Tailored Immune Responses: Novel Effector Helper T Cell Subsets in Protective Immunity

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Abstract: Differentiation of naive CD4+ cells into functionally distinct effector helper T cell subsets, characterised by distinct “cytokine signatures,” is a cardinal strategy employed by the mammalian immune system to efficiently deal with the rapidly evolving array of pathogenic microorganisms encountered by the host. Since the Th1/Th2 paradigm was first described by Mosmann and Coffman, research in the field of helper T cell biology has grown exponentially with seven functionally unique subsets having now been described. In this review, recent insights into the molecular mechanisms that govern differentiation and function of effector helper T cell subsets will be discussed in the context of microbial infections, with a focus on how these different helper T cell subsets orchestrate immune responses tailored to combat the nature of the pathogenic threat encountered.

Introduction

Bidirectional intercellular communication between innate and adaptive immune systems is crucial for success of immunity to microbial infection. The activation and fate of clonally selected cells of the adaptive immune system is strongly influenced by innate effector cells, and orchestration of adaptive responses to pathogenic microorganisms requires synergistic collaboration with the innate immune system to efficiently resolve infection. Via production of diverse pleiotropic cytokines, effector CD4+ T helper (Th) cells function to direct efficient immune reactions by dictating the actions of both innate and adaptive arms of the immune system. Through their ability to coordinate innate/adaptive effector cell activity, Th cells directly and/or indirectly influence almost every aspect of an immune response: they provide signals to help B cells undergo class switch recombination (CSR), affinity maturation and differentiation, perpetuate CD8+ T cell responses, regulate the recruitment and function of innate effector cells, and contract responses to resolve and/or adjust the magnitude of inflammation.

Pathogen-specific CD4+ T cells coordinate immune responses by differentiating into discrete subsets of effector Th cells defined by production of distinct cytokine “signatures.” The specific differentiated state of effector Th subsets is attributed to their expression of subset-specific transcription factors that programme subset-specific transcriptomes, whilst concomitantly suppressing alternative fates the precursor could have assumed [1]. Induction of these transcriptional programmes is predominantly determined by innate-immune-derived cytokines present during MHC-II-restricted T cell receptor (TCR)-mediated activation released into the “immunological synapse” by antigen-presenting cells, particularly by DCs (examples shown in Figure 1). DCs are themselves instructed to produce cytokines following detection of specific pathogen-associated molecular patterns (PAMPs) on foreign microbes through pattern recognition receptors (PRRs) during pathogen encounter in the periphery [2]. Thus, important information regarding the nature of the specific pathogens can be conveyed to developing effector helper T cells that subsequently differentiate into an effector programme equipped with a particular cytokine-secreting repertoire, thereby eliciting a pathogen-tailored immune response.

These views of helper T cell differentiation and function were first introduced by Mosmann and Coffman in 1986, who demonstrated that T cell clones were divisible into two subsets, termed Th1 and Th2, based on their mutually exclusive production of interferon (IFN)-γ or interleukin (IL)-4, -5, and -13, respectively [3]. This subdivision was of major significance as IFN-γ-producing Th1 cells were subsequently shown to be critical in host defences against intracellular pathogens by activating cell-mediated immunity, whilst Th2-driven responses were essential for efficient humoral responses against extracellular microbes. The Th1/Th2 paradigm served as a useful conceptual construct for understanding how Th1 cells controlled different arms of the immune system, and dysregulation of Th1/Th2 responses has since been implicated in the pathogenesis of many immune-related disorders such as autoimmune and allergic disease. Development of techniques such as multi-parameter flow cytometry and engineering of fate-mapping cytokine reporter mice has recently facilitated major progress in Th cell biology, with seven functionally unique Th subsets now described. These comprise Th1, Th2, Th17, follicular helper T cells (Tfh), inducible T regulatory cells (iTreg), and the most recently described and least well-characterized subsets, Th9 and Th19 cells, each of which is produced upon antigen presentation in the presence of specific cytokines or sets of cytokines (Figure 1). In this review, recent insights into the mechanisms that govern differentiation, migration, and function of effector Th cells will be discussed in the context of microbial infection, focusing on the contribution of

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emerging subsets of effector helper T cells, with less emphasis on TH1 and T H2 subsets, whose function has been well-established and is described elsewhere [4]. The function of Tregs in protective immunity will also not be discussed in this review as this has been the subject of recent comprehensive review elsewhere [5].

T Helper 1 (TH1) and T Helper 2 (TH2)

TH1 differentiation from naïve precursors is initiated by signal transducer and activator of transcription (STAT)-1 activation downstream of type 1 interferon, IFN-γ and IL-27 signalling, which induces expression of the TH1-specific master transcription factor T-bet [6–9]. This process enables activated CD4+ T cell responsiveness to DC-derived IL-12 via T-bet-mediated induction of the high-affinity IL-12 receptor beta 2 chain on the cell surface [10–12]. IL-12 signalling through STAT4, together with T-bet, directly transactivates the Ifng gene, which further promotes TH1 differentiation via STAT1 activation in an autoregulatory feedback loop [13–15]. T-bet also drives expression of the chemokine receptor CXCR3, which facilitates TH1 migration to inflamed sites of pathogen encounter where CXCL9, CXCL10, and/or CXCL11 are produced (Figure 1) [12,15–17]. TH1 cells orchestrate the cell-mediated cytotoxic response against intracellular pathogens principally via provision of IFNγ to enhance macrophage activation and promote antigen-specific cytotoxic T lymphocytes (CTLs). Classical infections controlled or cleared by effective TH1 responses include intracellular bacteria such as Listeria monocytogenes, Salmonella species, and Mycobacterium tuberculosis, intracellular parasites such as Leishmania donovani, and a number of viral pathogens [18–25]. In addition, TH1-derived production of the pro-inflammatory cytokine IL-21 has also been shown to be a key regulator of the long-term maintenance and functionality of antigen-specific CTLs important for protection against both acute and chronic infection with lymphocytic choriomeningitis virus (LCMV) [26–28].

Despite the appreciation of the existence of the TH2 subset for more than 25 years, the molecular mechanisms that govern TH2 differentiation remain controversial. Early reports demonstrated that the TH2 differentiation programme is set up via STAT6 activation downstream of IL-4 signalling, directly transactivating the TH2-lineage-specific transcription factor GATA-3 that in turn induces expression of the TH2-specific cytokine genes Il4, Il5, and Il13 [29–36]. However, recent studies suggest that TH2 cell induction may be far more complex than originally described, with reports that TH2 differentiation can occur independently of the STAT6/IL-4 axis [37] and may require additional cytokines including IL-2, IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) (reviewed in [4,38]). Nevertheless, production of IL-4, -5, and -13 by TH2 cells plays important roles in the activation and recruitment of basophils, induction of eosinophilia, regulation of antibody-dependent cell-mediated cytotoxicity (ADCC) mechanisms, and, by acting on resident cells at sites of inflammation, creates a hostile environment that favours extracellular microbial expulsion [4]. TH2 cells express the chemokine receptors CCR3, CCR4, and CCR8, and thus migrate to sites expressing their ligands in response to infection (Figure 1) [39,40]. Effective TH2 responses are required for host defence against extracellular parasites such as Schistosoma mansoni or Trichuris muris [41–44].

