Human T follicular helper cells in primary immunodeficiencies

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Purpose of review
To summarize our understanding of the biology of T follicular helper (Tfh) cells and how insights into this are being provided by the study of human monogenic immunological diseases.

Recent findings
Antibody production is a key feature of the vertebrate immune system. Antibodies neutralize and clear pathogens, thereby protecting against infectious diseases. Long-lived humoral immunity depends on help provided by Tfh cells, which support the differentiation of antigen-specific B cells into memory and plasma cells. Tfh cells are generated from naïve $CD4^+$ T cells following the receipt of inputs from various cell surface receptors. Although genetically modified mice have provided a great understanding of the requirements for generating Tfh cells, it is critical that the requirements for human Tfh cells are also established. This is being achieved by the systematic analysis of humans with monogenic mutations that cause primary immunodeficiencies characterized by impaired humoral immunity following infection or vaccination.

Summary
The elucidation of the mechanisms that regulate Tfh cell generation, differentiation and function should reveal targets for novel therapeutics that may offer opportunities to manipulate these cells to not only improve humoral immunity in the setting of primary immunodeficiencies but also temper their dysregulation in conditions of antibody-mediated autoimmunity.

Keywords
$CD4^+$ T cells, CXCR5, immunological memory, primary immunodeficiencies, T follicular helper cells, vaccination

INTRODUCTION

In healthy individuals, natural infection or immunization with approved vaccines usually generates protective immunity such that infectious disease from subsequent exposure is prevented. The ability of the mammalian immune system to generate such robust, efficient and specific immunity and memory relies on the flexibility of naïve $CD4^+$ T cells to differentiate into diverse subsets equipped with specific functions to eradicate pathogenic threats [1]. Th1, Th2 and Th17 cells have been implicated in providing immune-mediated protection against specific pathogens [intracellular viruses and mycobacteria (Th1), extracellular parasites (Th2) and fungi (Th17)] [1,2]. However, a cardinal feature of immunity following natural infection, and the success of most vaccines, is the generation of long-lived plasma cells and memory B cells selected to produce high-affinity neutralizing antibodies either constitutively or following reencounter with the initiating pathogen [3–5]. This process is dependent on T follicular helper (Tfh) cells, a specialized population of effector $CD4^+$ T cells. Here, we will review the biology of Tfh cells and discuss how defects in Tfh cell formation or function can contribute to the clinical features of human monogenic conditions.

T FOLLICULAR HELPER CELLS: DISCOVERY IN HUMAN LYMPHOID TISSUES

Tfh cells were first identified in human tonsils as $CD4^+$ T cells expressing the B-cell zone-homing

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KEY POINTS

- Analysis of human PIDs characterized by poor antibody responses provides an opportunity to dissect the molecular and cellular requirements for generating Tfh cells in humans.
- Loss-of-function mutations in *CD40LG*, *ICOS*, *SH2D1A*, *BTK* and *STAT3* compromise the generation of Tfh cells in humans.
- Tfh cells are required for the generation of long-lived humoral immunity to T-cell-dependent antigens.
- The rapid discovery of new gene mutations underlying novel human PIDs will facilitate the identification of novel regulators of human Tfh cell generation and function.

chemokine receptor CXCR5, but lacking the T-cell zone chemokine receptor CCR7, thus allowing them to reside in B-cell areas of secondary lymphoid tissues [6,7]. Beyond CXCR5, Tfh cells are typically identified by high levels of expression of the surface receptors, ICOS and PD-1, the transcriptional repressor, BCL6, and the cytokine, IL-21 [6–9]. The close proximity of Tfh cells to B cells facilitates T-dependent B-cell activation, expansion and differentiation. Indeed, $CD4^+CXCR5^+$ T cells are more efficient than $CD4^+CXCR5^-$ T cells at inducing class switching and Ab secretion by cocultured B cells through mechanisms predominantly involving CD40L, ICOS and IL-21 [6,7,10–12]. These initial descriptions resulted in the recognition that Tfh cells, rather than Th2 or another effector $CD4^+$ T-cell subset, were primarily responsible for mediating T-dependent B-cell differentiation and generating long-lived humoral immunity (Fig. 1a).

