Targeting T$_{FH}$ cells in human diseases and vaccination: rationale and practice

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Abstract

The identification of CD4$^+$ T cells localizing to B cell follicles has revolutionized the knowledge of how humoral immunity is generated. Follicular helper T (T$_{FH}$) cells support germinal center (GC) formation and regulate clonal selection and differentiation of memory and antibody-secreting B cells, thus controlling antibody affinity maturation and memory. T$_{FH}$ cells are essential in sustaining protective antibody responses necessary for pathogen clearance in infection and vaccine-mediated protection. Conversely, aberrant and excessive T$_{FH}$ cell responses mediate and sustain pathogenic antibodies to autoantigens, alloantigens, and allergens, facilitate lymphomagenesis, and even harbor viral reservoirs. T$_{FH}$ cell generation and function are determined by T cell antigen receptor (TCR), costimulation, and cytokine signals, together with specific metabolic and survival mechanisms. Such regulation is crucial to understanding disease pathogenesis and informing the development of emerging therapies for disease or novel approaches to boost vaccine efficacy.

T cell help is necessary to effectively mount antibody responses, a discovery dating back to 1968 (ref. 1). About 30 years later, a population of antigen-primed CD4+ T cells was characterized as expressing the chemokine receptor CXCR5, migrating to B cell follicles, and being superior at supporting cultured B cells for antibody production[2–5]. After another decade of research, and particularly with the identification of the lineage-directing master transcription factor BCL6 (refs. [6–8]), their identity as T_{FH} cells became widely accepted. Since then, ever-growing evidence from both experimental models and human studies has revealed a central role for T_{FH} cells in regulating protective antibody responses following infection or vaccination, as well as pathogenic antibody responses in autoimmunity, alloimmunity, and allergy[9–11]. In this Review, we summarize the critical functions of T_{FH} cells in human health and disease, and discuss how an understanding of the molecular and cellular mechanisms that regulate T_{FH} function can guide the development of therapeutic approaches to target T_{FH} cells in physiological and pathological conditions.

Quantification of T_{FH} cell numbers to assess T_{FH} differentiation, survival, and memory

T_{FH} cells are arguably the orchestrators of germinal center (GC) reactions, which are transient microanatomical structures that develop in secondary lymphoid organs (SLOs) in response to protein anti- gens. T_{FH} cells are specialized effector $CD4^+$ T cells that support both extrafollicular and GC B cell responses. Extrafollicular responses produce a short wave of antibodies with little affinity maturation. In contrast, GC B (B_{GC}) cells undergo extensive rounds of somatic hypermutation and selection that culminate in affinity maturation. Affinity-matured B_{GC} cells can bind more antigens and form cognate interactions with T_{FH} cells to receive positive selection signals, which then promotes differentiation into antibody-secreting (B_{AS}) or memory (B_{Mem}) B cells[12,13]. T_{FH} cells are induced in immune responses. As such, their numbers are often used as readouts to reflect T_{FH} cell differentiation, survival, or memory maintenance.

Among CD4+ T cells, GC-resident T_{FH} (GC- T_{FH}) cells show the highest expression of PD-1 and are usually characterized as CXCR5^{hi}PD-1^{hi}BCL6$^+$ (refs. [14–16]). They account for 10–30% of antigen-specific $CD4^+$ T cells in SLOs at the peak of mouse immunization or infection responses[16–19]. Given the difficulty of obtaining SLOs in humans, circulating T_{FH} (cT_{FH}) cells are enumerated in peripheral blood mononuclear cells (PBMCs), which typically constitute 5–15% (children) or 5–25% (adults) of total $CD4^+$ T cells[20,21]. cT_{FH} cells are heterogeneous, and CCR7^{lo}PD-1$^+$ICOS$^+$CD38$^+$ cT_{FH} cells are associated with active T_{FH} differentiation[21,22]. T_{FH} cell numbers are also indicative of defects in T_{FH} cell differentiation, which are commonly found in individuals with monogenic immunodeficiency caused by mutations in genes encoding T cell costimulatory receptors (*CD40*, *CD40LG*, and *ICOS*), cytokines and cytokine receptors (*IL21* and *IL21R*), signaling molecules (*SH2D1A*, encoding SAP), or transcription

factors (*STAT3*). These mutations result in reduced T_{FH} cells and lead to compromised humoral immune responses (reviewed in ref. [23]).

