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REVIEW

Th17 cytokines and vaccine-induced immunity

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Abstract T helper type 17 (Th17) cells are a distinct lineage of T cells that produce the effector molecules IL-17, IL-17F, IL-21, and IL-22. Although the role of Th17 cells in primary immune responses against infections is well documented, there is growing evidence that the Th17 lineage maybe critical for vaccine-induced memory immune responses against infectious diseases. Here, we summarize recent progress in our understanding of the role of IL-17 in vaccine-induced immunity.

Keywords Th17 · Infection · Vaccine-induced immunity

Abbreviations

IL	Interleukin
IFN-γ	Interferon gamma
MIP-2	Macrophage inflammatory protein-2
G-CSF	Granulocyte colony-stimulating factor
CXCR-3	Chemokine CXC motif receptor 3
KC	Keratinocyte chemoattractant

Introduction

Immunity induced by vaccination has contributed to the eradication of smallpox along with a decrease in the prevalence of diseases such as polio, measles, and rubella, making vaccination the most successful medical story in history. Despite the importance and success of vaccination, the mechanisms of generation of vaccine-induced cellular immunity are only just beginning to be explored. CD4+ T helper cells are important mediators of adaptive cellular immune responses. Based on the production of discrete cytokine profiles, they have been classified into T helper1 (Th1) and T helper 2 (Th2) subsets [76]. Th1 effectors produce Interferon-gamma (IFNγ) and regulate cellular immunity against intracellular infections whereas Th2 cells produce Interleukin (IL)-4, IL-5, and IL-13 and mediate humoral immunity against parasite infections. Recent compelling evidence has clearly changed this traditional paradigm of Th1/Th2 cell dichotomy to include a third subset of CD4 T cells referred to as T helper 17 (Th17) cells [36, 53, 83]. Th17 cells produce the cytokines IL-17A (IL-17) [36, 83] and IL-17F [53], as well as the cytokines IL-21 [50, 80] and IL-22 [15, 58]. This new Th17 cell lineage fills in some of the missing gaps in host immunity not fully explained by the Th1/Th2 paradigm. IL-17 plays a prominent role in tissue destruction associated with models of autoimmune diseases such as arthritis, multiple sclerosis, and colitis (reviewed in [23]). On the other hand, it is becoming apparent that IL-17 can play protective roles in immunity against infectious diseases, especially at the mucosa (reviewed in [48]). Since IL-23 was initially reported to act on memory or activated T cells that express the IL-23 receptor and produce IL-17 [81], it is likely that Th17 cells may have a role to play in vaccine-induced memory immune responses. Accordingly, recent advances have suggested a critical role for IL-17 in vaccine-induced protective cellular responses against several pathogens. In this review, we have summarized recent progress in our understanding of the role of IL-17 in protective vaccine-induced immunity against infectious diseases.

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Role of Th17 vaccine-induced immunity in bacterial infections

The intracellular pathogen *Mycobacterium tuberculosis* causes the disease tuberculosis, which kills more than two million people every year worldwide. The development of multidrug resistant bacteria along with increased incidence of HIV-associated tuberculosis has threatened the current control measures [25]. The only tuberculosis vaccine available, Bacille Calmette Guerin (BCG), has variable efficacy in different populations and has prompted the search for more effective vaccines against tuberculosis. The protective memory response to tuberculosis has conventionally been associated with the appearance of Th1 cells that produce IFNγ and activates macrophages to control *M. tuberculosis* [5, 17, 45]. However, focus on this population has not led to substantial improvement in our ability to vaccinate against this disease [19, 55]. That mycobacterial-exposed adults express high frequencies of IFNγ IL-17- and IL-22-producing cells of long-lived central memory phenotype [38, 94] suggests that Th17 cytokines along with Th1 cytokines may play important roles in memory responses to mycobacteria. Accordingly, although IL-17 and IL-23 are dispensable in the primary immune response to *M. tuberculosis* [7, 49], IL-23 and IL-17 appear to play a critical role in expression of vaccine-induced immunity against pulmonary tuberculosis [47]. Subcutaneous immunization with a *M. tuberculosis* peptide, Early Secretory Antigenic Protein ($ESAT6_{1-20}$) in adjuvant, generated both antigen-specific IL-17 and IFNγ responses [2, 47]. However, long-lasting IL-17-producing memory cells populated and persisted in the lungs, the primary site of *M. tuberculosis* infection, while IFN-γ-producing antigen-specific T cells persisted in the central lymphoid organs [47]. The IL-17-producing vaccine-induced antigen-specific cells populating the lungs expressed CCR4 chemokine receptor that has been associated with migration of cells into tissue [47]. Following challenge with pulmonary *M. tuberculosis* infection, accumulation of IL-17-producing cells occurred in the lungs prior to accumulation of IFNγ-producing antigen-specific T cells. The accumulation of IL-17-producing cells was accompanied by induction of chemokines, CXCL9, CXCL10, and CXCL11, which are known to induce migration of Th1 cells by binding to the receptor CXCR3 [2, 47]. That the chemokines CXCL9, CXCL10, and CXCL11 have IL-17-responsive elements suggests that IL-17 may directly induce these chemokines in the lung [47]. Induction of these CXCR3-ligating chemokine genes was defective following neutralization of IL-17 and correlated with a decrease in the accumulation of IFNγ-producing cells, suggesting a role for IL-17 in recruiting Th1 cells [47]. Moreover, the IFN-γ response that trailed IL-17 production was absent in IL-23-deficient mice, whereas it was restored following exogenous IL-17 treatment. These data suggest that the recall IFNγ response is dependent on IL-17 production following challenge with *M. tuberculosis* [47]. Further, antigen-specific cells from wild-type vaccinated mice when expanded in the presence of IL-23 in vitro and transferred into IL-23-deficient mice could confer protection following challenge with pulmonary *M. tuberculosis* [47]. These data, therefore, suggest that IL-23 is required during the priming and expansion of the protective memory responses rather than during the effector stage or recall responses. Following challenge, IL-17 is required for induction of chemokines and recruitment of Th1 cells for protection against *M. tuberculosis*.