T FOLLICULAR HELPER CELL SUBSETS

Although the initial characterization of Tfh cells at the cellular, molecular and functional levels involved the analysis of lymphoid tissues, extrapolation of these findings to settings of human disease is challenging as access to human lymphoid tissues is not always possible. This has necessitated the study of a small subset of CXCR5-expressing $CD4^+$ T cells in the peripheral blood. Although there have been some conflicting findings, the general consensus is that these cells are the circulating counterpart of Tfh cells in human secondary lymphoid tissues. Blood-derived $CD4^+CXCR5^+$ T cells express higher levels of ICOS, CD40L, PD-1, IL-21, IL-10, CXCL13 and BCL6 – which are all features of Tfh cells – following in-vitro stimulation, and are more efficient at inducing B-cell differentiation, than $CD4^+CXCR5^-$ T cells [13,14,15▪▪,16▪▪,17]. However, key differences exist between circulating Tfh cells and those in secondary lymphoid tissues, including substantially reduced expression of Bcl-6, ICOS and PD-1 by blood Tfh cells, and their expression of CCR7 [9,13,14,15▪▪,16▪▪,17]. This change in phenotype possibly coincides with the evolution of a lymphoid tissue Tfh cell into a quiescent memory-type cell following tissue egress and entry into the circulation.

The population of circulating $CD4^+CXCR5^+$ T cells is heterogeneous, with distinct subsets being identified according to differential expression of specific surface receptors. Morita *et al.* [14] originally reported the presence of subsets on the basis of the expression of CXCR3 and CCR6. Thus, they found Th1 ($CXCR3^+CCR6^-$), Th2 ($CXCR3^-CCR6^-$) and Th17-like ($CXCR3^-CCR6^+$) populations of Tfh cells. In addition to producing varying amounts of IL-21, Th1-like, Th2-like and Th17-like Tfh cells expressed signature features of conventional Th1 (i.e., *TBX21*; IFNγ), Th2 (*GATA3*; IL-4, IL-5, IL-13) and Th17 (*RORC*; IL-17A, IL-22) cells [14]. Notably, the Th2-Tfh and Th17-Tfh subsets produced higher levels of IL-21 and induced greater B-cell differentiation than Th1-like Tfh cells [14]. More recently, Locci *et al.* [15▪▪] and He *et al.* [16▪▪] fractionated circulating $CD4^+CXCR5^+$ T cells into subsets according to differential expression of PD-1 and CXCR3 or CCR7. This revealed that the $PD\text{-}1^+CXCR3^-$ and $PD\text{-}1^+CCR7^{lo}$ subsets of $CD4^+CXCR5^+$ T cells were enriched for IL-21-secreting cells and were most efficient at providing help for B-cell differentiation *in vitro* [15▪▪,16▪▪]. These findings led to the conclusion that circulating $CD4^+CXCR5^+$ T cells have most likely undergone a Tfh differentiation program *in vivo* and can be used as a surrogate or biomarker of lymphoid tissue Tfh cell activity *ex vivo*. Indeed, these studies successfully established correlations between the overabundance of Th2 and Th17-type or $PD\text{-}1^+CCR7^{lo}$ Tfh cells and severity of disease in the setting of some autoimmune conditions [14,16▪▪], as well as between the presence of $PD\text{-}1^+CXCR3^-$ Tfh cells and the ability to generate neutralizing Abs against HIV in infected individuals [15▪▪]. Thus, the identification of a circulating counterpart of Tfh cells has laid the foundation to more easily study these cells in human disease.

