

Jay K. Kolls

CD4⁺ T-cell subsets and host defense in the lung

Author's address

Jay K. Kolls¹

¹Children's Hospital of Pittsburgh, University of Pittsburgh, Pittsburgh, PA, USA.

Correspondence to:

Jay K. Kolls

Children's Hospital of Pittsburgh,
Department of Pediatrics RRB 8130,
4401 Penn Avenue
Pittsburgh, PA 15224, USA

Tel.: +1 412 7710

Fax: +1 412 7636

e-mail: jay.kolls@chp.edu

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Summary: CD4⁺ T-helper subsets are lineages of T cells that have effector function in the lung and control critical aspects of lung immunity. Depletion of these cells experimentally or by drugs or human immunodeficiency virus (HIV) infection in humans leads to the development of opportunistic infections as well as increased rates of bacteremia with certain bacterial pneumonias. Recently, it has been proposed that CD4⁺ T-cell subsets may also be excellent targets for mucosal vaccination to prevent pulmonary infections in susceptible hosts. Here, we review recent findings that increase our understanding of T-cell subsets and their effector cytokines in the context of pulmonary infection.

Keywords: CD4⁺ T cells, pneumonia, mucosal vaccination

Introduction

CD4⁺ T-helper (Th) cells are critical components of the adaptive immunity in the lung (Fig. 1). The development of these cells arises after priming with antigen presented by class II major histocompatibility complexes (termed signal 1). Further activation occurs after the engagement of costimulatory molecules and their receptors expressed on both antigen-presenting cells (APCs) and T cells (termed signal 2). This second signal is critical for both generating antigen-specific effector T cells as well as memory cells. Presentation of antigen in the absence of costimulation can result in T-cell anergy. Final effector function is due to cytokine/growth factor-directed CD4⁺ T-cell differentiation (signal 3). This latter aspect of differentiation is driven by lineage-specific transcription factors as well as changes in chromatin remodeling. The critical role of CD4⁺ T cells in lung immunity and pulmonary host defenses was clearly demonstrated by the high incidence of pulmonary infections as a complication of human immunodeficiency virus (HIV) infections/acquired immunodeficiency syndrome (1–3).

CD4⁺ T-helper subsets

Th2 cells

Th2 CD4⁺ T cells differentiate from naive precursors under the direction of the transcription factor GATA3 and signal

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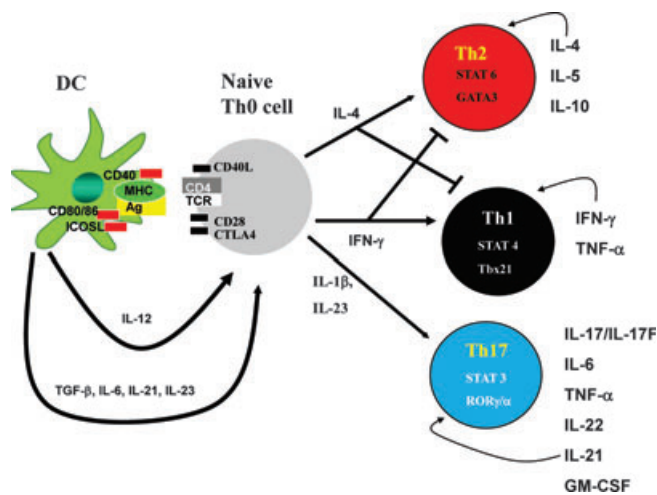