Further, proof of principle that IL-17 may be a good predictor of vaccination success against tuberculosis comes from studies that have used mycobacterial protective Antigen 85 (Ag85A) in the form of viral boosters (Modified vaccinia virus Ankara or adenoviral vectors) [102] or plasmid DNA boosters [87] to *Mycobacterium bovis* BCG vaccination. Mice [87] and cattle [102] that have been boosted following *M. bovis* BCG vaccination induced high expression of IL-17 and correlated with better protection when compared to *M. bovis* BCG vaccination alone. Interestingly, IL-17 was below the detection limit in mice primed with DNA Ag85A or vaccinated with *M. bovis* BCG alone [87]. The absence of IL-17 production in response to the *M. bovis* BCG vaccine alone and the lack of protective efficacy to pulmonary tuberculosis further suggest that generation of vaccine-induced IL-17 may be prerequisite for successful long-lasting memory immunity against tuberculosis. Further, addition of plasmids expressing IL-23 as adjuvants to DNA vaccines expressing protective *M. tuberculosis* Ag85B also increased Th1 immune response and improved protective immunity to *M. tuberculosis* challenge, compared to immunization with Ag85B DNA vaccine alone [108]. Although IL-17 responses were not studied in this model, it is probable that IL-23 also induced effective Th17 responses contributing to protection. These data suggest that IL-17 and IL-23 may be necessary in eliciting vaccine-induced immunity of both Th17 and Th1 phenotypes and may impact vaccine-design strategies against tuberculosis.

The role of IL-17 in vaccine-induced responses against extracellular bacteria such as *Streptococcus pneumoniae*, *Bordetella pertussis*, *Helicobactor pylori*, and *Pseudomonas aeruginosa* are just beginning to be elucidated. *S. pneumonia* causes one million deaths in children per year [105]. The use of vaccinations based on purified or conjugated capsular antigens clearly show that anticapsular antibodies protect humans against pneumococcal colonization and disease [10]. However, more recent studies have shown that protection against pneumococcal challenge can be mediated by antibody-independent mechanisms that are

CD4-dependent [64]. Protection following delivery of cell wall polysaccharides [63, 64] or pneumococcal whole-cell antigen [11] given mucosally in the adjuvant cholera toxin was found to induce IL-17 responses [64]. Further, neutralization of IL-17 or challenge of IL-17 Receptor gene-deficient immunized mice decreased protection and increased colonization following pneumococcal challenge [63, 64]. Interestingly, protection appears to be independent of Th1 and Th2 immunity, since mice lacking IFNγ or IL-4 were still able to elicit protection [63]. The cellular source of IL-17 in this model was identified to be CD4 T helper cells, since transfer of CD4 T cells into RAG-gene-deficient mice could confer protection following challenge with pneumococcal challenge [63]. Conversely, depletion of CD4 T cells reduced the levels of IL-17 production in restimulation in vitro assays. The mechanism of protection in this model was hypothesized to be mediated by IL-17-dependent enhancement of phagocytic killing of pneumococci by neutrophils [63]. In a model of primary infection and secondary challenge with a P1121 strain of *S. pneumonia*, protection was also shown to be dependent on CD4 T cell IL-17 production [114]. In this model, the primary *S. pneumococci* challenge generated CD4 Th17 memory cells via a TLR2-dependent pathway. Upon secondary challenge with *S. pneumococci*, the enhanced IL-17 responses mediated recruitment of monocytes, macrophages, neutrophils, and pathogen clearance. Further, tonsillar cells from children and adults, but not umbilical cord blood produced IL-17 following exposure to antigen [63], suggesting that the CD4 subset is likely a memory cell resulting from prior exposure to the pathogen. These data could well explain why HIV infection confers a 50-fold increased risk of *S. pneumococci* infection, which is inversely related to CD4 T-cell count [32].