REQUIREMENTS FOR T FOLLICULAR HELPER CELL FORMATION: INSIGHTS FROM MICE

The generation of Tfh cells results from a complex series of interactions initially between naïve $CD4^+$ T cells and dendritic cells (DCs) in the T-cell zone of

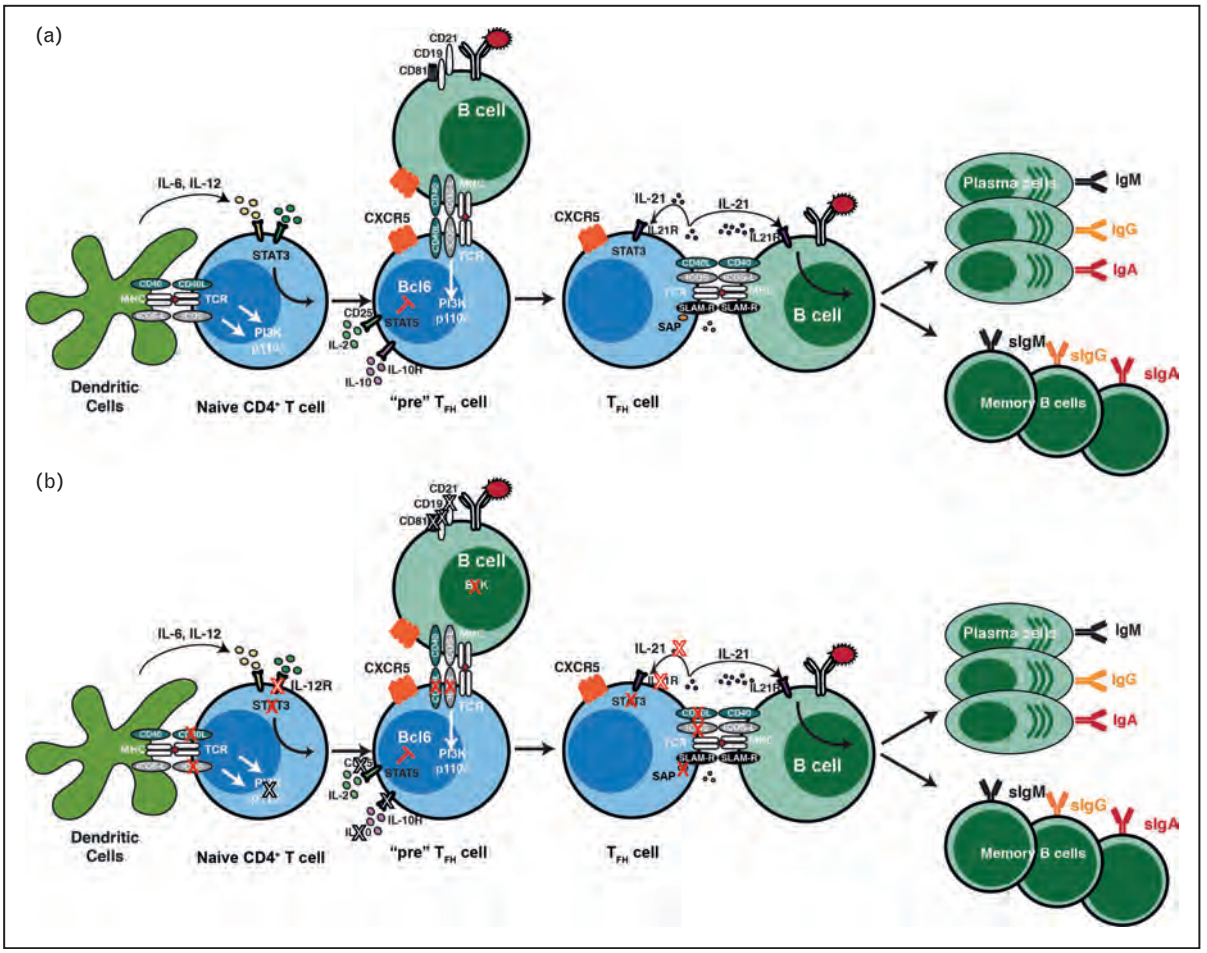

FIGURE 1. Pathways leading to T follicular helper (Tfh) cell development – effect of PID mutations. (a) Following encounter with dendritic cells (DCs), naïve CD4$^+$ T cells develop into activated pre-Tfh-type cells that migrate into B-cell follicles. Signals provided by B cell reinforce the 'Tfh program', resulting in the generation of differentiated Tfh cells, which can migrate into germinal centers and provide help to Ag-specific B cells for their differentiation into long-lived memory B cells and plasma cells. Multiple inputs from both DCs and B cells are required for Tfh formation, including cytokines and receptor/ligand interactions. Similarly, signals delivered through the IL-10/IL-10R and IL-2/CD25/STAT5 axes suppress Tfh formation. (b) Indicates where and how specific monogenic mutations can perturb Tfh formation. Mutations in *CD40LG, ICOS, STAT3, SAP* and *BTK* (resulting in a lack of B cells) (depicted by solid red crosses) have all been found to the compromise formation of Tfh cells in humans. Mutations in *IL12RB1* or *IL21/IL21R* (depicted by outline red crosses) may reduce Tfh cell numbers; however, more studies are required to definitively establish the roles of these genes in Tfh formation. Mutations in *PI3K p110d, CD19/CD21/CD81, IL10/IL10RB* and *CD25* (depicted by outline black crosses) may theoretically impact Tfh function, with PI3K p110d and CD19/CD21/CD81 being required for Tfh formation and IL10/IL10RB and CD25 signaling functioning to restrain Tfh formation. Thus, the latter mutations may contribute to aberrant Tfh generation and development in settings of monogenic autoimmune conditions.