Fig. 1. CD4⁺ T-helper cell differentiation. Antigen is presented by dendritic cells (DCs) by class II major histocompatibility complex (MHC) to naive CD4⁺ Th0 cells. Full T-cell activation requires a second signal consisting of the upregulation and expression of costimulatory molecules such as inducible costimulatory ligand (ICOSL), CD28, and cytotoxic T-lymphocyte antigen-4 (CTLA-4). T-cell differentiation is instructed by cytokine/growth factor signals. T-bet can be activated by STAT1 as well as bind to the IFN γ locus followed by induction of IL-12 β R2. IL-12 activates STAT4, which can further drive Th1 development. IFN γ can act in an autocrine manner to further augment Th1 differentiation. IL-4 induces Gata3, which further induces IL-4 and supports the differentiation of Th2 cells. Th2 cells produce IL-4, IL-5, and IL-13 as their effector cytokines. TGF β and IL-6 can induce ROR γ t expression as well as activation of STAT3. This leads to induction of the IL-23 receptor, rendering these cells responsive to IL-23, which is required for terminal differentiation of Th17 cells. Th17 cells produce the cytokines IL-17/IL-17F, IL-22, IL-21. IL-21 can act in an autocrine fashion to further the differentiation of the Th17 lineage.

transducer and activator of transcription 6 (STAT6) (4). Effector cytokines produced by Th2 cells include interleukin-4 (IL-4), IL-5, and IL-13. GATA3 binds directly to the *Il4* locus, and IL-4 and signaling via STAT6 is critical for further Th2 proliferation and lineage commitment (4). Th2 effector cytokines mediate immunity against infections with helminths (Fig. 2). Deletion of *Gata3* in mice results in embryonic lethal, but conditional deletion of *Gata3* in T cells confirms the essential role of this transcription factor in Th2 differentiation and the expulsion of helminths from the gastrointestinal tract (5). IL-5 is an essential growth factor for eosinophilopoiesis (6, 7). Mice with homozygous deletion of *Il5* have substantial reduction in both peripheral and bone marrow eosinophils (6, 7). In contrast, overexpression of IL-5 protein results in substantial eosinophilia in blood and tissues (8). IL-13 signals through a receptor complex of IL13RA1 and IL4R α and activates STAT6 signaling. These receptors are expressed on airway epithelium as well as airway smooth muscle. In bronchial epithelium, IL-13 is a

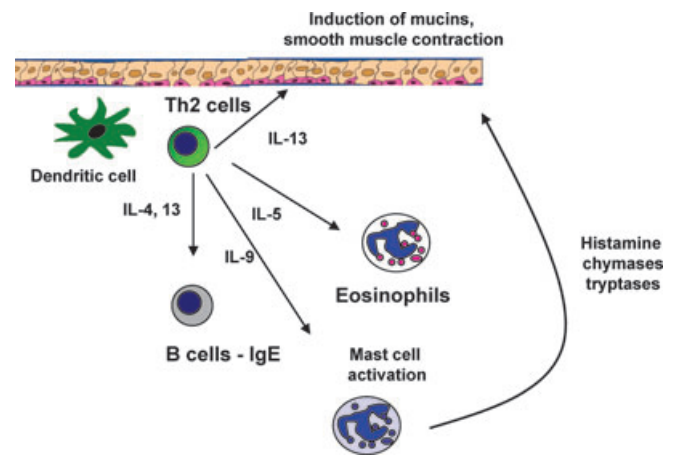


Fig. 2. Th2 cells and immunity at the mucosa. Allergen parasites or helminthic infection can induce TSLP and IL-25 in the lung epithelium. These cytokines can drive early IL-4 production leading to the differentiation of Th2 cells. IL-4 and IL-13 can drive the induction of IgE as well as stimulate epithelial cells to increase mucous production. IL-5 is a critical regulator of eosinophilopoiesis. Binding of IgE to Fc ϵ R on mast cells leads to their degranulation and release of chymases, tryptases, and histamines.

major factor in mucous production and goblet cell differentiation in the airway (9, 10). Moreover, IL-13 signaling via STAT6 in airway smooth muscle and in airway epithelium leads to airways hyperresponsiveness to methacholine (9, 10). These cells and effector cytokines have been implicated in diseases such as allergic rhinitis, atopic dermatitis, and asthma (11). Anti-IL-5 has been investigated in asthma and although initial studies did not show clear cut efficacy (12), but subgroups of patients with high sputum eosinophilia respond to IL-5 blockade (12). Similarly, initial studies with IL-13 blockade were also negative, but recently, studies suggest that by stratifying patients with IL-13 driven asthma (by assessing the serum level of periostin, an IL-13 regulated gene in lung epithelium) can identify subgroups who respond to anti-IL-13 (13). In addition to asthma, IL-13 has been implicated in fibrotic processes in the lung in response to drugs such as bleomycin (14–16).