The prevalence of whooping cough, caused by *B. pertussis*, has seen significant reductions in previous years due to immunization with whole cell pertussis vaccine (Pw) [33]. However, due to the adverse immunological reactions occasionally seen with Pw, it was substituted with an acellular pertussis vaccine (Pa) administered in alum as an adjuvant [34]. Although the safety of the Pa vaccine is higher, the protective memory elicited is lower [74]. Importantly, immunization with Pw, but not Pa, has been shown to induce populations of IL-17- and IFNγ-producing cells [8, 39]. Treatment with a neutralizing IL-17 antibody during challenge in Pw-immunized mice resulted in a reduction of protective efficacy, further substantiating the role of IL-17 in vaccine-induced immunity against *Bordetella* [39]. The induction of IL-17 responses in Pw-immunized mice was found to coincide with the ability of LPS in the Pw vaccine to induce Th17-polarizing cytokines IL-23, IL-1β, and TNF-α in DCs [39]. The induction of these cytokines was dependent on TLR4 signaling because TLR4-deficient mice had significantly lower IL-23 production as well as lower IL-17 responses and failed to protect following pathogen challenge [8, 39]. In this model, it is hypothesized that TLR4-dependent IL-17 may elicit cellular immunity to *B. pertussis* through a number of mechanisms including the recruitment of neutrophils mediated by induction of MIP-2, enhancement of antibacterial activity, and macrophage killing activity [39]. Therefore, IL-17 appears to be a major modulator of the memory response generating strong protection in response to Pw immunization, which is not observed in Pa immunization. Future attempts to improve the efficacy of Pa vaccines may thus benefit from incorporation of components that will effectively induce IL-17 vaccine-induced responses.

IL-17 also plays a role in vaccine-induced immunity against *Helicobacter* infections. *H. pylori* is a Gram-negative bacterium that inhabits the gastric mucosa. Approximately two thirds of the world's population is currently infected, and majority of the infected population are asymptomatic. However, infection can lead to development of peptic ulcer disease, gastric mucosa-associated lymphoid tissue lymphoma, and gastric cancer, leading to significant mortality and morbidity [13]. In the absence of any available vaccine and expensive antimicrobial therapies, infection with *H. pylori* for most of the infected population is a lifelong coexistence. In the mouse model, vaccinations with *H. pylori* lysate [21] or recombinant *H. pylori* urease [100] in cholera toxin adjuvant provides protection following challenge with *Helicobacteria*. Immunized mice rapidly accumulate CD4 T helper cells that produce IL-17 and IFNγ recruit neutrophils and clear the bacteria [21, 100]. In the *H. pylori* lysate vaccine model, the IL-17-producing cells accumulate in the gastric mucosa 1 week prior to accumulation of IFNγ-producing CD4 T cells, suggesting that IL-17-producing cells can induce chemokines to recruit Th1 cells. Further, accumulation of IL-17-producing CD4 T cells coincided with increased induction of myeloperoxidase and neutrophil recruitment, while depletion of IL-17 abrogated neutrophil recruitment and vaccine-induced protection [21]. Further proof for a role for vaccine-induce IL-17 in this model was shown when unimmunized mice were protected by exogenous delivery of IL-17 [21]. In the *H. pylori* urease vaccine model, both IL-17 and IFNγ responses appeared in the gastric mucosa at the same time, and the IL-17-dependent protective mechanism was hypothesized to be mediated by induction of neutrophil recruiting chemokines such as KC and MIP2 [100]. These results suggest IL-17 acts by recruiting neutrophils that are critical for clearance of the pathogen, but the resulting inflammation may be a likely cause of the pathology that is associated with protection.

P. aeruginosa is the third most common isolated Gram-negative bacterium in the setting of hospital-acquired pneumonia and is a significant pathogen for immunocompromised patients [70]. Vaccine development against *P. aeruginosa* is hampered by the inability to achieve broad protection across different LPS "O" antigen serotypes. Development of an attenuated *P. aeruginosa* strain PA01 as a vaccine prevents against lethal pneumonia challenge with the parental strain but not against LPS-heterologous strains [85]. However, recent advancements in the design of a live-attenuated PA14 strain as a vaccine protects against not only the parental strain but also against LPS-heterologous strains of *P. aeruginosa* [86]. The mechanism of protection in this model was shown to be induction of IL-17 by CD4 T cells, resulting in rapid recruitment of neutrophils and bacterial clearance [86]. Vaccine efficacy was diminished with deletion of IL-17 and absence of IL-17 receptor [86]. This is consistent with other extracellular vaccine models that suggest that IL-17-mediated vaccine protection is mediated by neutrophil recruitment and bacterial killing.