Apart from T_{FH} cell differentiation, T_{FH} cell survival mechanisms also shape T_{FH} cell numbers. Two major programmed cell death pathways, namely ferroptosis and pyroptosis, have been described as fundamental in controlling T_{FH} cell survival. Unlike non-T_{FH} effector $CD4^+$ T cells, T_{FH} cells experience persistent TCR stimulation through their interactions with B_{GC} cells, which increases the production of reactive oxygen species and exaggerates lipid peroxidation. Therefore, T_{FH} cells are susceptible to ferroptosis, iron-dependent lipid-peroxidation-induced cell death[24]. Ferroptosis regulates T_{FH} cell numbers and function in protein immunization in mice and humans[24]. In contrast, pyroptosis specifically controls T_{FH} survival in the gut, during inflammation, and in response to tissue damage[25,26]. Hence, diverse cell-death pathways appear to control T_{FH} cell survival, depending on physiopathological contexts[27].

$CXCR5^+$ memory T_{FH} cells are superior to $CXCR5^-$ memory $CD4^+$ T cells in providing B cell help and thus accelerating humoral recall immune responses. The relative contributions of circulating versus resident memory T_{FH} cells in recall immune responses are not well understood, but both memory populations can significantly rein- force T_{FH} cell function. In a stepwise T_{FH} differentiation model[28,29], $CXCR5^+PD-1^+ICOS^+$ precursor T_{FH} (pre-T_{FH}) cells are generated at the inter-follicular zone and the T–B border, where they support antigen-primed B cell expansion and extrafollicular responses[14,30,31]. It has been suggested that cT_{FH} cells are predominantly generated from pre-T_{FH} cells, whereas GC-T_{FH} cells may contribute more to resident memory T_{FH} cells[21,32,33] (Fig. 1a). Collectively, strong T_{FH} differentiation, better T_{FH} survival and superior memory maintenance can promote T_{FH} cell numbers and function, which are associated with potent antibody responses.

Qualitative assessment of T_{FH} function: heterogeneity, plasticity, and specificity

T_{FH} cells comprise a heterogeneous pool of cells that differ not only in differentiation stages (such as pre-T_{FH}, GC-T_{FH}, or memory T_{FH}) (Fig. 1a), but also in diverse functions that are closely associated with secretion of specific cytokines. For example, human cT_{FH} cells are often classified by the expression of different surface homing receptors as $CXCR3^+CCR6^-$ $cT_{FH}1$, $CXCR3^-CCR6^-$ $cT_{FH}2$, and $CXCR3^-CCR6^+$ $cT_{FH}17$ subsets that produce type 1 helper T (T_H1), T_H2, and T_H17 effector cytokines, respectively[20]. Up to 50–60% of GC-T_{FH} cells produce the signature cytokine IL-21, but expression of other cytokines varies, including IL-2, IFN-γ, IL-4, and IL-10 (refs. [8,34–37]). T_{FH} cell-derived cytokines direct extrafollicular and GC antibody isotype class-switching (IFN-γ for IgG2a, IL-4 for IgG1, IL-4 and IL-13 for IgE, IL-10 and

IL-21 suppressing IgE)[38–41], modulate B_{GC} differentiation (IL-21 for B_{AS}, IL-4 and IL-9 for B_{Mem})[42–45], and perform specific functions (dopamine facilitates productive T_{FH}– B_{GC} interactions[46], and granzyme B and perforin direct killing of B cells[47]). New technologies that integrate single-cell and spatial information promise a better understanding of T_{FH} cell heterogeneity.

The high degree of heterogeneity in T_{FH} cells can be partially explained by the high plasticity of T_{FH} cells. T_{FH} cells show positive epigenetic hallmarks on key transcription factor genes (*TBX21*, *GATA3*, and *RORC*) and can be repolarized into T_H1, T_H2, or T_H17 cells[48]. One key function of the T_{FH}-defining transcription factor BCL6 is suppressing gene expression networks that dictate other effector T_H cell programs[7,8,49]. Compared with high BCL6 expression in GC-T_{FH} cells, memory T_{FH} cells, including cT_{FH} cells, express much lower levels of BCL6 and are thus ready to re-differentiate into non-T_{FH} effectors. Intriguingly, a small fraction of GC-T_{FH} cells were found to upregulate FOXP3 in the late GC response in mice, a mechanism proposed to facilitate GC contraction[50]. Conversely, FOXP3$^+$ regulatory T (T_{reg}) and IL-17$^+$ T_H17 cells can *trans*-differentiate into GC-T_{FH} cells in gut Payer's patches (PPs)[51,52]. Therefore, T_{FH} cell plasticity needs to be accounted for when assessing T_{FH} cell function and should thus be assessed dynamically.

The relationship between T_{FH} cell functional heterogeneity and antigen specificity is an important but not well-understood question. T_{FH} cell differentiation is influenced but not dictated by antigen bio- availability and TCR specificity because monoclonal CD4$^+$ T cells (for example, OT-II or SMARTA T cells with transgenic TCR) generate both T_{FH} and non-T_{FH} effectors. In mouse models, T_{FH} differentiation requires intermediate to high TCR signal strength and is promoted by antigen persistence[17,18,53–55]. However, further increases in TCR affinity may favor T_H1 differentiation by upregulating the CD25–BLIMP1 axis that antagonizes T_{FH} differentiation[56,57].