Th2 cells also facilitate B-cell differentiation and antibody responses to T-cell-dependent protein antigens (17), including the development of an anti-immunoglobulin E (IgE) response. It has been recently recognized that Th2 cell differentiation is not only regulated by IL-4 but also several cytokines produced by the lung epithelium, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 (17). It has been shown recently that polymorphism in both IL-33 and its receptors ST2 is associated with asthma, strongly implicating Th2 cells and specifically the IL-33 ST2 signaling pathway in this disease.

Th1 cells

Th1 cells, initially described by Mossman and Coffman (18), were defined by their ability to express interferon- γ (IFN- γ). Differentiation of CD4⁺ Th1 cells is controlled by the transcription factors T-bet (19) and STAT4. Differentiation is controlled by IL-12p70, which is a heterodimer of IL-12p35 and IL-12p40 subunits (20). However, both *in vitro* and *in vivo* Th1 differentiation can be independent of IL-12. One critical IL-12-independent pathway is through the induction of type I interferons that can facilitate Th1 differentiation in certain situations (21). IFN- γ which signals via a receptor complex consisting of two IFN- γ R1 and two IFN- γ R2 chains can signal in an autocrine paracrine fashion to further amplify Th1 differentiation and lineage commitment. IFN- γ receptors are expressed on a wide variety of cells including myeloid cells including macrophages and dendritic cells (DCs), as well as structural cells in the lung such as epithelial cells and fibroblasts (22). IFN- γ R1 and IFN- γ R2 activate Janus-associated kinases 1 and 2 (JAK1/2), which phosphorylates STAT1. STAT1 undergoes homodimerization and translocation to the nucleus and binds to DNA-encoded γ -activated sequences that ultimately control gene transcription (22).

IFN- γ is critical for mediating immunity and host resistance to many intracellular infections including *Mycobacterium tuberculosis*, *Listeria monocytogenes*, and *Salmonella typhimurium*. Patients with mutations IL-12p40, IFN- γ , or receptors for IL-12 or IFN- γ have increased susceptibility to intracellular infections due to these organisms (23, 24). Patients with IFN- γ receptor mutations can develop disseminated infection with bacillus Calmette-Guerin (BCG) that is resistant to antibiotics and IFN- γ therapy (23, 25). Patients with IL-12p40 mutations can also develop BCG or *S. typhimurium* infection, but theoretically can respond to IFN- γ (24). Thus, there is strong evidence that this pathway is essential for human control of these intracellular pathogens.

Th17 cells

Th17 cells are recently described effector lineage of T-helper cells that produces IL-17A, IL-17F, IL-21, IL-22, and IL-26 (the latter expressed in human cells). Th17 cells differentiate under control of the transcription factors retinoid orphan receptor- γ (ROR γ), ROR- α , and STAT3 (26, 27). It was initially believed that one of the critical instructional cytokines for Th17 differentiation was IL-23 (28); however, IL-23 receptors are not expressed on naive CD4⁺ T cells. Landmark studies published in 2005 showed that these cells develop

independently of STAT4 or STAT6 as well as T-bet or GATA3 (29, 30). These data implicated that Th17 cells were a distinct CD4⁺ T-cell lineage (29, 30). Th17 differentiation can occur with stimulation with transforming growth factor- β (TGF- β) and IL-6 (31–33). Moreover, this cytokine/growth factor combination induces the expression of IL-23R (31–33). Signaling via IL-23 allows terminal differentiation and expansion of Th17 cells (34). Another critical effector cytokine produced by Th17 cells is IL-22, which is controlled by IL-23 as well as the transcription factor the arylhydrocarbon receptor (Ahr) (35). IL-21 (36, 37) can function in an autocrine manner to further expand Th17 differentiation (Fig. 1). It has been recently shown *in vivo* that TGF β activation and thus differentiation of Th17 cells requires activation of latent TGF β by α v integrins (38, 39). This pathway is not required for IL-17 production by $\gamma\delta$ T cells (38, 39).