Collectively, all these reports (Table 1) demonstrate that IL-17 has a critical role in vaccine-induced immunity against bacterial infections, primarily by induction of chemokines to recruit protective Th1 cells, neutrophils, macrophages, and enhanced phagocytic killing (Fig. 1). Further, it is likely that the induction of antimicrobials by IL-17 augments clearance of pathogens [7, 115]. Therefore, targeting IL-17 is an area of active research and likely to impact future vaccine design against bacterial infections.

Th17 vaccine-induced immunity and fungal infections

Candida albicans is a commensal fungal organism of the oral cavity and gastrointestinal tract. However, under settings of immunodeficiency such as HIV, *Candida* can become pathogenic. To date, there is no vaccine available for *Candida*. Protection against the disseminated form of the disease caused by *Candida* requires IL-17-mediated recruitment and expansion of neutrophils for pathogen control [42]. The oral form of the disease as in oropharyngeal candidiasis (OPC), occurs in >90% of HIV+ individuals [24]. In humans, the production of Th17 cytokines correlates with protection against *Candida* infections, since patients with chronic mucocutaneous candidiasis produced significantly lower amounts of IL-17 and IL-22 in vitro following antigen stimulation when

Table 1 Vaccine models where IL-17 is required for protection against infectious diseases

Pathogen	Vaccine	Organ	Mechanism/reference
M. tuberculosis	Peptide vaccine	Lung	Recruitment of CD4 Th1 cells [47]
M. tuberculosis	Subunit vaccine	Lung	[2]
M. tuberculosis	Viral vectors expressing protective antigen	Lung	[38]
M. tuberculosis	Primary BCG vaccination followed by booster with viral vectors expressing protective antigens	Lung	[102]
M. tuberculosis	Primary BCG vaccination followed by booster with DNA vaccine encoding protective antigens	Lung	[87]
M. tuberculosis	DNA vaccine expressing protective antigen and expressing IL-23	Lung	[108]
S. pneumoniae	Pneumococcal whole-cell antigen	Lung	[11]
S. pneumoniae	Cell wall polysaccharides	Lung	Monocyte, macrophage, and neutrophil recruitment and phagocytic killing [63, 64]
S. pneumoniae	Live organism	Lung	Monocyte, macrophage, and neutophil recruitment [114]
B. pertussis	Whole cell vaccine	Lung	Neutrophil recruitment and enhanced phagocytic killing [39]
H. pylori	*H. pylori* lysate and recombinant urease	Gut	Recruitment of neutrophils, monocytes and macrophages and enhanced killing activity [21, 100]
P. aeruginosa	Live organism	Lung	Recruitment of neutrophils and increased bacterial clearance [86]
Rhesus rotavirus	Recombinant antigen	Intestine	[72, 95, 99]
Influenza virus	DNA vaccine expressing protective antigen and IL-23	Lung	[106, 107]
Eimeria acervulina	Recombinant antigen and IL-17	Intestine	[22]