lymphoid tissues and subsequently between these DC-primed CD4$^+$ T cells and B cells within follicles. Indeed, B cells are required for the generation of Tfh cells as these cells fail to form in B-cell-deficient mice [18,19]. These interactions involve numerous cell surface receptors (CD28, ICOS, CD40L, SLAM family members) and cytokines (e.g., IL-6, IL-12, IL-21, IL-27) coupled to their associated signaling pathways (SAP, STAT1 or STAT3) (Fig. 1a). Although Tfh cells are reduced in many gene-targeted mice, no single cytokine or signaling pathway is essential, indicating that substantial redundancy exists between these pathways (reviewed in [20–23]).

Interactions between these ligands and receptors leads to the induction of key transcription factors that imprint upon the activated CD4$^+$ T cells a Tfh fate. Bcl-6 was the first, and perhaps the best characterized, transcription factor identified as being required for Tfh cell formation. Bcl-6 functions as a transcriptional repressor. By suppressing

the expression of *Tbx21*, *Gata3* and *Rorc*, Bcl-6 prevents the commitment of naïve $CD4^+$ T cells into Th1, Th2 and Th17 cells, respectively [20–26]. However, it is possible that Bcl-6 also contributes to Tfh formation by inducing expression of relevant target genes [20,23]. Interestingly, some signature features of Tfh cells – such as CXCR5 and IL-21 – were not induced by Bcl-6 alone [20–22]. This led to the discovery of additional transcription factors – Ascl2, BATF, cMAF, IRF4, STAT3 – that work cooperatively or sequentially to induce Tfh cells [20–23]. Thus, BATF can induce Bcl-6 and cMAF [27], with cMAF inducing CXCR5 and IL-21 [28–30], whereas STAT3-activating cytokines can directly induce IL-21, as well as cMAF and possibly BATF [29,31]. Interestingly, the E3 ubiquitin ligase Itch is important for inducing or maintaining Bcl-6 expression in Tfh cells and is thus required for Tfh formation [32]. Recently, Ascl2 was also found to play a critical role in inducing CXCR5 and regulating trafficking of Tfh cells [33].

