytokines, it has been found that significant portions of human T_H17 cells are also able to coexpress the T_H1 signature cytokine IFNγ.²² The significance of these dual producers has yet to be determined, however, they can be distinguished from single producers based upon expression of chemokine receptors.⁵⁸

T_H17 Cells in Pulmonary Immunity

Development of T_H17 cells has been shown to be critical in mounting an effective immune response to a wide range of pathogens within the lung (Fig. 3). These pathogens are commonly extracellular bacteria or fungi. Much of the data supporting these statements are based upon infection of murine strains deficient in T_H17 promoting or effector cytokines.

In research performed by Jay Kolls and colleagues, it was found that the T_H17 effector cytokine IL-17A plays a critical role in host defense to pulmonary infection with *Klebsiella pneumoniae*. Resulting in 100% mortality and increased bacterial dissemination in IL-17RA deficient mice following challenge.^{59,60} This group additionally found that IL-22 was also crucial for host defense to *K. pneumoniae* by acting in synergy with IL-17A, and also by promoting maintenance of the pulmonary epithelium.⁵²

Pulmonary challenge with *Mycoplasma pneumoniae* also results in the upregulation of CD4⁺ T-cell produced IL-17A. This production of IL-17A is dependent on the T_H17 terminal differentiation cytokine IL-23, and blocking antibodies to the IL-23p19 subunit decreased IL-17A production. This decrease in IL-17A production correlated with a decreased bacterial clearance and host defense.⁶¹

Interestingly, T_H17 cells can play other important roles than those found in primary infection. Although both a T_H1 and T_H17 response develop during a primary challenge with *Mycobacterium tuberculosis*, the absence or neutralization of T_H17 dependent IL-23 has been shown to have no effect on immune-mediated protection.^{62,63} Despite this finding, it was discovered that the memory T_H17 cells formed following protective vaccination against *M. tuberculosis* were critical for an accelerated and increased recall response to a secondary challenge. It was proposed that memory T_H17 responses increased the effectiveness of the vaccine through IL-17A-dependent production of chemokines for the recruitment of T_H1 cells and pulmonary immunity.^{64,65} This is a significant finding and could be applied to increase the efficacy of other vaccines.

Aside from extracellular bacteria, T_H17 cells have been found to be critical in providing protection against fungal infections. One such finding demonstrated a significant upregulation of

IL-17A in response to challenge with the fungi *Candida albicans*. Abrogation of IL-17A or its receptor led to a dramatically increased rate of mortality in mice following challenge.⁶⁶

Caveats to these experiments include that T_H17 cells are not the sole producers of IL-17A. In addition to CD4⁺ T-cells, IL-17A production has been found in $\gamma\delta$ T-cells,^{67,68} NKT cells⁶⁹ and CD8⁺ T-cells.^{70,71} IL-17A message has also been found in monocytes, neutrophils and eosinophils.⁷² Therefore, it is possible that the protective immunity observed could be attributed to these other cellular sources. Lineage specific deletions of cytokines and receptors must be performed in order to define the protective source as T_H17 cells.

Evidence testing the importance of T_H17 cells in the pulmonary immunity of humans has proven to be much more difficult to determine. However, two studies suggest that they may be just as important as was found in the mouse.

Memory T_H17 cells can readily be found in human peripheral blood following stimulation with either heat-killed *M. tuberculosis* or *C. albicans*.⁵⁸ Suggesting that these cells were at one time important for mounting an immune response to these pathogens. Additional insight into the importance of T_H17 cells in human pulmonary immunity came from an unlikely source. Hyper IgE syndrome (HIES) or Job's syndrome is characterized by frequent and severe fungal and bacterial pulmonary infections.⁷³ The genetic cause for HIES was recently determined to be due to a dominant negative mutation in the STAT3 molecule.^{74,75} STAT3 signaling is known to be critical in T_H17 development and one study found that HIES patients exhibited an inability to differentiate naive CD4⁺ T-cells into T_H17 cells, but not other T-helper lineages, suggesting their significance in pulmonary immunity to a range of pathogens that chronically infect these patients.⁷⁶

Several of the pathogens discussed above are opportunistic and can normally be cleared by immuno-competent individuals. Therefore, it may not be necessary to manipulate the T_H17 response to promote immunity. However, these diseases can become life threatening in immuno-compromised individuals, therefore making manipulation more desirable.