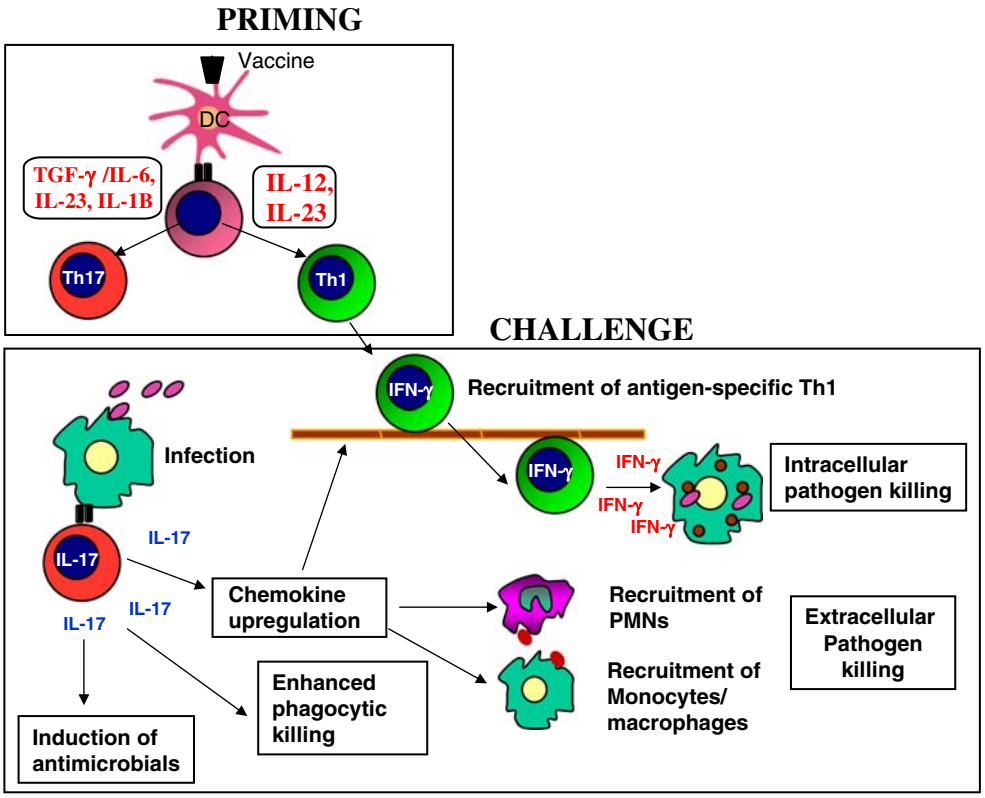

Fig. 1 Model of vaccine-induced IL-17 in conferring protection against pathogens. Vaccination induces the generation of both Th1 and Th17 cells. Following challenge with pathogen, there is an expansion of memory Th17 cells and the production of IL-17. IL-17 can upregulate CXCR3 ligands resulting in augmented recruitment of Th1 effectors, production of IFNγ, leading to control of intracellular pathogens. Vaccine-induced IL-17 can augment chemokine induction by epithelial cells, resulting in recruitment of neutrophils, monocytes, and macrophages and enhance killing in phagocytic cells. IL-17 also augments induction of antimicrobial peptides important for control of extracellular pathogens

compared to healthy individuals [28]. Moreover, patients with autosomal dominant hyper-IgE syndrome (Job's Syndrome) resulting from a mutation in the STAT-*3* gene do not generate Th17 responses and are extremely susceptible to bacterial infections such as *S. aureus* and mucocutaneous fungal infections caused by *Candida* species [75]. Further, human studies have shown that memory CD4 cells specific for *C. albicans* are mainly IL-17- [1, 116] and IL-22-producing cells [60]. In a mouse model of OPC, it has been shown that Th17 cells and IL-17 receptor signaling are necessary for host protection [16]. In this model, protection appears to be independent of the Th1 pathway [16]. Further, it has been shown that IL-23 and IL-17 are protective in pulmonary models of fungal infections with *Pneumocystis carnii* [89] and *Aspergillus fumigatus* [103], because IL-23KO mice or neutralization of the IL-23/IL-17 axis resulted in impaired clearance of the pathogen. However, the induction of IL-17 during fungal inflammations appears to be a double-edged sword since the heightened Th17 responses against *C. albicans* and *A. fumigatus* can also mediate severe tissue pathology [113]. These studies suggest that design of vaccines for *Candida* will likely target the generation of IL-17-vaccine-induced responses for rapid recruitment of neutrophils and fungal clearance. However, the pathological consequence of protection will also need to be thoroughly evaluated, prior to targeting IL-17 in vaccine-induced immunity against fungal infections.

Th17 vaccine-induced immunity against viral infections

Documentation of the presence of viral-specific Th17 cells in HIV infection [112], *cytomegalovirus*-specific IL-17-producing cells in humans [112] and in mice [6] suggests that Th17 cells may have a role to play in recall responses to viral infections. Much of what is known about the mechanism of IL-17 in vaccine-induced immunity to viruses is using a well-characterized mouse model of rotavirus. Rotavirus is a gastrointestinal virus that accounts for approximately 600,000 deaths worldwide in children [82]. Live attenuated virus vaccines are now being licensed worldwide and are shown to be highly protective in controlled clinical trials [90, 101]. However, second-generation component vaccines are being explored to circumvent possible side effects that have been associated with live rotavirus vaccines [77]. One of the second generation vaccines is an *Escherichia coli* expressed VP6, the protein that comprises the intermediate capsid layer of the rotavirus particle. Administration of VP6 mucosally in an *E. coli* heat-labile toxin or a cholera toxin-derivative adjuvant generates both IL-17 and IFNγ producing memory CD4 cells, is protective and reduces fecal shedding [72, 95, 99]. When compared to VP-6 vaccination, vaccination with the live rotavirus, although protective, induced lower levels of IFNγ and IL-17 responses [99]. Interestingly, following vaccination with VP6 and challenge with rotavirus, both IFNγ-receptor-deficient mice and IL-17-