The subdivision of T cells into TH1 and TH2 subsets has utility in understanding how the adaptive immune system tailors responsiveness to different types of pathogens by directing the activation of distinct immune components. Clearly however, the TH1 and TH2 subdivision is an oversimplification as the substantial pathogen diversity warrants more than two broad types of immune response. Therefore, it is perhaps surprising that it was only relatively recently that the TH1 and TH2 paradigm has
been expanded to definitively include additional subsets of T cells and the role of these subsets in responses to distinct microbial challenges has been interrogated. In the remainder of this review, these emerging subsets of T cells and their role in protective immunity will be described.

**T Helper 17 (Th17)**

It was not until 2005 that a third major population of effector Th17 cells was described on the basis of the observation that peripheral CD4+ T cells could differentiate into a distinct lineage in a GATA-3- and T-bet-independent fashion [45,46]. Early reports suggested that these cells did not produce molecules commonly associated with Th1 or Th2 subsets but characteristically expressed the highly pro-inflammatory cytokines IL-17A and IL-17F, and were subsequently designated Th17. However, later studies have demonstrated that subsets of Th17 cells can produce IFN-γ, IL-4, or IL-13 under certain circumstances [47–50], although the function of Th17-derived IFN-γ, IL-4, or IL-13 has yet to be explored in infectious models. Th17 differentiation requires IL-6 and is also promoted by the otherwise immunosuppressive cytokine TGF-β1 (Figure 1) [51–53]. Signal transduction downstream of these cytokines, including STAT3 activation downstream of the IL-6 receptor, induces expression of the Th17-lineage-specific transcription factor RORγt, which directly transcribes the Th17-lineage-specific cytokines Il17a and Il17f (Figure 1) [54–57]. IL-6-mediated induction of IL-21 during Th17 cell differentiation is reported to reinforce Th17-lineage commitment via STAT3 activation downstream of the IL-21 receptor in an autocrine manner [53,58,59]. It has also been reported that autocrine TGF-β1 promotes Th17 cell differentiation *in vivo* [60]. Whilst addition of IL-6 and TGF-β1 into naive CD4+ T cell cultures does indeed drive Th17 cell differentiation, the *in vivo* requirements of Th17 cell differentiation are far more complex than these *in vitro* conditions. Recent reports suggest that Th17 cell differentiation can be induced independent of TGF-β1 signalling when driven by the inflammatory cytokines IL-6, IL-1β, and IL-23 [61]. Th17 cells induced independently of TGF-β1 (termed Th17β23, owing to their requirement for IL-23) appear to possess more inflammatory characteristics than conventional TGF-β1-driven Th17 cells (Th17β). Furthermore, it has also recently been shown that IL-6 and TGF-β3 drive differentiation of Th17 cells that are functionally and molecularly distinct to the conventional Th17β cell [62]. Thus, it is likely that Th17 cells in any given response may comprise a heterogeneous population of distinct types of Th17 cells that arise in discrete cytokine microenvironments, possess distinct but similar transcriptomes, and subsequently possess different cytokine-secreting repertoires and functions. However, these hypotheses remain to be extensively tested. Characteristically, Th17 cells express the chemokine receptor CCR6 and their homing is thereby regulated by the CCR6 ligand, CCL20, at sites of infection [63]. The near ubiquitous expression of the IL-17 receptor on non-haematopoietic cells facilitates the broad physiological functions of Th17 cells during inflammation. Through the induction of the inflammatory chemotactic factors CXCL1, CXCL2, CXCL5, and CXCL23 at sites of inflammation via production of IL-17A/F, IL-22, and GM-CSF, Th17-mediated responses are dominated by the inflammatory and phagocytic functions of neutrophils [64]. Other Th17-mediated functions include induction of antimicrobial peptides (including S100 proteins and β-defensins), promotion of granulopoiesis via induction of G-CSF, and enhancement of monocyte and neutrophil activation to promote their phagocytic activity [64].

Whilst Th17 cells represent, in most cases, the major source of adaptive IL-17 during microbial infection, IL-17 elicted from non-Th17 cell sources can also be a determining factor in host defence. Invariant natural killer T (iNKT) cells, natural killer (NK) cells, γδ-T cells, and type 3 innate lymphoid cells (ILC3) (including lymphoid tissue-inducer (LTI) cells) have all been shown to produce protective innate-derived IL-17 in response to infection [65–68]. The importance of IL-17 derived from non-Th17 cell origins has recently been reviewed elsewhere [69,70].

Recent gene-knockout studies have demonstrated the vital importance of IL-17-mediated inflammatory responses for host defences at epithelial barriers, particularly against notoriously persistent extracellular bacteria and fungi (Figure 2, panel A). Seminal work by Ye et al. demonstrated the critical importance of IL-17 for protective immunity in a murine model of extracellular bacterial infection using *Klebsiella pneumoniae* in IL-17R-deficient mice [71]. These mice were more susceptible to intranasal *K. pneumoniae* infection relative to WT counterparts, which correlated with significant delay in neutrophil recruitment into the alveolar space and heightened dissemination of bacteria into the circulation [71]. Numerous studies that followed confirmed the essential role of IL-17 in host protection against *K. pneumoniae* [71–73]. Mice infected with other extracellular bacteria including *Citrobacter rodentium* [74,75], *Bordetella pertussis* [76,77], *Porphyromonas gingivalis* [78,79], or *Streptococcus pneumoniae* [80,81], for example, also mount protective IL-17 responses, and disruption of IL-17A or its receptor leads to exacerbated bacterial burden or dissemination, increased disease susceptibility resulting from defective induction of CXC chemokines, and impaired neutrophil recruitment to sites of bacterial inoculation. Mice with deficiencies in IL-23, a cytokine axis critical for the stabilisation of the Th17 phenotype [82], also display exacerbated pathology associated with numerous extracellular bacterial infections. IL-23p19-deficient mice, akin to IL-17−/− or IL-17R-deficient mice, also fail to effectively mount protective IL-17 responses to *C. rodentium* [52] and *K. pneumoniae* [72] infections. In the absence of these components of the Th17– and IL-17-producing innate cell response, bacterial clearance is impeded, leading to augmented bacterial dissemination and disease susceptibility associated with reduced early IL-17-mediated neutrophil infiltration. Importantly, administration of recombinant IL-17 into IL-23-deficient infected mice restored neutrophilia at sites of inoculation [72], demonstrating the critical importance of the IL-23/IL-17 axis in host defence against various extracellular bacterial infections. Whilst these studies strongly implicate a protective role for the IL-23/IL-17 axis in protection against extracellular infections at epithelial surfaces, the precise cellular origin of IL-17 remains controversial. In the context of *C. rodentium* infection, both an early innate and late adaptive source of IL-17 is thought to be crucial to host protection. Interestingly, IL-17 responses early during *C. rodentium* infection were shown to be elicited from a specialised subset of CD4+ T cells present within the lamina propria (LP) [83]. Differentiation of these cells was dependent on the innate immune sensor receptors NOD-1 and NOD-2, which were shown to regulate intestinal DC-derived IL-6 and subsequent differentiation of these LP-resident “early” Th17 cells. Importantly, NOD-1/NOD-2 deficiency did not alter IL-17A production during the late “adaptive” phase of infection suggesting that these sensors specifically regulate early CD4+ T-cell-derived IL-17. Thus, based on their rapid induction and distinct dependency on NOD-1/NOD-2, these early Th17 cells were termed innate Th17 (ThIN17) cells. ThIN17-derived early IL-17 is not restricted to *C. rodentium* infection as the same study demonstrated that these cells also contribute to defence against *S. typhimurium*, another attaching and effacing bacterium [83]. Other
cells, including γδ-T and ILC3 cells, have also been shown to produce IL-17 following extracellular bacterial challenge [83–86]. Thus, it will be important to delineate the source of IL-17 in the context of extracellular bacterial infections to fully understand the function of T\textsubscript{H}17 cells in these settings. Experiments where IL-17 is specifically deleted in the T cell compartment will be required to obtain this information.