T-follicular helper cells

T-follicular helper (Tfh) cells are a subgroup of CD4⁺ T cells that are located in the B-cell follicle region of secondary lymphoid tissues including lymph nodes and bronchial-associated lymphoid tissues in the lung. These cells regulate T-cell-dependent B-cell activation through the expression of CD40L and IL-21 (40). These cells develop under the control of the transcription factor Bcl-6 (40) and are also regulated by inducible costimulatory (ICOS).

T-regulatory cells

Treg differentiation is controlled by the transcription factors Foxp3 and STAT5 (41). These cells are essential for mediating tolerance to inhaled antigen in the lung (41). Deletion of these cells or abrogating their effector molecules, which include IL-10 and TGF β , prevent airways sensitization to allergen as well as allergic inflammation (41). These cells can suppress the effector activity of many T-helper subsets and can be thymically derived (natural Tregs) or induced in the periphery (iTregs). An exhaustive review of these cells is beyond the scope of this chapter, but the reader is referred to excellent thorough reviews (41, 42) of these cells if they seek a more in-depth description of these cells.

Non-CD4⁺ T-helper cell sources of Th1/Th2/Th17 effector cytokines in the lung

Other cells in the lung exist that produce many of the same effector cytokines as Th1, Th2, or Th17 cells including innate lymphoid cells, natural killer (NK) cells, and $\gamma\delta$ T

cells. $\gamma\delta$ T cells are resident in mucosal sites including the lung and can produce IFN- γ , IL-4, IL-17, and IL-22 (43). These cells are a major source of early IL-17 after pulmonary infection, and IL-17 production by these cells is regulated by IL-23 and IL-1 β (44–48). $\gamma\delta$ T-cell production of IL-10 has been shown to play a key counter-inflammatory role in some pulmonary infections such as *Pneumocystis* infection (49).

NK cells can also produce IL-4, IFN γ , and IL-17 (50) in the lung in response to infection, allergen, or ozone. One population that has been extensively studied is a population that expresses an invariant T-cell receptor (iNKT cells) that recognizes a galactolipid *Sphingomonas*, α -galactosylceramide (51, 52). These cells are elevated in the bronchial alveolar lavage fluid of patients with asthma (53, 54). NKT cells produce IFN γ in response to *S. pneumoniae* pulmonary infection (55) and IL-17 in response to *Escherichia coli* lipopolysaccharide (LPS) (50). These cells also produce IL-17, and this response is critical for airways hyperresponsiveness to ozone (56). NK cells can develop under the control of IL-15 and express antiviral molecules such as IFN- γ as well as cytotoxic molecules (57).

Innate lymphoid cells are also important sources of effector cytokines in the lung. These cells are defined by the lack of lineage markers and T-cell receptors, but they require IL-7 signaling for their development. Thus, these cells are present in recombination-activating gene 1 (RAG1) or RAG2^{-/-} mice, but are lacking in RAG2, common γ chain (γ c) double deleted mice (58, 59). Retinoid orphan receptor γ t (ROR γ t)-expressing cells are critical for the formation of secondary lymphoid tissues (via regulation of lymphotoxin expression) and play critical roles in mucosal immunology in the gastrointestinal tract through the production of IL-17 and IL-22 (60). Type 2 ILCs produce IL-5 and IL-13 and participate in the clearance of helminths from the gastrointestinal tract (61). These cells appear to be regulated by IL-25 (IL-17E) as well as IL-33, a member of the IL-1 family. Recently, it has been demonstrated that a population of ILCs produce IL-13 in response to IL-33 induced by viral infection, and these cells mediate in part viral induced exacerbation of allergic disease in the lung (62). These cells can also be activated by protease allergens to drive eosinophilic airways inflammation as well as airways hyperresponsiveness (58) under control of IL-33 and TSLP. Thus, these cells recapitulate many aspects of CD4⁺ T-cell immunity in that there are subsets that express similar effector molecules, yet these cells are activated early and their activation is independent of TCR stimulation.