T_H17 Responses in the Elderly

It is of interest to note that respiratory tract infections are the leading cause of morbidity and mortality within the elderly population of the US.⁷⁷ A number of the disease causing pathogens require T_H17 responses for proper clearance and immunity.^{52,77,78} Although a number of factors contribute to this decreased pulmonary immunity,⁷⁷ it would be of interest to investigate the capacity of aged mice and elderly humans to mount protective T_H17 responses. To the best of our knowledge this is a question that remains to be investigated. In addition to extracellular bacteria and fungi, T_H17 cells are also getting more attention for a different role they may be playing during viral infections.

T_H17 Responses in HIV and AIDS

Uncontrolled HIV infection can be characterized by systemic activation of the immune system and eventual decline in CD4⁺ T-cell numbers, resulting in AIDS. Despite being in early stages of investigation,

a number of recent advances suggest that T_H17 cells may play a role in this series of events and represent a new therapeutic option.

It has been proposed that the systemic immune activation observed in progressive HIV infection can be attributed to microbial translocation from the gastrointestinal (GI) tract.⁷⁹ There is also a selective depletion of CD4⁺ T-cells from the GI tract of HIV infected individuals. This depletion occurs within the blood as well, but not in the lungs of HIV infected individuals, and significantly higher numbers and higher functioning HIV-specific CD4⁺ T-cells were found in the lung as compared to the blood.⁸⁰ When the subsets of remaining CD4⁺ T-cells were examined in HIV infected individuals and SIV-infected macaques, it was found that the T_H17 subset appeared to be selectively depleted in the GI tract and blood. This apparent selective depletion of T_H17 cells correlated with increased susceptibility to enteric bacterial dissemination,⁸¹ increased plasma viral levels and AIDS progression.⁸² This suggests that T_H17 cells play an important role in maintaining the gut epithelial barrier and preventing microbial translocation and subsequent systemic immune activation during HIV infection. In support of this, it was found that following HAART, there was a partial reconstitution of GI CD4⁺ T-cells and an increase in polyfunctional anti-HIV responses, both of which correlated with a reappearance of T_H17 cells.⁸³ Additionally, it was found that in SIV infection of sooty mangabeys, which do not succumb to AIDS, there was no selective depletion of T_H17 cells.⁸⁴

Therefore, it appears that T_H17 cells may play a significant but indirect role in HIV progression by preventing microbial translocation and subsequent systemic immune activation. Further studies must be conducted to verify that T_H17 cells are directly responsible for preventing GI microbial translocation and by what mechanism this is accomplished. One excellent candidate cytokine that may aid in this process is IL-22, which has demonstrated to be important in maintaining gut and lung epithelial barrier function, repair and production of antimicrobial peptides.^{52,53} It is possible that manipulation of the T_H17 response could aid in restricting systemic immune activation and permit an increased anti-HIV immune response. This data should also be taken into account when developing potential HIV vaccines, in which it may be useful to elicit an increased T_H17 memory response. It would also be of significant interest to determine the mechanisms by which T_H17 cells appear to be selectively depleted during HIV infection and develop therapies for its prevention.

Whether T_H17 cells play a more direct role in anti-HIV responses has yet to be determined. One report found HIV-specific T_H17 cells present in the peripheral blood of patients during early stages of infection, but these cells were reduced or non-detectable in chronic or non-progressive patients.⁸⁵ Conversely, another study found that in early HIV infected patients T_H17 cells were only specific for bacterial or fungal antigens, and not viral antigens.⁸⁴ Therefore, further studies must be conducted to clarify the specificities of T_H17 cells in response to HIV infection and any role they may have in directly targeting the virus.

T_H17 Cells in Pulmonary Disease

T_H17 cells are well known for the pathogenic role they play in autoimmune diseases. However, in the context of the lung, T_H17

cells are becoming increasingly linked to many other diseases including allergic asthma, transplant rejection, COPD and cystic fibrosis (Fig. 3). Evidence for the involvement of T_H17 cells is based upon mouse models and samples obtained from human patients.