The importance of IL-17-driven inflammation in the context of antifungal host defence has also been established. In mice and men, pathogen-specific T\textsubscript{H}17 responses have been shown to confer protection against the dimorphic filamentous fungus \textit{Candida albicans} [87]. Within the memory CD4\textsuperscript{+} T cell pool of healthy volunteers, \textit{Candida}-specific T\textsubscript{H} cells are enriched within the T\textsubscript{H}17 subset and significantly heightened numbers of IL-17-producing cells in peripheral leukocytes of acute \textit{Candida}-infected patients have been documented compared to healthy controls upon restimulation with \textit{Candida} antigens [88]. Moreover, chronic mucocutaneous candidiasis patients have diminished numbers of IL-17A-producing cells within the peripheral leukocyte pool compared with acutely infected patients and healthy controls [89]. These data, and observations that patients with autosomal dominant hyper-IgE syndrome, characterised by defects in T\textsubscript{H}17 differentiation due to mutations in the T\textsubscript{H}17-polarising transcription factor STAT3 [90–92], are more susceptible to \textit{Candida} and other fungal infections, support an important role for the T\textsubscript{H}17 response in effective antifungal immunity [93]. More detailed analyses of the functional role of IL-17 in fungal immunity have come from murine models of experimental fungal infection. In line with human studies, mice with a deficiency in IL-17A or its receptor are more susceptible to experimental fungal infection.

![Diagram of novel T\textsubscript{H} subsets in inflammation](image)

**Figure 2. Novel T\textsubscript{H} subsets in inflammation.** (A) T\textsubscript{H}17 and T\textsubscript{H}22 cells have overlapping functions in the mouse. Via production of the inflammatory mediators IL-17A, IL-17F, GMCSF (T\textsubscript{H}17), and IL-22 (T\textsubscript{H}22), these T\textsubscript{H} subsets mediate protective immunity against extracellular pathogens intimately associated with mucosal barriers. (B) T\textsubscript{H}9-cell-derived IL-9 may play an important role in antiparasitic immunity via mediating mast cell activation and mastocytosis, increasing the chemotactic potential of an inflammatory site via regulation of inflammatory chemokine production, and promote basophil and eosinophil function.

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A role for the IL-17 axis in antifungal immunity in mice was first described in 2004, in a study in which intravenous infection of IL-17R-deficient mice with Candida led to decreased survival rates and augmented fungal burden in the kidney [94]. In a model of oropharyngeal candidiasis (known as “thrush”), mice with deletions in IL-23p19, IL-17RA, or IL-17RC developed exacerbated thrush lesions associated with augmented fungal burdens, whilst mice deficient in Th1 effector cytokines IFN-γ or TNF-α were resistant to oral infection [95–97]. Critical requirements for the IL-23/IL-17 axis in protective immunity have also been described in murine models of dermal candidiasis [98]. Collectively, these studies demonstrate that the IL-17 response is essential for protective immunity against disseminated, skin, or mucosal Candida infection. Whilst it was believed that Th17 cells represent the major cellular source of protective IL-17 against Candida, a recent study demonstrated that ILC3-derived IL-17 was the critical source of this cytokine in an oropharyngeal candidiasis infection model [67]. Immunity to oropharyngeal infection with Candida was not altered in Rag-deficient or T-cell-deficient animals, suggesting that Th17-cell-derived IL-17 was not an important component of host defence. Antibody-mediated depletion of all ILCs, as well as deletion of ILC3 cells using Rorc-deficient mice, led to enhanced susceptibility to Candida infection, implicating ILC3 cells as the crucial cellular source of protective IL-17 in this model. Further investigation is required to determine the contribution of innate and adaptive sources of IL-17 in other models of primary Candida infection or other fungal pathogen models where IL-17 has been shown to confer protection including Cryptococcus neoformans [99], Aspergillus fumigatus [100], and Pneumocystis carinii [101]. Taken together, recent data have suggested that both Th17– and innate-cell-derived IL-17 play important roles in the context of extracellular bacterial and fungal infections. It is likely that innate IL-17 is crucial as the first line of defence whilst the pathogen-specific Th17 cell response plays more prominent roles during the late phase of infection and in recall challenges. In support, Th17 cell recall responses are required for effective clearance of Candida and K. pneumoniae infection [102,103]. Th17 responses have also been shown to be required for vaccine-induced protection to the endemic fungal pathogens Coccidioides posadasii, Histoplasma capsulatum, and Blastomyces dermatitidis [104] as well as a number of other mucosa-associated pathogens [105].