receptor-deficient are protected, similar to wild-type mice [95]. This suggests that IL-17 or IFNγ can compensate for absence of the other. Further, in this model, IFNγ can inhibit rotavirus replication and may have a more direct role in protection, whereas IL-17 may play a more indirect role by impacting cell recruitment [73]. In addition, vaccination with DNA constructs encoding influenza virus hemagglutinin (HA) and IL-23 maintained cellular responses over longer periods of time [106] and cleared more virus when challenged with influenza virus than HA constructs alone [107]. These studies support a role for IL-23 and IL-17 in generating effective long-lived vaccine-induced immunity against viruses.

Th17 vaccine-induced immunity in parasitic infections

The role of IL-17 in vaccine-induced immunity against parasites is underexplored. Evidence that IL-17 may be important in parasite infection models is demonstrated by induction of IL-17 in response to *Eimeria maxima*, *Nippostrongylus brasiliensis*, and *Leishmania donovani* [41, 59, 84]. Moreover, production of IL-17 and IL-22 in human subjects showed a strong and independent association with protection against Kala alzhar, the disease caused by *L. donovani* [84]. Inclusion of IL-17 as a coadjuvant along with a purified recombinant protein vaccine could reduce oocyte shedding and induce protection in chickens against the parasite, *Eimeria acervulina* [22]. These studies suggest that IL-17 may have an important role in induction of Th17 memory responses and could be a potential target in the design of effective vaccines against parasites. In contrast, IL-17 has been implicated in tissue pathology associated with schistosomiasis [91, 92]. Therefore, future research on the protective versus pathological role of IL-17 in parasitic models should be carefully studied prior to targeting IL-17 in vaccine strategies against parasitic infections.

Innate receptors and adjuvants involved in generation of Th17-immune responses

The differentiation of naïve T cells into Th17 cells depends on exposure to TGF-β and IL-6, while IL-23 is required to further stabilize and maintain the commitment of Th17 cells to this lineage (reviewed in [51]). These polarizing cytokines induce the expression of the transcription factors RORγt and RORα on newly activated T cells and allow Th17 differentiation [44, 111]. RORγt also induces the expression of IL-23 receptors on newly primed T cells and expands their responsiveness to IL-23 to sustain Th17-lineage-specific responses. The gp-130-Stat3 pathway has been shown to be critical for Th17 development [14, 20, 75]. IL-1β and TNF-α have also been suggested as additional co-factors required for the differentiation of Th17 cells [97]. More recently, IL-21 has been shown to amplify Th17 cell generation in an autocrine manner [50, 80]. Overinduction of Th17 cells can impact tissue destruction due to expression of inflammatory signals. Therefore, the generation of Th17 cells appears to be tightly regulated. Cytokines such as IL-27 [9, 96], those belonging to Th1 (IFNγ) and Th2 pathways (IL-4) [36, 83], and IL-2 [54] negatively regulate the induction of Th17 cells, thereby maintaining a fine balance in the host.

Adjuvants and pathogen products largely act through pattern recognition receptors (PRR) on antigen-presenting cells. Several of the Th17 polarizing cytokines such as IL-23, TGF-β, IL-6, and IL-1β are induced in dendritic cells when activated by components of adjuvants. Some of the best-studied examples are TLR ligands due to their ability to stimulate robust pro-inflammatory and immune effector responses. TLR2 ligands such as peptidoglycan [97] and lipoteichoic acid [88] induce IL-23 but not IL-12 in human monocyte-derived DCs. Other TLR2 agonists such as Pam2C can inhibit IL-12 production induced by other TLR agonists and enhance IL-23 production in monocyte-derived DCs [31]. Single treatment with other TLR ligands such as the TLR4 ligand, LPS, and TLR7/8 ligand, resimiquoid (R848), can also induce IL-23, IL-6, and IL-1β in human monocyte-derived DCs and result in differentiation of Th17 cells [61, 88]. Neither of these TLR ligands alone induced significant IL-12, except when combined with IFNγ or other TLR ligands. For example, co-treatment of DCs with LPS and R848 result in induction of both IL-12 and IL-23 and predominant Th1 responses [88]. The combination of the ligands for nucleotide-binding oligomerization domain (NOD2) such as muramyldipetide (MDP) along with TLR1/2 and TLR2/6 preferentially induced IL-23 rather than IL-12 [31] and promoted IL-17 responses but not IFNγ responses [97]. Interestingly, MDP by itself was unable to induce IL-23, IL-1β in APCs, or Th17 responses [97]. Further, a combination of TLR2 ligand Pam2C along with β-glucan receptor/dectin-1 ligand, β-glucan, induced high levels of IL-23, whereas β-glucan in association with R848 induced high levels of IL-12 [31]. Therefore, it is becoming apparent that different TLR agonists based on the cellular expression of their receptors and presence of other ligands induce different T-cell polarizing cytokines that can impact the generation of downstream T-cell effector function. Therefore, depending upon the pathogen target, inclusion of the right combination of adjuvants will allow us to induce Th1 and/or Th17 responses in vaccine strategies.