Allergic asthma has long been considered to be a T_H2 driven disease of the lung, characterized by elevated IgE levels and eosinophilic infiltration.^{86,87} However, it was found that IL-17A message or cytokine was elevated in the bronchoalveolar lavage (BAL) and serum of some asthmatic patients,^{88,89} and increased levels of IL-17A correlated to increased severity of airway hypersensitivity.⁹⁰ Upon reexamination of asthmatic classifications, it appears that several forms of asthma are not characterized by an eosinophilic infiltrate, but rather by a neutrophilic infiltrate, such as in cases of non-atopic asthma and a severe form of steroid-resistant atopic asthma.⁹¹ Neutrophilic infiltration is characteristic of a T_H17 response, as many T_H17 effector cytokines can induce granulopoiesis and chemokine gradients suitable for neutrophil recruitment. Mouse models of airway hypersensitivity responses (AHR) have greatly varied in their dependence on T_H17 cells, most likely due to different sensitization and challenge protocols. However, these differences may also mimic the variability observed in human asthmatic disease.⁹¹ In support of this, a recent paper identified that in one model of AHR, T_H17 polarized cells and not T_H2 polarized cells, mediated a steroid-resistant form of AHR correlating to the neutrophilic infiltrate observed in steroid-resistant asthmatic patients.⁹² Therefore, it is likely that T_H17 cells drive some types of allergic asthma, while T_H2 responses dominate the rest.

Recent evidence suggests that acute and chronic rejection of lung transplants are partially mediated through T_H17 cells. Measurements of cytokine levels in the BAL of patients that received a lung transplant revealed an upregulation of IL-17A message which also correlated with a neutrophilic infiltrate.⁹³ Further studies identified T_H17 responses to be responsible for autoimmune or alloimmune rejection in rat models of lung transplantation, as well as, correlates in human patients.^{94,95} Taken together, current data suggests that T_H17 cells may mediate rejection through an autoimmune or alloimmune response, however, a more clearly defined role for T_H17 cells in lung transplant rejection must be investigated.

Chronic obstructive pulmonary disease (COPD) is another inflammatory lung disease in which the T_H17 pathway is shedding new light on its pathogenesis.⁹⁶ Although direct evidence is lacking, there appears to be a strong correlation of the involvement of a T_H17 response. In COPD patients, there is an association between a CD4⁺ T-cell airway infiltrate and a neutrophil airway infiltrate.⁹⁷ It is highly possible that the CD4⁺ T-cells could be recruiting the neutrophils through secretion of IL-17A. Additionally, through a number of genetic approaches, it has been found that there is an association of COPD susceptibility and gene polymorphisms in the T_H17 effector cytokine IL-17F and the T_H17 regulatory cytokine IL-27.^{98,99} This evidence for the involvement of T_H17 cells in COPD is still in early stages of understanding, however, the characteristics of COPD pathogenesis are fitting with the known pathways of T_H17 cells.⁹⁶

Cystic fibrosis (CF) is the most prevalent lethal genetic mutation in the Caucasian population, and is characterized by chronic pulmonary inflammation and progressive loss of lung function.¹⁰⁰ Mutations causing CF are known to occur in the cystic fibrosis transmembrane conductance regulator (*cftr*), which is important in anion transport across epithelial cells. These *cftr* mutations cause a disruption in a thin layer of fluid bathing the pulmonary epithelium, resulting in reduced clearance of inhaled particles and colonization with bacteria such as *Pseudomonas aeruginosa*.¹⁰⁰ An innate immune response composed of granulocytes, as well as an adaptive immune response ensues both of which correlate to an increase severity of lung disease in CF patients.¹⁰¹ Discovery of the T_H17 lineage has provided insight to this disease causing immune response in pioneering work performed by Jay Kolls and colleagues.¹⁰² A mouse model of *P. aeruginosa* pulmonary colonization found an induction of the IL-23 and IL-17A pathway, leading to a predominantly neutrophilic infiltrate.¹⁰³ More directly, measurements of cytokine levels in the sputum of CF patients demonstrated increase levels of IL-17A, IL-17F and IL-23,¹⁰⁴ leading to a hypothesis that CF lung pathology is T_H17 driven.¹⁰² Further research must be performed to determine the exact mechanism by which *P. aeruginosa* in CF patients leads to a disease causing T_H17 immune response. However, these findings can lead to significant advancements in therapies used to alleviate pulmonary disease in CF patients.