As outlined above, host defences against intracellular pathogens are classically considered to be coordinated by the Th11 domain. However, recent data have also revealed a potential role for Th17 cells in the context of intracellular microbial infection [106]. Pulmonary infection of mice with the intracellular bacterium Francisella tularensis induced a protective Th17 response [107,108], and deletion of the IL-23/IL-17A axis, but not IL-17F or IL-22, increased susceptibility to pulmonary tularemia [108]. Interestingly, the reported biological function of IL-17A in this model was induction of IL-12 and IFN-γ production from APCs, subsequently promoting antigen-specific Th1 responses [108]. The ability of IL-17A to regulate Th1 responses in the context of microbial infections is not limited to the tularemia model, with reports that IL-17 can influence adaptive immunity to pulmonary Chlamydia muridarum [109] and Mycobacterium bovis BCG [110] infection via similar mechanisms. However, impaired Th17-mediated neutrophil recruitment is also likely to contribute to these observed phenotypes [108]. Indeed, IL-23/Th17-dependent neutrophil responses are important components of protective immunity to other intracellular bacteria infections such as Mycoplasma pneumoniae [111] and Salmonella enterica serotype Typhimurium [112,113]. Taken together, these studies suggest that Th17 cells function in concert with Th11 cells to efficiently resolve some intracellular bacterial infections. The molecular and cellular basis of how these pathogens elicit Th17 cell responses despite induction of a priming environment dominated by Th11-polarising cytokines that antagonize Th17 differentiation remains to be determined. Owing to inherent differences in PRR stimulation by various bacteria, it is possible that certain bacteria effectively induce potent IL-12/IFN-γ responses, whilst other bacterial pathogens require an additional IL-17-dependent mechanism for host IL-12/IFN-γ production to resolve intracellular infection. Moreover, the requirement of neutrophil responses to intracellular infection poses something of a paradox, as these cells are not thought to elicit robust effector responses against intracellular pathogens. However, it may be that Th17-driven neutrophil responses in these settings are active against the extracellular phase of a pathogen’s life cycle, i.e., trans-epithelial bacterial entry.

Virus-specific IL-17-producing CD4+ T cells have also been detected in mice following herpes simplex virus (HSV) [114], Theiler’s murine encephalomyelitis virus (TEMV) [115], and vaccinia virus (VV) [116] infection, among others [20], albeit at a lower magnitude than the prototypic antiviral Th1 response. In these settings, Th17 responses appear to be detrimental to the host as IL-17-deficiency, or neutralisation of IL-17A, reduced HSV-induced stromal keratitis [114]. Furthermore, neutralisation of IL-17 during chronic TEMV infection increased viral clearance and enhanced cytotoxic T cell responses [115], and neutralisation of IL-17 during VV infection decreased the size of primary and satellite lesions, and promoted viral clearance [116]. Nevertheless, protective roles have also been ascribed to Th17-derived cytokines in some viral infections [117]. This precarious balance of protective versus harmful effector functions of Th17 cells has also been observed in the context of parasitic infections where IL-17 has been shown to promote host defence against intestinal Toxoplasmab gondii infection at the expense of heightened immunopathology [118] and contribute to control of S. mansoni infection in the lung [119], yet enhance immunopathology associated with schistosomiasis when mice are infected with S. mansoni in the liver [120,121].

Thus, it is apparent that the Th17 subset is predominantly associated with host defence against extracellular pathogens via IL-17-mediated mobilisation of neutrophil responses. Th17 cells may also serve as an important adjunct to Th1 responses in certain intracellular infections by inducing neutrophil responses that may function during the extracellular life cycle of intracellular pathogens, or by influencing APCs to promote Th1 polarisation in some intracellular bacterial infections that fail to induce efficient pathogen-specific Th1 cell differentiation. Conversely, the Th17 response can be detrimental to the host in that it can contribute to viral persistence and promote pathological inflammation associated with parasitic infection, which thereby presents these cells as a potential therapeutic target to limit pathology in these settings.

Given that optimal immunity to extracellular infections is highly dependent on the function of antibodies, the critical importance of generating pathogen-specific memory Th17 cells in vaccine development under conditions where a primary live infection elicits protective Th17 cell responses is often overlooked. Further study of this system will facilitate design of vaccines that result in improved memory Th17 cell development in synergy with robust antibody responses such that both humoral and cell-mediated arms of the immune system enter into immunological memory. Such strategies are indeed currently under development as is evident by promising vaccine candidates that elicit pathogen-specific Th17 cells in the context of anti–S. pneumoniae immunity [122].
T Helper 22 (TH22)

Recently, a subset of human TH cells dedicated to production of the cytokine IL-22 has been described and proposed to be a separate lineage of TH cell, designated TH22 [123,124]. IL-22 is a pro-inflammatory member of the IL-10 family of cytokines that appears to be particularly important for driving inflammatory responses at cutaneous and mucosal surfaces [125]. TH22 differentiation from naive precursors has been reported to be IL-6- and TNF-α-dependent [123] (Figure 1). Expression of the IL-22 receptor is restricted to stromal cells of the skin, intestine, liver, kidney, pancreas, and lung [128,129], implicating TH22 cells as an important mediator of the interaction of the immune system with the non-hematopoietic environment. TH22 cells have been shown to express the chemokine receptors CCR4, CCR6, and CCR10 [123,124], the ligands of which are known to regulate homing to these organs.

In mice, IL-22 elicited from TH cells appears to be restricted to the TH17 cell subset [130], with very few studies having detected bona fide IL-17-/- TH22 cells in vivo. For this reason, evaluating the function of this potential novel human T cell subset using murine models of microbial infections currently presents significant challenges. The TH22 response clearly shares many similar features with the TH17 response, as evidenced by the lack of obvious divergence between these responses in mice, and the common reliance on AhR signalling for aspects of their function [126]. Akin to the function of other TH17-derived cytokines, IL-22 ligation with its receptor markedly induces expression of multiple antimicrobial peptides including the S100 proteins S100A7–A9, β-defensins, the intestinal antimicrobial peptides RegIII-β and -γ, and stimulates production of protective mucus elicited from goblet cells (Figure 2, panel A) [129–132]. IL-22 also upregulates expression of the inflammatory chemokines CXCL1, CXCL2, and CXCL5, which act in synergy with IL-17 to induce a chemotactic environment that promotes neutrophilia at sites of infection [133]. In addition to its antimicrobial and pro-inflammatory effects, IL-22 also plays an important role in tissue regeneration and wound healing by promoting epithelial cell proliferation and inducing expression of anti-apoptotic proteins [134].

IL-22 appears to play a dichotomous role in host defence depending on the nature of the pathogen and site of infection. Protective functions of IL-22 have been described in the context of extracellular pathogen infection of the lung and intestine including K. pneumoniae and C. rodentium. In most cases, IL-22 was essential for control of bacterial replication and dissemination, most likely in part due to the ability of this cytokine to potently induce antimicrobial peptide production by epithelial cells at these barrier surfaces [132,133]. In line with the protective role for TH17 responses in antifungal immunity, IL-22-producing CD4+ T cells have also been detected within the Candida-specific memory T cell pool of healthy patients [135], and are defective in patients with chronic mucocutaneous candidiasis [89,136,137]. However, the function of IL-22 in experimental C. albicans infection remains controversial [98,138]. In a murine model of oropharyngeal candidiasis, host defence was predominantly mediated by IL-17, not IL-22 [96], whilst protective immunity to C. albicans infected intragastrically was dependent on IL-22-mediated production of antimicrobial peptides including S100A8 and S100A9, which prevented yeast dissemination to the kidneys and stomach [139].