Although many factors are important for promoting Tfh cell genesis, a smaller number of molecules that restrain Tfh formation have been identified. The most potent repressor of Tfh cells is Blimp-1, which is induced by IL-2/STAT5 signaling and represses Bcl-6 [24,34] (Fig. 1a). IL-10 also ameliorates Tfh formation; however, the molecular pathway(s) underpinning this effect are unknown [22] (Fig. 1a). Another regulator is the transcription factor FoxP1, which suppresses Tfh formation by inhibiting expression of ICOS and IL-21 [35]. Interestingly, Itch can associate with FoxP1 to promote its degradation, thereby relieving FoxP1-mediated negative regulation of Tfh cells [32]. Thus, akin to Bcl-6 and Blimp-1, Itch and FoxP1 may function as another Yin-Yang pair acting as diametric opposites to promote and impede, respectively, Tfh formation [32,35]. Overall, positive and negative signals from various inputs operate to dynamically and intrinsically fine-tune Tfh formation and function, thereby ensuring the induction of a Tfh response that is appropriate for the current pathogenic threat. Dysregulation of this process appears to cause diseases as diverse as autoimmunity, immunodeficiency or malignancy characterized by the overrepresentation, reduction or transformation, respectively, of Tfh cells [21,22].

REQUIREMENTS FOR T FOLLICULAR HELPER CELL FORMATION: INSIGHTS FROM HUMAN PRIMARY IMMUNODEFICIENCIES

The vast majority of our knowledge on the requirements for generating Tfh cells has come from the analysis of gene-targeted or naturally occurring mutant mice. Although it is highly likely that these requirements are mirrored in human Tfh cells, in light of the numerous differences between human and murine immune systems [36], it is important to confirm that this is indeed the case. The study of humans with monogenic immunological diseases such as primary immunodeficiencies (PIDs) has established some of the requirements for generating human Tfh cells.

Hyper IgM (HIGM) syndrome is characterized by normal to elevated serum IgM, but severely reduced IgG, IgA and IgE resulting in increased susceptibility to infection by a range of pathogens. HIGM can be caused by mutations in *CD40LG* or *CD40*, underscoring the requirement for CD40/CD40L signaling for immunoglobulin isotype switching. Mutations in *ICOS* manifest as adult-onset common variable immunodeficiency, whereas mutations in *SH2D1A* (encoding SAP) cause X-linked lymphoproliferative disease (XLP) with progressive hypogammaglobulinemia being a key clinical trait [37]. Common features of these PIDs are impaired humoral immune responses associated with defects in generating memory B cells and a deficit of well-formed germinal centers in secondary lymphoid tissues [37]. In contrast, X-linked agammaglobulinemia (XLA) is caused by *BTK* mutations, resulting in an almost complete loss of B cells due to a block at the pre-B-cell stage of development and a severe reduction in levels of all serum immunoglobulin isotypes [37].

The predominant expression of CD40L, ICOS and SAP by T cells pointed to the $CD4^+$ T cell-intrinsic nature of defective humoral immunity in HIGM, ICOS deficiency and XLP, respectively. Analysis of peripheral blood samples revealed that the frequencies of circulating $CD4^+CXCR5^+$ T cells are dramatically decreased in CD40L-deficient and ICOS-deficient individuals [38] (Fig. 1b). These findings indicate that signals received via CD40/CD40L and ICOS/ICOS-L are required not only for B-cell differentiation but also for the generation, maintenance and survival of human Tfh cells [38]. XLA patients also had few circulating $CD4^+CXCR5^+$ T cells [39] (Fig. 1b), reminiscent of B-cell deficient mice [18]. The fact that a similar reduction was found in common variable immunodeficiency patients with reduced peripheral B cells [39] implied that the Tfh cell deficit was primarily due to the absence of B cells rather than a lack of BTK, which can function in some myeloid cells that persist in XLA patients. This establishes the importance of B cells for the induction or maintenance of Tfh cells in humans and infers important roles for ICOS/ICOS-L and CD40L/CD40 interactions between B cells and $CD4^+$ T cells in these processes. Unlike patients with

mutations in *CD40LG*, *ICOS* or *BTK* [38,39], XLP patients have normal numbers of circulating Tfh-like cells [16▪▪,19,40]. However, SAP-deficient $CD4^+$ T cells failed to acquire functional attributes of Tfh cells *in vitro* [19,40,41].