T_H17 Cells in Gut Diseases

Like the lungs, the gut is constantly exposed to a plethora of exogenous antigens, and a balance must be maintained to control immune responses. Similar to pulmonary diseases, there is also a growing body of evidence implicating T_H17 responses to inflammatory diseases in the gut. Evidence linking T_H17 responses include elevated levels of IL-17A and IL-22 in patients suffering from inflammatory bowel disease (IBD).^{105,106} A recent report also demonstrated an increased susceptibility and resistance to IBD was linked to polymorphisms in the IL-23 gene locus.^{107,108} In support of this, abrogation of IL-23 in mouse models of IBD greatly attenuates disease.^{109,110} It is likely that direct evidence further implicating T_H17 cells in the pathogenesis of inflammatory diseases of the gut will be discovered in the near future.

Manipulation of T_H17 Responses

As discussed above, T_H17 cells are involved in immunity to a range of pathogens, but can also contribute to pathogenesis in a number of immune-mediated diseases, all within the pulmonary environment. Therefore it is highly desirable to effectively manipulate T_H17 cells to promote responses in the former and inhibit responses in the latter (Fig. 4).

As reviewed, a number of targets exist to either promote or inhibit T_H17 responses. It must be decided whether to target the differentiation, maintenance or effector pathways. Further investigation must be conducted into which target results in the best therapeutic efficacy. Additionally, these cells have many diverse functions, some of which presumably have yet to be defined, and therefore inhibiting the development of the lineage rather than a

specific effector molecule may have adverse effects. A number of T_H17 effector molecules also have different regulatory mechanisms such as the negative feedback loop that exists in the production of IL-17A,¹¹¹ and therefore a partial blockade of IL-17A could eventually lead to increased or prolonged production. Therefore regulation of a targeted T_H17 molecule must be taken into consideration when designing a therapy.

The method of manipulation will also have a tremendous impact on the efficacy of a therapy. A number of therapeutic options are available for manipulation of a T_H17 response. These options include the use of drugs, or more directly immune-based therapies such as recombinant cytokines, monoclonal antibodies and DNA-based approaches. Each of these strategies has advantages and disadvantages. It also must be taken into account that the target of these therapies is the pulmonary mucosal environment, thereby adding another layer of discrimination. We have summarized some of the current data regarding the use of these agents as therapies, and their ability to be effectively targeted to the lungs (Table 1).

Drugs are an attractive and popular method to manipulate immune responses. A number of immunostimulatory and immunosuppressive drugs are available and are currently being used to treat pathogen and immune mediated diseases discussed above. These drugs are effective, yet also have numerous disadvantages. It is common for these drugs to have many undesirable side effects, ranging from nausea to death. Frequently these drugs will also be exceedingly expensive and require multiple treatments. Pulmonary delivery of such drugs is also not always feasible and will often result in undesirable systemic effects.

Currently no recombinant cytokines have been approved for use within the US to treat pulmonary disease. Data is still lacking on the effectiveness of using recombinant cytokines to treat pulmonary disorders, despite demonstrating effectiveness in the treatment of other diseases. Therefore it is possible that recombinant cytokines would make a good target for the manipulation of T_H17 responses. However, a number of discouraging questions remain regarding the stability of recombinant cytokines and their ability to be targeted to only the lung and not at significant levels systemically.¹¹²

Monoclonal antibodies are known for their exquisite specificity, which is a highly desirable characteristic of a potential therapy. However, the use of monoclonal antibodies in the treatment of human diseases, in particular pulmonary diseases, can have a number of drawbacks. Monoclonal antibodies are generally created using rodents, and therefore when introduced into a human would create an undesirable anti-rodent immune response. This would also greatly decrease the efficiency if multiple treatments were required. It is possible for these antibodies to be “humanized” to reduce their reactivity, this is however an expensive process and cannot guarantee that an anti-ideotypic response would not occur. Also, there is currently no effective method to target monoclonal antibodies to the lung.¹¹³

The use of DNA-based approaches for the manipulation of pulmonary T_H17 responses has a large number of advantages. Production of the DNA and vectors is significantly more cost