CD4⁺ T-cell effector cytokines in the lung

Type 2 effectors

Both IL-4 and IL-13 activates STAT6 signaling in a variety of lung cells including alveolar macrophages, fibroblasts, airway smooth muscle, and airway epithelium. In macrophages, activation of STAT6 leads to what is termed 'alternative macrophage activation' which is characterized by the expression of arginase 1, YM1, YM2, and the macrophage mannose receptor (63). It has been shown that IL-4 treatment of macrophages can reduce their phagocytic ability, but IL-4 stimulation of macrophages can augment the clearance of apoptotic neutrophils (63). Alternatively, activated macrophages (AAMs) play regulatory roles in helminth infection and can reduce immunopathology (63). It has also been shown that AAMs have augmented dectin-1 expression as well as the macrophage mannose receptor. Given that both of these receptors are critical in recognizing the fungal carbohydrates β 1,3 glucan and mannan, respectively, AAMs may have greater fungicidal activity. Induction of AAMs may also be exploited by pathogens to allow their survival. For example, *Francisella tularensis*, a virulent pathogen in the lung, can prolong its intracellular survival via induction of AAMs (64).

As mentioned earlier, IL-13 induction of STAT6 signaling induces several mucin genes in airway epithelium including *Muc5ac* and *Muc5b* as well as inducing goblet cell hyperplasia (65). These signaling effects are critical for host defenses against helminths such as *Nippostrongylus brasiliensis* (66). During viral infection, airways mucins can prevent viral spread; however, in infants this can also contribute to airway obstruction. The role of IL-13 in viral infection is complex, and IL-13 is not necessarily beneficial to the host. For example, IL-13 has recently been shown to increase the susceptibility of epithelial cells to infection with rhinovirus (67).

Type 1 effectors

There are several mechanisms by which IFN- γ controls lung immunity to intracellular pathogens. IFN- γ can prime macrophages to enhance their intracellular microbicidal activity (68) in a process termed classical activation of macrophages (69). IFN- γ priming augments Toll-like receptor (TLR) signaling (70). IFN- γ also increases microbicidal activity via the induction of inducible nitric oxide synthase, which regulates the production of reactive nitrogen intermediates (71, 72). IFN- γ can also increase the production of reactive oxygen species. These activities may explain the therapeutic benefit of IFN- γ in patients with

chronic granulomatous disease due to mutations in NADPH oxidase (73–75).

IFN- γ signaling also upregulates class II major histocompatibility complex molecules and costimulatory molecules such as CD80 and CD86, which can augment antigen presentation to naive T cells. IFN- γ 's first observed activity was its ability to suppress viral replication in many target cells including macrophages, fibroblasts, and lung epithelial cells (76). This occurs in part through the induction of many antiviral genes such as MxA (77). However, other respiratory viruses including severe acute respiratory syndrome coronavirus are controlled by IFN γ through mechanisms that are independent of MxA (78).

IFN- γ signaling in lung structural cells including fibroblasts and epithelium cells induces several chemokine ligands for CXCR3, including CXCL9, CXCL10, and CXCL11. CXCR3 is expressed on Th1 cells, and thus, the induction of these chemokines by IFN- γ may be a critical mechanism to increase the recruitment of Th1 cells. Moreover, these chemokines are critical for granuloma formation, which is essential for control of many intracellular pathogens such as *M. tuberculosis* (79, 80). Systemic and aerosolized IFN- γ has been investigated for the potential adjunctive treatment of tuberculosis. A recent meta-analysis showed that IFN- γ was well tolerated and associated with higher sputum sterilization rates (81); however, definitive randomized control trials are lacking to make firm conclusions on the efficacy of this cytokine.