Autosomal dominant hyper-IgE syndrome is caused by mutations in *STAT3* [37]. Although affected patients do not exhibit hypogammaglobulinemia *per se*, they do have impaired functional Ab responses and a reduction in circulating memory B cells [37,42,43]. Consistent with such defects in humoral immunity, these patients have a deficiency in circulating $CD4^+CXCR5^+$ T cells (Fig. 1b), and their naïve $CD4^+$ T cells fail to differentiate into Tfh-like cells following in-vitro stimulation [17,44]. STAT3 is activated by cytokines including IL-6, IL-12, IL-21 and IL-27, which contribute to Tfh cell generation in mice [20–23]. Thus, these cytokines probably combine to control human Tfh differentiation in a STAT3-dependent manner. Signal integration between multiple cytokines is likely required because the frequencies of circulating $CD4^+CXCR5^+$ T cells in patients with loss-of-function mutations in *IL21R* [45▪▪] or *IL21* [46▪] are not significantly reduced compared to normal controls (Fig. 1b). Similarly, although IL-12 is the main inducer of human Tfh-like cells *in vitro* [47,48], we found that mutations in *IL12RB1*, encoding IL-12Rβ1, caused only a mild (and nonsignificant) reduction in circulating Tfh cells [44]. Despite this, another study observed fewer circulating Tfh cells (Fig. 1b), memory B cells and lower avidity Ab responses to tetanus in patients with *IL12RB1* mutations [49▪] even though these patients had normal levels of serum IgG against tetanus toxoid, rubella, Epstein-Barr virus, cytomegalovirus and varicella virus [49▪] As the defect in circulating $CD4^+CXCR5^+$ T cells improved with age [49▪], IL-12 signaling may only be required for Tfh cells early in life. Overall, it would appear that in the absence of IL-12 or IL-21 signaling, compensatory mechanisms involving other cytokines function to preserve Tfh cell differentiation.

Analyses of monogenic PIDs revealed requirements for B cells, *CD40LG*, *ICOS*, *SH2D1A*, *STAT3* and possibly *IL12RB1* in Tfh formation, function and maintenance (Fig. 1b). They also provided further correlative evidence that circulating Tfh cells are related to bona-fide Tfh cells, as a deficit of peripheral $CD4^+CXCR5^+$ T cells paralleled a deficiency of germinal centers and presumably lymphoid tissue Tfh cells. However, the fact that circulating Tfh cells were present in normal frequencies in XLP patients highlights the need to consider alternative approaches to interrogate Tfh function in PIDs beyond merely phenotyping. Extending this analysis to quantify subsets (using CCR7/PD-1 or CXCR3/CCR6 expression), coupled with in-vitro analysis of function (e.g., expression of IL-21, inducing B-cell differentiation), may provide more robust read-outs of the status of Tfh cells and their function in the setting of PIDs.

WHAT ELSE CAN PRIMARY IMMUNODEFICIENCIES TEACH US ABOUT HUMAN T FOLLICULAR HELPER CELLS?

The studies detailed above can be examples, and inspirations, for future investigations using other monogenic immunological diseases that could inform us of the pathways that converge to yield human Tfh cells, as well as identifying novel targets to modulate human Tfh formation in the settings of autoimmunity or PID. For instance, the role of B cells in inducing Tfh cells could be further refined by studying humans with loss-of-function mutations in molecules that play important roles in regulating B-cell function, such as *CD19* [50], *CD81* [51] and *CD21* [52] (Fig. 1b). Similarly, studies have revealed that ICOS signals via the PI3 kinase pathway to generate Tfh cells in mice [53,54]. Humans with gain-of-function mutations in *PI3CKD*, encoding the p110δ subunit of PI3 kinase, have poor humoral immunity to T-dependent and T-independent Ags [55▪▪,56▪▪]. Although this likely results from a B-cell-intrinsic defect [56▪▪], it is possible that dysregulated ICOS signaling in $CD4^+$ T cells impacts Tfh cell development, further compromising Ab responses (Fig. 1b). Gain-of-function mutations in *STAT1* were originally reported to cause chronic mucocutaneous candidiasis [57]. However, it is now evident that these mutations result in a broad and variable clinical phenotype, with some patients presenting with defects in generating protective Ab responses [37,57]. These patients will provide an opportunity to address the role of aberrant STAT1 signaling in human Tfh cells and may resolve conflicting results regarding the importance of STAT1 in murine Tfh cells [58–60].