Numerous studies have suggested that immunity to intracellular pathogens or parasites does not appear to rely on IL-22. Host defence against the intracellular pathogens Mycobacterium avium and L. monocytogenes, or the parasitic pathogen S. mansoni, is IL-22-independent [140–142]. Unlike K. pneumoniae and C. rodentium, these pathogens are not intimately associated with mucosal or cutaneous barriers, which may underlie the redundant role of IL-22 in these settings. Furthermore, IL-22 has been shown to be detrimental in a murine model of oral T. gondii infection [140,145]. In this model, IL-23 promoted development of ileitis in an IL-22-dependent manner. Whilst no difference in protozoan burden was documented between WT and IL-22-deficient mice, WT mice succumbed to infection due to intestinal necrosis, whereas IL-22-deficient mice displayed increased survival rates with only minor inflammation evident. Flow cytometric analyses implicated CD4+ T cells in the lamina propria as the major source of IL-22, which contributed to T. gondii-induced panileitis principally via an immune response directed against gut microbiota rather than the protozoan pathogen. These data suggest that particular microbial agents can induce detrimental IL-22-mediated pathogenic inflammation [143]. On the contrary, studies have implicated protective roles for IL-22 in certain intracellular pathogen infections including experimental influenza and dengue infectious models [144–146]. Despite reports that IL-22 deficiency or neutralisation does not alter the outcome of M. tuberculosis infection in mice [140,147], Scriba et al. demonstrated that a substantial proportion of mycobacteria-specific TH cells from healthy M. tuberculosis–exposed individuals produce IL-22 and are distinct from TH17 and TH1 cells, implicating IL-22 as an important cytokine axis in human anti-mycobacterial immunity [148]. These reported differences between mice and men in M. tuberculosis infection support the notion that CD4+-T-cell-derived IL-22 plays a more prominent role in the human than murine immune system, at least under certain circumstances.

Despite the fact that TH22 cells are not clearly distinguishable from the TH17 subset in mice, experiments to specifically evaluate the significance of T-cell-derived IL-22 in models of microbial infection have been performed. TH22 cells have been detected in experimental coxsackievirus-B3-induced myocarditis where they appear to exacerbate acute viral-induced myocarditis associated with increased cardiac viral replication, heightened cardiomyopathy, and reduced survival rates [149]. More recently, a study by Basu et al. demonstrated, for the first time, the function and protective efficacy of TH22-derived IL-22 in the context of microbial infection [150]. Significant expansion of IL-22-producing CD4+ T cells that lacked expression of IL-17A occurred in the colonic lamina propria during the late phase of C. rodentium infection, implicating TH22 cells as the predominant TH cell subset mediating host protection to this enteropathogenic bacterium. Infection of IL-6-deficient mice led to profound defects in lamina-propria-resident TH22 cell numbers, but not IL-22 production from other cells, relative to IL-23-deficient mice or WT counterparts, illustrating the importance of IL-6 in regulating TH22 differentiation. The importance of T-cell-derived IL-22 in protective immunity to this pathogen was reflected in the marked decline in survival of mice treated with neutralising antibodies to IL-22 administered after the peak of the innate immune response. Moreover, adoptive transfer of in vitro-generated TH22 cells, but not in vitro-generated TH17 cells, into IL22-/- mice rescued recipient mice from pathogenic inflammation [150]. These experiments are the first to definitively demonstrate the existence and function of TH22 cells during enteropathogenic bacterial infection. Further detailed studies are required to explore the function of TH1-cell-derived IL-22 in other infectious models.
It is important to appreciate that, similar to other TH-cell-derived cytokines, IL-22 production is not restricted to the CD4+ T cell compartment. Various other cells, including γδ-T, NKT, and CD8+, have the ability to produce IL-22 that participates in host defence against microbes [70,132,139,142]. More specifically, ILC3s have been shown to be a dominant innate source of IL-22 during infection [125]. Thus, in order to delineate the function of TH22-derived IL-22 in the context of microbial infection, mice with T-cell-specific deletions of IL-22 will be required. However, to our knowledge, these reagents have yet to be developed.

Given the recent discovery of the TH22 subset, limited studies have been carried out to date regarding the function of TH22 cells in host defence to microbes. As discussed above, current data suggest that the TH22 subset, in most cases, has overlapping functions with the TH17 lineage in mice, in contrast to the human system where IL-22-secreting T cells potentially form a distinct lineage. It is now important to dissect how and why certain infections elicit IL-22 responses that are favoured over IL-17-mediated immunity in humans. The results of such studies may provide crucial insights into how the balance of TH22/TH17 cells defends against certain pathogens and may lead to the development of vaccines tailored to particular microbial threats.

### T Helper 9 (TH9)

IL-9 represents one of the most understudied cytokines in the field of TH cell biology despite its diverse biological effects on numerous cell types of myeloid, lymphoid, and stromal origin [151]. IL-9 was first associated with TH2-mediated responses following reports that IL-9 expression in T cells was high in TH2-prone BALB/c mice relative to the TH1-prone C57Bl/6 mouse strain during the course of L. major infection [152]. Subsequent studies implicated a protective role for IL-9 in TH2-driven responses during murine parasitic infections [153,154], with IL-9 levels in mesenteric lymph nodes correlating with expansion of TH2 cell populations and a requirement of IL-9 for CSR to the “type-2” antibody isotypes IgG1 and IgE [155,156]. Furthermore, an in vivo requirement for IL-4, a crucial mediator of TH2 differentiation, for induction of IL-9 expression by T cells was later demonstrated in L. major–infected BALB/c mice [152]. The results of these studies led to the classification of IL-9 as a TH2-derived pro-inflammatory cytokine. However, despite the clear association between IL-9 and TH2 responses, recent reports of high-level IL-9 production in macrophage– and neutrophil-dominated inflammatory settings were counter to previous conceptions that IL-9 was elicited from TH2 cells [157]. These findings have recently been reconciled with the discovery that naïve T cell priming in the presence of IL-4 and TGF-β drives differentiation of a functionally disparate subset of IL-9-secreting TH1 cells, designated TH9 (Figure 1) [158,159]. Subsequent studies have called into question the requirement of IL-4 in TH9 differentiation with reports that IL-4R signalling induces expression of suppressor of cytokine signalling (SOCS) family member cytokine-induced SH-2 protein (CIS), which inhibits STAT3/STAT6 signalling and subsequent TH9 cell differentiation [160]. Indeed, IL-9 production in T cells has been shown to be independent of IL-4 when activated in the presence of TGF-β1 and 1-α [161]. In vivo, the molecular requirements for TH9 cell induction may involve many additional stimuli including IL-23, TSLP, 1,25-dihydroxvitamin D3, programmed cell death ligand (PD-L) 2, cyclooxygenase (COX)-2, and tumor necrosis factor receptor superfamily member 4 (OX40) [162]. Furthermore, data suggesting that the TH9 programme is unstable and highly prone to plasticity have raised questions as to whether this IL-9-secreting CD4+ T cell indeed represents a distinct differentiation lineage. These aspects of TH9 cell biology will not be discussed in this review but have been recently reviewed elsewhere [162,163]. Differentiation of this subset is thought to require the transcription factors PU.1, IRF4, and BATF [164–166]. We have recently examined chemokine receptor expression by TH9 cells and have shown that these cells express a broad range of trafficking receptors, including CCR3, CCR6, and CXCR3 [167]. Notably, these receptors are also characteristically expressed by other TH cell subsets (TH2, TH17, and TH11, respectively) suggesting that TH9 cells have the capability of being recruited to, and contributing to multiple, functionally distinct forms of inflammatory lesions. Whilst CCR4 expression by TH9 cells generated in our models was not detected, recent work has suggested that these cells also express CCR4 and CCR8, which would presumably allow these cells to traffic to cutaneous sites of inflammation [168].