Zymosan, a β-glucan-containing preparation of yeast cell walls, is a potent producer of IL-23 and IL-10 in DCs and induces potent Th17 responses [31]. The β-glucan

receptor (Dectin-1) is one of the major PRRs involved in stimulation of DCs by zymosan. Accordingly, β-glucan also induced IL-23 and IL-10 in DCs and generated robust Th17 responses [31]. The dectin1 agonist, curdlan, functions via Dectin 1-Syk-CARD9 signaling pathway to promote DC maturation and secretion of Th17-polarizing cytokines IL-23, TNF-α, and IL-6 and generated effective Th17 and Th1 responses [57]. Furthermore, the macrophage mannose receptor has been recently shown to induce IL-17 production in T cells response to *Candida* mannan [98]. Since some of these responses are synergistic with TLR signaling, it is likely that a combination of adjuvants functioning via different pathways may improve vaccine strategies to induce both Th17 and Th1 responses.

DNA booster vaccines expressing *M. tuberculosis* Ag85A in the adjuvant vaxfectin induced higher levels of IL-17 from both CD4 and CD8 cells when compared to unvaccinated or BCG-alone-immunized groups [87]. The IL-17 responses induced by vaxfectin coincided with high induction of IL-6, suggesting that including adjuvants that drive Th17 polarizing cytokines, may substantially improve Th17 responses and benefit vaccine design.

Cholera toxin (CT), a major enterotoxin produced by *Vibrio cholerae*, is a potent mucosal immunogen and adjuvant that is protective in several models [30]. The type of T-helper responses induced by the adjuvant CT is controversial and may well depend on the route of immunization and nature of the bystander antigen. For example, some studies have proposed that CT promotes a strong Th2 bias because it induces the production of IL-4, IL-5, and IL-10 but poor Th1 responses [4, 30, 66, 109, 110]. Additionally, CT and other *E. coli* enterotoxins have been shown to inhibit IL-12, DC differentiation, and IFNγ production [52, 93]. However, other studies have shown that mucosal immunization of antigens in CT induces a mixed Th1/Th2 profile [26, 29, 43]. Most recently, mucosal immunization of bystander antigens in CT has been shown to be a strong inducer of Th17 responses [56, 71]. Further the IL-17-driving ability of CT was dependent on induction of IL-6 and was mediated by the β-subunit of CT. CT-induced Th17 responses induce chemokines and result in recruitment of neutrophils. The effect of CT in driving Th17 responses was also evident in Th1- or Th2-favored condition of respiratory virus infection [56]. Genetically detoxified Pertussis toxin also possesses adjuvant properties and can enhance Th1 and Th17 responses by induction of IL-12, IL-1β, and IL-23 in dendritic cells via engagement of TLR4/TLR2 [79]. In light of these studies, the robust IL-17 responses seen in protective vaccine-induced models of *Streptococcus* [62–64], *H. pylori* [21, 100], and rotavirus [72] indicate that inclusion of CT or variants of other enterotoxins may result in strong IL-17 vaccine-induced immunity.

The delivery of antigenic peptide in an adjuvant that contains Monophospho lipid A (MPL), a TLR4 stimulant [27], trehalose dimycolate (TDM), and the small cationic

Table 2 Innate receptors and adjuvants involved in generation of Th17 polarizing cytokines