Humans have also been identified with mutations in *IL10* or *IL10RB1*, which cause early-onset severe inflammatory bowel disease [61], and *IL2RA* (CD25) or *STAT5B*, causing not only severe multisystemic autoimmune manifestations due to defects in homeostasis of regulatory T cells (Tregs) but also immune deficiency characterized by recurrent microbial infections [62]. As signaling via IL-10/IL-10R and CD25/STAT5 pathways constrain Tfh development in mice [22,23,34], the assessment of Tfh-type cells in these patients could provide important insights into the role of these cytokine signals in regulating human Tfh cells

(Fig. 1a,b). Interestingly, mutations in *ITCH* yield an autoimmune phenotype similar to patients with defects in *IL2RA* or *STAT5B* [62]. Although the development of autoimmune manifestations in patients with *IL10*, *IL10RB1*, *IL2RA*, *STAT5B* or *ITCH* mutations are probably due to impaired Treg function, aspects of these conditions may also involve dysregulated Tfh cells, as noted for other systemic (polygenic) autoimmune conditions [21,22]. It will be important to determine whether *ITCH* is a positive or negative regulator of human Tfh cells, as it was recently shown to promote Tfh formation in mice [32].

In addition to conventional Tfh cells that promote T-dependent B-cell responses, a subset of Tfh cells – T follicular regulatory (Tfr) cells – has been identified that suppresses humoral immunity by acting on either Ag-specific B cells or Tfh cells themselves [21,22]. Although the exact mechanisms underlying Tfr-mediated repression remains to be completely elucidated they are likely to be similar to those utilized by conventional Tregs, such as cytokines (IL-10, TGFβ, IL-35) and surface receptors (CTLA4) [62]. As more studies are being performed to dissect Tfr cells in mice and understand how they restrain humoral immunity, PIDs represent a resource to extrapolate some of these findings to humans, thereby revealing the biology of human Tfr cells. Thus, individuals with mutations in *IL10*/*IL10R*, *IL2RA*/*STAT5B*, *ITCH* and *FOXP3* [61,62], as well as the recent identification of individuals with heterozygous mutations in *CTLA4* [63▪▪], who all develop severe autoimmunity, provide invaluable opportunities to determine the molecular requirements for the generation and function of human Tfr cells. Interestingly, Meffre and colleagues recently reported that patients with IPEX due to *FOXP3* mutations have an increased frequency of circulating Tfh-like cells and these cells had greater expression of the Tfh-related molecules *BCL6*, *PD1*, *ICOS* and *IL21* than corresponding normal cells [64▪▪]. Whether these differences result from a lack of Tfr cells themselves, a lack of regulation of Tfh cells by Tfr cells or both remains to be determined, but this study reveals the potential insights that can be gained by analyzing Tfh, and potentially Tfr, cells in patients with monogenic autoimmune diseases, akin to our improved knowledge of human Tfh cells from analyzing PIDs.

CONCLUSION

Studies of genetically modified or naturally occurring mutant mice have led to a stunning increase in our understanding of the factors required for the generation, function and regulation of Tfh cells. Although many of these processes are likely to be conserved in other mammalian species, in order to develop rational strategies for treating various immunological dyscrasias it remains important to study Tfh biology in humans. Analyses of patients with monogenic PIDs or autoimmune diseases have indeed yielded critical insights into human Tfh cells and have provided the framework for continued investigation of these cells in different immune settings. With the exponential increase in discovery of novel genes causing PIDs by next-generation sequencing [65], it is clear that many examples of monogenic immunological diseases will arise providing fertile ground for future studies and greater knowledge of the molecular and cellular regulation of human Tfh cells.

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Conflicts of interest

There are no conflicts of interest.

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- ▪ of special interest
- ▪▪ of outstanding interest

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