Type 17 effectors

IL-17 can signal in human bronchial epithelium (HBE) to induced chemokine ligands for CXCR2, such as CXCL8, CXCL1, and the granulopoietic growth factor G-CSF (82, 83). Like other cell systems, HBE responses to IL-17 or IL-17F are augmented by TNF- α (82, 84). HBE cells express IL-17RA, IL-17RC, as well as IL-22R and IL-10R2, the receptors for IL-22 (82, 83) (Fig. 3). As IL-17A and IL-17F can be coexpressed in the same cell, it has been reported that these two IL-17 family members can form three cytokines including IL-17A homodimers, IL-17A/F heterodimers, which have intermediate activity compared with IL-17A homodimers, and IL-17F homodimers, which have the least potent activity (85). One mechanism by which IL-17 increases the production of chemokines is through increasing mRNA stability of this transcript (86, 87) resulting in augmented protein production. A similar mechanism has also been reported for IL-17-mediated increases in G-CSF production (88).

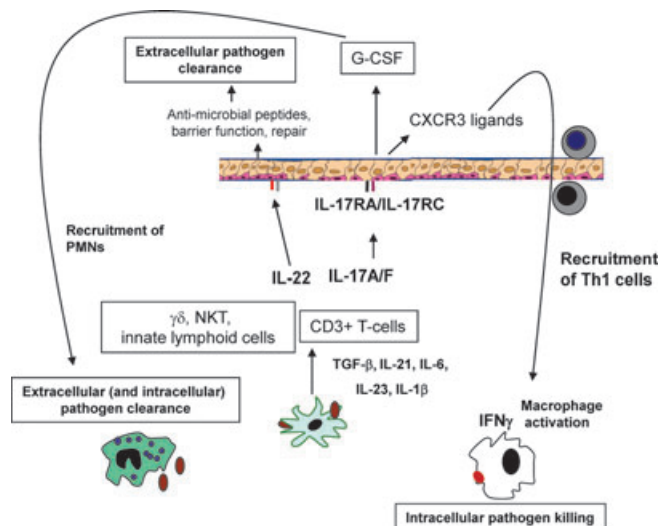


Fig. 3. Th17 cytokines in mucosal immunity. Many infections, including those caused by fungi and bacteria, can activate dendritic cells and macrophages to produce interleukin-6 (IL-6), IL-23, and IL-1 β . IL-23 and IL-1 β can drive IL-17 production by both innate lymphoid cells and $\gamma\delta$ T cells. Differentiation of Th17 cells requires TGF β , and a critical mechanism of activation of TGF β in the lung is through the activation of this growth factor by α integrins. IL-17 and IL-22 can signal to the epithelium to augment G-CSF as well as ligands for CXCR2 that mediate the recruitment of neutrophils. These two cytokines also induce the expression of anti-microbial proteins such as lipocalin-2 and β -defensins. IL-22 can also augment epithelial repair. After vaccination, Th17 cells through the production of IL-17 can also induce ligands for CXCR3 that increase the recruitment of IFN γ -producing Th1 cells, which can also help control intracellular pathogen growth.

IL-17RA is ubiquitously expressed on many cells including myeloid cells; however, these cells express very little IL-17RC. Thus, IL-17A and IL-17F have limited activity on myeloid cells. It has been reported that IL-17 can enhance IL-12p70 in alveolar macrophages (89) as well CCL2, CCL3, GM-CSF, IL-1 β , and IL-9 in CD4⁺ T cells (90). Th17 cells express IL-17RA and IL-17RE, which form a receptor complex for IL-17C (91–93). IL-17C can increase the production of IL-17 by these cells (91–93). IL-17C can be expressed in lung epithelium (unpublished observations) and thus can serve as a feed forward mechanism by which the epithelium could influence interstitial T-cell responses. In addition to regulating neutrophil recruitment in the lung (94), IL-17 augments apical bicarbonate transport in HBE cells (95). Carbonate anion regulates the activity of β defensins (96), and this may be an important mechanism of IL-17's net anti-microbial effect in the lung mucosa.