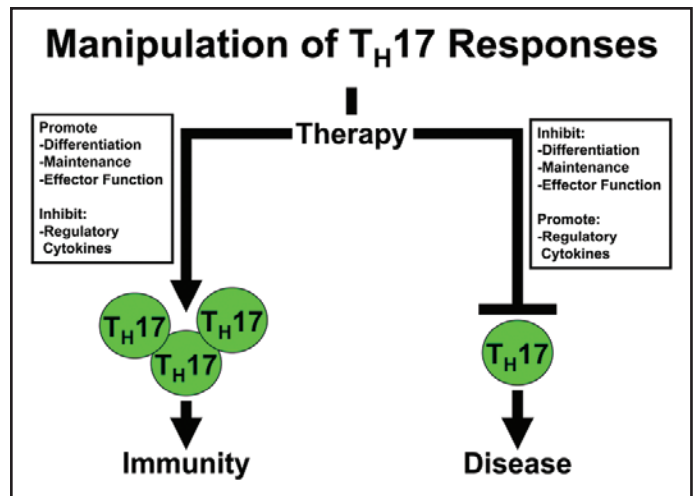


Figure 4. Manipulation of T_H17 responses. In the manipulation of T_H17 cells, it would be desirable to promote responses that enhance immunity or inhibit responses to prevent disease. Various developmental, maintenance, regulatory and effector pathways exist for therapeutic targeting.

effective than other approaches, and their efficiency has been demonstrated in a number of other disease settings. DNA and vectors are also easy to manipulate for the incorporation of a number of targets. In addition, there is an ample variety of vectors available ranging from those with a high immunogenicity to naked-DNA with almost no natural immunogenicity (Table 2). Thereby providing methods to effectively induce a T_H17 response when influencing immunity, or suppress T_H17-mediated disease without potentially worsening the inflammatory state by enhancing immune activation. Furthermore, many of these vectors were derived from viruses that primarily infect mucosal surfaces, and would therefore efficiently target therapies to the pulmonary environment. It is also possible to make use of naked-DNA loaded into liposomes, which can then be effectively targeted with different specificities in the lung.^{114,115}

There are disadvantages to the use of DNA-based vaccination including theoretical safety issues regarding integration, autoimmunity and transfer of antibiotic resistance.^{114,115} However, these are highly unlikely outcomes, and resolutions are already being put into place to monitor and prevent these undesirable side effect.^{114,115} Additionally, although clinical trials have thus far demonstrated that the use of DNA-based vaccination approaches is well tolerated and safe, they have not yet demonstrated a similar potent immunogenicity as observed in mice. In spite of this, it is believed that immunogenicity will be improved with advancements in, plasmid and gene optimization, formulation of standard and immune plasmid adjuvants and delivery methods.^{114,115} Therefore we believe that DNA-based approaches hold a promising future in the development of T_H17 manipulating techniques.

In addition to these DNA-based approaches, it is possible to use different adjuvants and co-immunizations to help improve the efficacy of vaccination. Recent pioneering work by Bin Wang

Table 1 Potential therapeutic options for the manipulation of T_H17 responses

	Advantages	Disadvantages	Pulmonary delivery
Drugs	Potent, many available	Potential toxicity, side effects, expensive, systemic	Varies
Recombinant Cytokines	Effective, many available	Transient, systemic, high doses required	Difficult, degradation issues
Antibodies	Highly specific	Immuno-reactive, systemic, must be humanized, requires large doses	Not yet possible, degradation and delivery issues
DNA-based	Cheap, effective, specifically target, easy to manipulate	Theoretical safety concerns with integration and autoimmunity, immunogenicity translation to humans	Varies, some mucosa-tropic vectors

Potential methods of manipulation include the use of drugs, recombinant cytokines, antibodies and DNA-based approaches. All methods come with various advantages and disadvantages. They must also be considered on their ability to target the lungs.

Table 2 Methods of DNA-based approaches for manipulation of T_H17 responses

DNA therapy and vaccination approaches	
Non-Viral Transfection: Inefficient, safe, transient	Viral Transduction: Efficient, less safe, some stable integration
↓	↓
Naked DNA: Direct injection of plasmid, very safe, poor efficiency, cheap, frequent boost, can be engulfed by pulmonary APCs	Pox Virus: Elicits strong immune response, safe, stable, good mucosal delivery
Lipofection: Enhanced cell entry, no toxicity, transient, can target to pulmonary APCs	Adenovirus: Very immuno-potent, easily manipulated, infects pulmonary mucosa
Electroporation: Difficult, alternative	Alpha virus Replicon: Capable of transducing DC's, induces both adaptive and innate immune response.
	VSV/HSV: Infects mucosal surfaces
	Listeria/Salmonella: Infects APCs, low immunogenicity in humans, mucosa tropic

A number of different options exist for the use of DNA-based approaches for the manipulation of T_H17 responses. These options vary in the ability to stimulate the immune system, as well as their ability to target a pulmonary environment. A number of these could be used to manipulate T_H17 immunity and disease.