Given that the description of this new TH9 cell subset came years after initial studies into the role of IL-9 in the context of microbial infections were carried out, the function of IL-9 in protective immunity will be discussed with conjecture on the role of bona fide TH9 cells in these settings. Studies using IL-9 transgenic (IL-9tg) mice have emphasized the importance of this cytokine in the control of certain intestinal parasitic infections (summarized in Figure 2, panel B). Following infection with Trichinella spiralis or T. muris, IL-9tg mice developed enhanced intestinal mastocytosis and augmented pathogen-specific IgG1 responses, which led to rapid parasitic expulsion from the gut [153,154]. Furthermore, treatment of mice with neutralising antibodies to IL-9 during the course of T. muris infection diminished immunity to this pathogen [168]. In line with the protective phenotypes observed in IL-9tg mice, a specific role for TH9-derived IL-9 in protective immunity to intestinal nematode infection was more recently assessed using mice in which TGF-β signalling, a crucial mediator of TH9 differentiation, was specifically deleted in the CD4+ T cell compartment. Infection of these mice with T. muris augmented worm burden and reduced IL-9 but not IL-13 production in mesenteric lymph nodes [159]. In these models, IL-9 appears to predominantly function via activation of mast cells, the inflammatory mediators from which promote seminal processes required for effective parasite expulsion such as induction of eosinophilia, increased intestinal permeability and contractility, and mucus production. Recent work by Licona-Limon et al. using novel IL-9 reporter mice (termed Interleukin Nine Fluorescent Reporter: INFER) and newly generated IL-9-deficient mice on a BALB/c background has revealed a critical and nonredundant role for TH9 and IL-9 in host defence to N. brasiliensis [169]. GFP reporter activity was detected in CD4+ T cells and type-2 ILCs (ILC2: a known prominent source of IL-9 in numerous type-2 models) in both lungs and mediastinal lymph nodes during the course of N. brasiliensis infection. IL-9-GFP detection in CD4+ T cells in the lung peaked early and declined during the course of infection, whilst IL-9-GFP+ ILC2s were detectable early and remained present throughout, suggesting a transient window of CD4+T-cell-derived IL-9 in this model. Adoptive transfer of TH9 cells into IL-9-deficient mice led to enhanced worm expulsion, demonstrating that TH9-derived IL-9 was an important contributor to IL-9-dependent immunity in this model. The results of this study also elucidated numerous other unknown aspects of TH9 cell biology including the demonstration of functional differences between TH9 and TH17 cells in host protection, despite prior reports concluding that the function of these two subsets in other models substantially overlapped. Surprisingly, transferred TH9 cells, but not TH17 cells, into infected Rag2-deficient hosts decreased worm burden. TH9-mediated protection correlated with increased...
numbers of mast cells and basophils in lungs and spleens of infected mice, implicating these innate effectors as the key responding cell types to IL-9-mediated immunity. Moreover, recent findings by Turner et al., using IL-9 fate mapper reporter mice (termed IL-9-creR26R<sup>YFP</sup> mice, which permanently label cells with enhanced YFP (eYFP) that have expressed IL-9 irrespective of their current IL-9 expression status) and IL-9R-deficient animals, support the notion that IL-9 plays a critical role in host defence against <i>N. brasiliensis</i> infection [170]. Both eYFP<sup>+</sup> CD4<sup>+</sup> T cells and ILC2 cells were detected in the lung during infection. IL-9 in this setting was demonstrated to positively regulate IL-5 and IL-13 responses, likely ILC2-derived IL-5 and IL-13 as T<sub>HF</sub>2 cell numbers were unchanged in IL-9R-deficient animals, promote ILC2 cell survival, drive lung tissue repair, and mechanisms and promote eosinophil recruitment and alternative activation of macrophages. These studies highlight the critical importance of both T<sub>HF</sub>9 and ILC2-derived IL-9 in host defence to <i>N. brasiliensis</i>. However, the results of these studies are not in keeping with a prior study, which demonstrated that IL-9-deficient mice on a mixed genetic background (129×C57Bl/6 F<sub>2</sub>) control effectively infection with <i>N. brasiliensis</i> [171]. The conflicting results of these studies warrant further investigation of IL-9 function in anti-<i>N. brasiliensis</i> immunity but suggest that the overall importance of IL-9 depends on complex multi-genetic factors. IL-9 deficiency, using 129×C57Bl/6 (F<sub>2</sub>) R<sup>B9</sup> mice backcrossed six times onto a BALB/c background, led to modest reductions in mast cell numbers but did not alter the outcome of infection with the flagellated intestinal protozoan parasite <i>Giardia lamblia</i> [172]. Moreover, IL-4 has been shown to control intestinal parasitic infections in an IL-5/IL-9/IL-13 triple-knockout mouse [173]. Collectively, these data suggest that the T<sub>HF</sub>9 subset may serve as an important adjunct to the T<sub>HF</sub>2 response in certain parasitic infections; however, it appears to be superfluous in certain circumstances where T<sub>HF</sub>2 responses suffice. In support, IL-9 has been shown to precede and regulate T<sub>HF</sub>2-associated cytokine responses in certain parasitic infections [169,170]. Given that T<sub>HF</sub>9 cells have been reported to produce the CCR4 ligands CCL17 and CCL22 [164], and studies that demonstrate that IL-9 can induce expression of the inflammatory chemokine CCL11 by smooth muscle cells [174,175], early IL-9 responses may be important for amplifying CCR3<sup>+</sup> eosinophil [176] or CCR3<sup>+</sup>/CCR4<sup>+</sup> T<sub>HF</sub>2 cell responses at sites of microbial infection.