IL-17RA is required for host resistance to the extracellular pathogens *K. pneumoniae* (94), and in this model IL-17RA regulates the local production of CXCR2 ligands as well

granulopoiesis through the regulation of G-CSF. IL-17RA is dispensable for the control of intracellular pathogen *M. tuberculosis* (83), but is required for the control of the intracellular pathogen *F. tularensis* (89). In this latter model, IL-17 regulates IL-12p70 production by macrophages, which subsequently generates the Th1 CD4⁺ T-cell response ultimately controls the pathogen (89).

IL-22 signals through STAT3 in HBE cells. IL-22 increases the clonogenic potential of HBE cells in colony assays (83) and also augments repair to puncture injury in confluent HBE cells (97) (Fig. 3). IL-17 and IL-22 stimulation of HBE and mouse tracheal epithelial cells induces several antimicrobial genes in lung epithelium including lipocalin 2 (83) and regenerating islet-derived protein 3- γ (98). Neutralization of IL-22 during experimental *K. pneumoniae* lung infection results in rapid bacteremia, which substantially increases mortality in this model. IL-22 is regulated by IL-23 and recombinant IL-22 can rescue IL-23-deficient mice (83). A complication pneumonia is acute lung injury which can be exacerbated by mechanical ventilation also called ventilation-induced lung injury (VILI). In a model of VILI, recombinant IL-22 has also been shown to decrease lung leak and improve lung fluid dynamics (99). These data support a potential therapeutic role of IL-22 in diseases such as severe pneumonia or acute respiratory distress syndrome.

In primary infection, early sources of IL-17 and IL-22 can be from innate lymphoid cells (100), NK or NKT cells (100, 101), or $\gamma\delta$ T cells (44, 45, 102). However, CD4⁺ Th17 cells can be elicited in the lung by vaccination. Vaccine-induced Th17 responses have been shown to play protective roles in a diverse set of organisms including both intracellular and extracellular bacteria as well as fungi. For example, Th17 cells induced by vaccination with the antigen from *M. tuberculosis* ESAT-6 induce a population of Th17 cells that augment the local production of ligands for CXCR3 in the lung and result in substantial enhanced recruitment of

protective Th1 cells (103). Fungal-specific Th17 cells have also been shown to be critical for vaccine-induced protection against *Coccidioides posadasii*, *Histoplasma capsulatum*, and *Blas-tomyces dermatitidis* infection (104). In this setting, the protection against fungal challenge was dependent on IL-17 regulation of neutrophil recruitment.

Vaccination of whole-cell polysaccharides of *S. pneumoniae* has been shown to induce IL-17 in the lung, and this IL-17 response has been shown to mediate serotype-independent immunity (105). Chen et al. (48) have also shown that Th17 cells elicited by *K. pneumoniae* vaccination recognize conserved outer membrane proteins in the cell wall of the bacteria and these antigens could also provide serotype-independent immunity. Th17 cells conferred heterologous protection against multiple serotypes of the organism (48). Thus, in two models of important human extracellular pathogens, Th17 cells are capable of mediating serotype-independent immunity which may advance vaccine approaches against these pathogens. It still remains to be defined which specific aspects of Th17 function that are required for protection. There needs to be better understanding of factors important in generating mucosal Th17 cells in the lung such as adjuvants, factor regulating proliferation, homing, and survival. More research is needed to also define the contributions of IL-22, IL-17F, and IL-17A/F heterodimers.

Conclusions

CD4⁺ T cells play critical roles in lung immunity, and these cells are impacted by many drugs as well as by HIV infection. CD4⁺ T cells are critical targets to achieve therapeutic vaccines against both bacterial and fungal pathogens. Future work will be required to understand the induction and survival of these cells in the lung and how they can be manipulated therapeutically.

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