Table 3 Current clinical trials in which therapies may be targeting T_H17 responses

	Target	Disease	Outcome	T _H 17 Manipulation
ABT-874/J695	IL-12/IL-23 p40	Crohn's Disease	Increase in response rates	Reduction in serum IL-17A protein
anti-IL-12p40	IL-12/IL-23 p40	Psoriasis	75% improvement in the psoriasis area and severity index	Unknown, decrease in IL-23p19 mRNA
Tocilizumab/MRA	IL-6R	Arthritis	Abrupt reduction in disease activity, improvements in disease score	Mouse models demonstrate inhibition of T _H 17 differentiation

A number of clinical trials exist which are aimed at treating diseases that have recently been determined to be strongly associated with the T_H17 pathway. The drug name, target, response and effect on the T_H17 pathway are summarized.

and colleagues has demonstrated that co-administration of a protein with DNA coding for that protein can confer protection to allergic and autoimmune diseases through the induction of regulatory T-cells.¹¹⁶⁻¹¹⁸ This group additionally demonstrated the converse that regulatory T-cells could be inhibited and the immunogenicity of vaccination increased through use of an inhibitor of regulatory T-cells as an adjuvant.¹¹⁹ Thereby demonstrating the vast range of combinatorial possibilities with DNA-based vaccination approaches to either increase or suppress immune responses.

Other vaccination strategies aside from solely DNA-based and protein-based approaches may also be useful in influencing

the responses of T_H17 cells. In pathogen-mediated pulmonary diseases, it is possible that use of live, attenuated or killed micro-organisms in combination with T_H17 promoting or inhibiting mediators would be efficient in protecting the host. These T_H17 mediators could be in the form of protein or DNA.

Current Clinical Trials

Current clinical trials exist which are aimed at providing therapy to diseases that are now believed to be at least partially driven by T_H17 responses. These trials involve non-pulmonary diseases, including Crohn's disease, psoriasis and arthritis. However, it is difficult to determine whether or not the therapies are directly

targeting the T_H17 pathway or if they are working by inhibiting other immune functions (Table 3).

One such treatment is the use of monoclonal antibodies to IL-12p40 in patients suffering from Crohn's disease¹²⁰ or psoriasis.^{121,122} These antibodies block a shared subunit of the T_H1 promoting cytokine IL-12, as well as, the T_H17 promoting cytokine IL-23. Although these treatments demonstrate promising results,¹²³ it is not known as to whether the effects are due to a downregulation of T_H1 cells, T_H17 cells, or both. However, one such study found a reduction of IL-17A production in the gut of patients receiving the anti-IL-12p40 therapy,¹²⁴ suggesting at least a partial effect on the T_H17 lineage.

Another therapy is the blockade of IL-6 signaling by a humanized anti-IL-6 receptor antibody, which has shown great promise in the treatment of juvenile idiopathic arthritis¹²⁵⁻¹²⁷ and rheumatoid arthritis.¹²⁸⁻¹³¹ Since it is known that IL-6 can help drive the differentiation of human T_H17 cells, it is predicted that one mechanism of this therapy is through inhibition of the T_H17 pathway. However, IL-6 is a cytokine with many different functions and therefore it is again difficult to determine exactly what role it is playing in arthritis. Although, one recent study of murine arthritis found that an IL-6R blocking antibody prevented arthritis onset by blocking the differentiation of T_H17 cells.¹³² Further data will need to be collected from these clinical trials before a conclusion can be drawn in humans.

Conclusion

We have reviewed the T_H17 pathway and the roles this pathway is known to play in pathogen-mediated and immune-mediated pulmonary diseases. The T_H17 pathway presents a number of targets for its promotion and suppression. Research must be conducted to further define the human T_H17 pathway, as well as, to identify feasible targets for manipulation in each disease setting. This can be accomplished using previously established primate models of pulmonary diseases discussed above,¹³³⁻¹³⁶ which have thus far not been utilized to investigate contributions and potential manipulations of the T_H17 pathway. These investigations are possible as many commercially available reagents for human T_H17 research are highly cross-reactive and can often be used interchangeably with primates. Although a number of different methods for manipulation exist, we propose that the use of combinatorial DNA-based approaches is the most promising and versatile mechanism available.

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