The function of IL-9 in other microbial infections is less well-defined with conflicting conclusions having been reached to date. Following reports that bronchial secretions from infants with respiratory syncitial virus (RSV) bronchiolitis contained high levels of IL-9 [177], the function of IL-9 was specifically investigated in a murine model of RSV vaccination and infection. Antibody-mediated neutralisation of IL-9 in these models resulted in enhanced viral clearance from the lungs and had varied effects on pathology depending on the timing of IL-9 depletion [178]. In contrast to the detrimental roles of IL-9 in the RSV model, prophylactic administration of recombinant IL-9 into mice infected with a lethal dose of <i>Pseudomonas aeruginosa</i> enhanced survival via suppression of inflammatory cytokines including IFN-γ and TNF-α, and induction of the immunomodulatory cytokine IL-10 [179]. Endogenous IL-9 induction was detected in spleens of mice infected with sublethal, but not lethal, doses of <i>P. aeruginosa</i>; however, the precise cellular source of this IL-9 was not explored [179]. IL-9-secreting CD4<sup>+</sup> T cells have also been detected in humans with <i>M. tuberculosis</i> infection [180]; however, the functional significance of these cells in immunity to this pathogen has yet to be investigated. It is clear that a great deal of work is still required to delineate the function of T<sub>HF</sub>9 cells in the context of microbial infections. Owing to the recent discovery of this subset, the majority of studies investigating the function of IL-9 in protective immunity have utilised systemic means of IL-9 blockade/neutralisation such as the use of antibody-mediated neutralisation or IL-9-deficient mice. However, IL-9 can be elicited from multiple cell types including ILCs, mast cells, Tregs, and natural killer T cells [181]. Therefore, more refined studies making use of the recently described INFER mice [169], which will be useful to detect IL-9-expressing cells in real time, IL-9 fate-mapping reporter mice [182], or mice with specific deletions of the Th9-specific transcription factor PU.1 in the T cell compartment [164] are required to determine which microbial infections elicit a Th9 response and whether this response is protective.

Taken together, it appears that the Th9 response may play significant roles in immunity to certain intestinal parasites and contribute to host protection via amplification of the infectious site’s chemotactic potential and mediating mast cell and basophil activation. Although less well-understood, the Th9 response may also participate in a diverse array of other infections; however, it appears that these cells play a role supplementary to T<sub>HF</sub>2 cells and in some cases a potentially detrimental role in host defences.

**T Follicular Helper (Th9)**

The Th<sub>H1</sub>, Th<sub>H2</sub>, Th<sub>H17</sub>, Th<sub>H22</sub>, and Th<sub>H9</sub> subsets represent populations of effector helper T cells that contribute to immune responses at peripheral sites of infection. Within secondary lymphoid organs, populations of effector CD4<sup>+</sup> T cells interact with clonally selected B cells to produce humoral immunity by providing crucial signals that regulate B-cell survival, proliferation, affinity maturation, CSR, and differentiation into memory B- or long-lived plasma cells. This molecular cross-talk between B and T cells occurs in two waves: first at the T-B border where CD4<sup>+</sup>-T-cell-derived cytokines instruct developing B cells to switch to an appropriate isotype and actively form specialised structures known as germinal centres (GC), followed by further interactions within the GC that ultimately determine the quality of antibody response generated [183]. Prior to 2004, CD4<sup>+</sup> T cells localised to GCs were thought to be a branch of canonically derived Th<sub>H1</sub> or Th<sub>H2</sub> cells that migrated into B cell follicles to coordinate CSR to IgG<sub>λ</sub> and IgG<sub>1</sub> via IFN-γ and IL-4, respectively [184]. However, recent data have demonstrated that these GC-localised T cells, now referred to as follicular Th<sub>H</sub> cells (T<sub>HF</sub>), are in fact a distinct differentiation lineage (Figure 1 and Figure 3). These cells are characterised by expression of the lineage-specific master regulator Bcl6 (the transcription factor c-Maf is also crucial to this subset) [185–187], as well as the ability to produce a range of cytokines including IL-4, IFN-γ, IL-21, and IL-17A [188–195] (Figure 1 and Figure 3). T<sub>HF</sub> cells are also characterised by high expression of the chemokine receptor CXCR5, which mediates migration into B cell follicles and GCs that are rich in CXCL13 [196,197].

Current models of T<sub>HF</sub> cell development describe a “two-wave” theory of differentiation: DC-instructed commitment to the T<sub>HF</sub> cell lineage (i.e., pre-T<sub>HF</sub> cell differentiation) followed by B-cell-instructed consolidation of the T<sub>HF</sub> cell programme (i.e., GC-resident T<sub>HF</sub> cells) [198]. It is thought that T<sub>HF</sub> cells selectively differentiate from naïve precursors with the highest affinity to any given antigen [199], consistent with reports that the magnitude of Th9 cell generation is dependent on the dose of Ag made available to the T cell during its interaction with a DC [200]. STAT3 activation downstream of IL-6 and/or IL-21 has also been shown to promote early commitment to the Th<sub>HF</sub> cell lineage via induction of their master transcriptional regulator Bcl6 [193,201,202].
Interestingly, STAT1 and STAT4 activation downstream of IL-6 and IL-12 signalling respectively, two transcription factors known to promote TH1 cell differentiation, have been shown to induce expression of Bcl6 in CD4\(^{+}\) T cells [203–206]. Nakayamada et al. provided evidence that TH1 cell differentiation may occur through a TH1-cell-like transitional state where T-bet and Bcl6 are co-expressed in the same cell [207]. T-bet was shown to eventually outcompete Bcl6 function when IL-12 signalling persists leading to downregulation of Bcl6, and TH1 lineage fate commitment then predominating [207]. Thus, the earliest events of DC-instructed commitment to the TH1 cell lineage are complex and likely involve the integration of numerous microenvironmental signals [208]. Importantly, differentiation of TH1 cells appears to be independent of the other known effector subsets as mice deficient in genes critical to TH1, TH2, and TH17 lineage development do not display marked differences in TH1 cell induction [193]. Early TH1 cell commitment is coupled with the downregulation of CCR7 (the ligands of which are in highest concentration in T cell zones of SLOs) and upregulation of CXCR5 (the ligands of which are in highest concentration in the B cell zones), facilitating the movement of the pre-TH1 cell to the T-B border [209]. Stable interactions with cognate B cells at this border are required for the terminal differentiation of the TH1 cell programme and are coupled with further upregulation of Bcl6, thus promoting expression of genes required for B cell help, and CXCR5, which facilitates the migration of these cells deeper into the B cell zone and into GCs where they execute their predominant effector function [198,210].