Adjuvant	Receptor binding	Cytokines produced in APCs	T-helper responses	Reference
Peptidoglycan (PGN)	TLR2	IL-23, IL1β	IL-17	[97]
Lipoteichoic acid (LTA)	TLR2	IL-23		[88]
LPS	TLR4	IL-23, IL-6, IL-1β, IL-12	IL-17 and IFNγ	[88, 104]
Resimiquod (R848)	TLR 7/8	IL-23, IL-6, IL-1β		[88]
Pam2C	TLR2/6	IL-23		[31]
Pam3C	TLR1/2	IL-23		[31]
LPS + R848	TLR4/7/8	IL-12, IL-23	IL-17 and IFNγ	[88]
Muramyldipeptide (MDP) + Pam2c or Pam 3C	Nucleotide-binding oligomerization domain 2 (NOD2), TLR 1/2/6	IL-23	IL-17	[31, 97]
β-glucan	Dectin 1	IL-23, IL-10	IL-17	[31]
Candida mannan	Macrophage Mannose Receptor	IL-23, IL-1β	IL-17	[98]
curdlan	Dectin-1	IL-23,IL-12p40 IL-10, IL-2, IL-6	IL-17, IFNγ	[57]
Cholera toxin	Cell surface gangliosides	Inhibits IL-12	IL-17, IFNγ, IL-4, IL-5	[56, 65, 109]
Pertussis toxin	TLR2/TLR4	IL-12, IL-1b, IL-23	IL-17,IFNγ	[79]
Trehalose dibehenate (TDB)	Syk-Card9 pathway	IL-6, IL-1β, IL-23, IL-12	IL-17, IFNγ	[104]
Dimethyl dioctadecylammonium bromide (DDA)	Unknown	IL-23, IL-12, TGF-β, IL-6, IL-1β	IL-17, IFNγ	[47]
CFA	MyD88 dependent	TGF-β, IL6, IL-23	IL-17	[18, 53, 67]

liposome dimethyl dioctadecylammonium bromide (DDA), induces both Th17 and Th1 responses in vitro and in vivo [47]. It was shown that DDA was the component of this vaccine adjuvant that could effectively induce TGF-β and Th17 responses in vitro, while MPL/TDM could induce IL-1β and IL-6, IL-12 and IL-23 [47]. TDM requires the macrophage receptor with collagenous structure, TLR2, and CD14 for induction of pro-inflammatory cytokines in macrophages [12]. The less toxic and synthetic variant of TDM called trehalose dibehenate (TDB) also induced robust Th1 and Th17 responses and activated APCs to produce IL-6, IL-1β, IL-23, and IL-12 via the Sky-Card9-dependent activation pathway [104]. Further, when DDA was used as a delivery vehicle with TDB, the adjuvant induced both Th1 and Th17 responses in vivo [46, 104] and elicited protective immunity when challenged with *M. tuberculosis, Chlamydia trachomitis*, and malaria parasite *Plasmodium* [3, 104]. This suggests that this adjuvant combination may function via both TLR-dependent and TLR-independent pathways in the induction of protective Th17 responses against a variety of infectious diseases (Table 2).

Inclusion of cytokines as coadjuvants could be very effective to generate specific T-helper cell subsets in vaccine strategies. For example, the inclusion of pro-inflammatory cytokines such as TNF-α, IL-1, and IL-6 as coadjuvants in generation of T-helper cells that can produce IL-17 have potent in vivo cognate activity [69]. Further co-administration of IL-23 expression plasmid in a hepatitis C virus (HCV) prime-boost immunization-enhanced HCV-specific CD4 and CD8+ T cells that produce IFNγ- as well as HCV-specific cytotoxic T-lymphocytic activity [35, 37, 68]. Incorporation of IL-23 in influenza virus HA cleared more viruses following challenge with influenza [107]. Further, that plasmids expressing IL-23 when used as cytokine adjuvants to DNA vaccines expressing *M. tuberculosis* Ag85B increased the Th1 immune response and improved protective immunity to *M. tuberculosis* challenge [108]. These studies provide scope for development for cytokine coadjuvants in generation of effective protective Th17 vaccine-induced immunity against a variety of infectious diseases.

IL-17 is a key player in the inflammation and tissue destruction associated with autoimmune disease models such as experimental autoimmune encephalitis [53] and collagen-induced arthritis [78]. Most autoimmune models deliver antigens in adjuvants such as Complete Freund's Adjuvant (CFA) [53] or pertussis toxin [40] to induce the inflammatory disease phenotype. Although CFA was initially thought to drive potent Th1 responses, it is now clear that CFA can drive differentiation of naïve T cells into Th17 pathway [53]. Further, pertussis toxin can also induce pathogenic Th17 inflammatory responses in the setting of vaccination [40]. These studies highlight the need for detailed characterization of adjuvants prior to inclusion into vaccine strategies since the delicate balance of IL-17 in vaccine-induced immunity may define tissue destruction or immune protection.

Summary and outlook

Compelling and accumulating evidence suggests that Th17 cells have evolved to mediate protective immunity against a variety of pathogens, primarily at mucosal sites. Importantly, the emerging evidence that Th17 cells are crucial players in generation of vaccine-induced protective responses against a variety of pathogens suggests that the incorporation of this knowledge into the design of current and future vaccines against infectious diseases will be a fruitful area of future research. It is also becoming apparent that the fine balance between protection and pathological manifestations of Th17 responses will define the final outcome of vaccine-induced IL-17 responses. Understanding the mechanism of induction and maintenance of IL-17 vaccine-induced responses has the potential to substantially impact vaccine strategies against infectious diseases.

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