Given that the ability of the host to generate high-affinity neutralising/opsonising antibody responses via the GC reaction is seminal for defence against a number of ever-evolving pathogens, the function of TFH cells is predominantly associated with regulating the process of antibody affinity maturation through governance of selection, survival, proliferation, and differentiation of high-affinity, pathogen-specific B cell clones. TFH cells execute these effector functions via the wealth of cell-surface and soluble proteins that they express (reviewed in [184]) including CD40L, which interacts with GC-B-cell-expressed CD40 and imprints an anti-apoptotic transcriptome in the responding B cell [211,212], and IL-21, which promotes the proliferation of GC B cells [190,213,214] and drives their differentiation toward the plasma cell compartment in both mice [215] and humans [216–218]. Therefore, it is perhaps of no surprise that individuals with mutations in these factors exhibit defects in generating isotype-switched high-affinity antibody responses and, thus, are more prone to opportunistic infection. For instance, patients with
mutations in Cd46g develop the primary immunodeficiency hyper-
immunoglobulin M syndrome, characterised by a severe deficit in
GC development and lack of circulating isotype-switched immu-
noglobulin, and, subsequently, an increase in susceptibility to
recurrent bacterial infections and are unresponsive to vaccination
[214,219,220]. Similarly, patients with a mutation in the SLAM-
associated protein (SAP), encoding gene Sh2d1a, a signalling
protein expressed by TFH cells critically required for the formation
of stable T:B conjugates [221,222] and GC-TFH cell expression of
IL-4 [223], develop X-linked lymphoproliferative (XLP) disease
characterised by an increased susceptibility to a number of
pathogens (particularly Epstein-Barr virus infection, which can be
fatal in children) due to abortive B cell responses [224].

Central to the ability of the host to generate an effective
antibody response to a given pathogen is the decision of B cells to
class switch to an appropriate antibody isotype for maximum
effector function tailored to the nature of the microbe. CSR to
IgG2A is driven by IFN-γ and plays a vital role in the neutralisation
of viruses, complement fixation, and opsonisation of microbes via
the Fc portion. Conversely, IL-4-mediated CSR to IgG1 and IgE is
essential for antibody-mediated cell cytotoxicity mechanisms in
the context of antiparasitic immunity. Despite the clear demonstration
by multiple groups that differentiation of Bcl6+ TFH cells in vivo is
dependent of Tfh1, Tfh2, or Tfh17 differentiation pathways
[185–187], how Tfh1 cells could coordinate CSR during particular
infections via IL-4 and/or IFN-γ posed something of a paradox.
Using reporter systems and other elegant approaches, recent work
has revealed that TFH cells can differentiate in a variety of priming
environments and that the cytokine milieu present during T cell
activation likely favours the production of IFN-γ or IL-4 by TFH
cells in the context of Tfh1– or Tfh2-polarising infections,
respectively (Figure 3). Using 4get/KN2 dual reporter mice,
which faithfully report cells that have actively transcribed from the
Il4 locus and cells actively producing IL-4 [225], Reinhardt and
colleagues demonstrated that during infection with the type-2
pathogens L. major or N. brasiliensis, IL-4 production in draining
lymph nodes was restricted to bona fide TFH cells that were
phenotypically and functionally distinct from canonical Tfh2 cells
[226]. TFH-cell-derived IL-4 is important for driving parasite-
specific IgG1 responses as GC B cells sorted from IL-4-producing
TFH/GC B cell conjugates were actively undergoing CSR to IgG1
[226]. Using IFN-γ reporter mice, the same study demonstrated that
GC B cells sorted from IFN-γ-producing TFH/GC B cell
conjugates were actively undergoing CSR to IgG2A [226].
Consistent with these observations, Lutjhe and colleagues recently
reported that TFH cells favoured the production of IFN-γ
following influenza infection [227]. However, development of
IgG2A+ (C57Bl/6 mouse equivalent of IgG2A) GC B cells was
unperturbed in chimeric mice reconstituted with IFN-γ-deficient
CD4+ T cells [227]. Collectively, the results of these studies suggest
that TFH-derived IL-4 is seminal for generation of pathogen-
specific IgG1 antibody responses whilst TFH-derived IFN-γ is not
essential for an IgG2A antibody response, although it may play a
supplementary role in this process.

Much controversy still exists in the field of TFH cell biology
regarding the origin of these cells during pathogen encounter.
Although it is apparent that the differentiation programmes of T
helper cells with B-cell helper function are separate to other
effector TFH cell subsets as described above, studies have
demonstrated that non-TFH effector T cells can re-differentiate
to the TFH lineage [229,229] (Figure 3). TFH cells have been
described to arise from Tfh1 in the context of LCMV infection
[230]; from Tfh2 cells in the context of Heligmosomoides polygyrus, S.
manusoni, and N. brasiliensis infection [185,226,229]; and from Tfh17
cells in the Peyer’s patches, which was shown to be critical for
antigen-specific IgA responses [231]. From a clinical perspective,
the ability of the host to generate high-quality antibody responses
governs the success of most currently available vaccination
strategies; therefore, understanding the differentiation pathways
and cytokine-secreting repertoires of TFH cells under different
immunising conditions is imperative to the design of vaccines that
generate high-affinity antibody responses with the appropriate
dominant antibody isotype tailored to the nature of the microbe
of interest. The recent development of cytokine fate-mapping
reporter mice and generation of Bcl6 and IL-21 reporter mice
that faithfully map TFH cells during an immune response
[227,229] should facilitate the collection of important information
regarding these processes.

Concluding Remarks

The adaptive immune response has a broad array of strategies
to combat infectious, potentially pathogenic agents. One of the
most important strategies utilised is to tailor the immune response
to combat particular classes of microbial agents, and TFH cell
subsets play a crucial role in this process. Significant progress has
dramatically improved our understanding of TFH cell biology with
a number of new subsets recently being identified and discussed in
this review, as well as emerging effector TFH cell phenotypes, such
as granzyme B-expressing cytolytic CD4+ T cells found in certain
viral infections [232] and subsets of TFH cells dedicated to
production of IL-21 [233], that have not yet been shown
to be a distinct effector lineage and are not discussed here but
warrant further study. Together, these cells give the adaptive
immune response the potential to deliver antigen-specific inflam-
atory responses that instruct and complement pathogen-tailored
innate inflammatory responses. These TFH responses differentially
combat extracellular pathogens, enhance cell-mediated immunity
required to combat intracellular pathogens, and promote humoral
immunity to produce antibodies that target pathogens. Future
challenges include further dissection of this system to identify other
potentially important subsets and identifying ways in which to
utilise this knowledge to develop better strategies to combat
infectious pathogens. Ascertain that such knowledge will be crucial
for determining whether future vaccination strategies: i) elicit
robust TFH cell responses with the appropriate cytokine-secreting
reertoire to induce an antibody response tailored to the nature of
the pathogen; and ii) activate the appropriate components of the
innate immune system that induce a priming microenvironment
driving differentiation of the desired pathogen-specific-effector TFH
cell subset and/or -CTL activation such that upon contact with
the live pathogen, all aspects of the adaptive immune system are
armed to promote effective, pathogen-tailored clearance of the
infectious agent. Thus, increased understanding of the complex
dynamics of TFH differentiation in the context of microbial
infections should lead to improved vaccine efficacy for a wide
range of human pathogens.

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