Altogether, we propose six essential elements to assess the role of T_{FH} cells quantitatively and qualitatively in physiological and pathological conditions: differentiation, survival, memory, heterogeneity, plasticity, and specificity (Fig. 1b).

Protective functions of T_{FH} cells in infection, vaccination, and cancer

T_{FH} cells can support at least four types of immune protection against infection (Fig. 2a). First, T_{FH} cells support the production of protective antibodies that inhibit pathogen replication, promote pathogen clearance, and drive antibody affinity maturation to improve pathogen neutralization. In chronic infection of lymphocytic choriomeningitis virus (LCMV) in mice, sustaining T_{FH} cells and GCs are instrumental in producing LCMV-specific antibodies. Given

that T_{FH} cell maintenance is procured by follicular dendritic cell (FDC)-derived IL-6, defects in IL-6 signaling consequently impair LCMV-specific antibody production and result in prolonged viremia[58]. Similarly, compromised antibody responses and failure of pathogen clearance were observed in T_{FH} cell-deficient mice exposed to hepatitis B virus or the intestinal pathogen *Citrobacter rodentium*[59,60], illustrating the role of T_{FH} cells in controlling pathogen clearance.

T_{FH} cells also orchestrate humoral immunity to resolve infections in humans. In people with acute coronavirus disease 2019 (COVID-19) and convalescent individuals with the disease, increased $CCR7^{lo}PD-1^+ICOS^+CD38^+$ cT_{FH} cells (suggestive of active T_{FH} differentiation) are associated with the production of neutralizing antibodies to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[61–64]. Conversely, the delayed development of neutralizing antibodies observed in deceased people who had COVID- 19 corresponds to defective T_{FH} cells and GCs in post-mortem SLOs[65]. Discrepancies were noted regarding T_{FH} heterogeneity in COVID-19, with either $cT_{FH}1$ or $cT_{FH}17$ proposed as a correlative for protective humoral immunity[61,62,64]. Functional heterogeneity in T_{FH} cells has also been reported in other human infections, such as $CXCR3^-$ cT_{FH} ($cT_{FH}2$ and $cT_{FH}17$) cells that correlated with broadly neutralizing antibodies in human immunodeficiency virus (HIV) infection[66]. Therefore, measuring both active T_{FH} differentiation and the relevant T_{FH} subset is crucial when assessing T_{FH} function in human infections.

Second, T_{FH} cells foster the generation of B_{Mem} cells that can rap- idly respond and differentiate upon reinfection. According to the current model, B_{Mem} cells experience fewer rounds of selection than B_{AS} cells in GC responses, as their emergence occurs at the earlier stages of the response. This is thought to ensure that B_{Mem} cells maintain a diverse range of antigen affinities and specificities and thus provide broad protection upon reinfection, especially by a heterologous pathogen[67]. Indeed, a commonality in immunodeficient individuals with impaired T_{FH} function is a decrease in the B_{Mem} compartment, especially isotype-switched B_{Mem} cells[23]. New animal studies are required to assess to what extent T_{FH} function regulates B_{Mem} cells and how it influences the outcome of reinfection.

Thirdly, and in addition to supporting humoral immunity, T_{FH} cells confer another layer of protection by sustaining $CD8^+$ T cell-mediated cytotoxicity. During chronic LCMV infection in mice, IL-21 secreted by $CD4^+$ T_H cells is essential to prevent effector T cells from adopting an exhausted phenotype, characterized by high and sustained expression of inhibitory receptors such as PD-1, progressive loss of effector functions, poor self-renewal, and deceased memory recall[68]. Mechanistically, IL-21 promotes the generation of $TCF1^+CXCR5^+CD8^+$ T cells[69], which locate in proximity to SLO follicles and possess stem-like self-renewal features[70,71].

Because chronic infection redirects T_H differentiation toward T_{FH} cells[53], it is tempting to conceive T_{FH} cells as the major resource of IL-21 that protects $CD8^+$ T cells from exhaustion and dysfunction in chronic infection. This hypothesis needs to be formally tested by an experimental system that can separate the role of T_{FH} cell-derived IL-21 in supporting $CD8^+$ T cells from other T_{FH} cell-mediated controls of viremia.

Lastly, T_{FH} cells in mucosal-associated lymphoid tissue (MALT) regulate the quantity and quality of IgA. IgA is critical in maintaining bacterial symbiosis and modulating respiratory and gastrointestinal-tract infections[72]. For example, PD-1-deficient mice exhibit an aberrant expansion of T_{FH} cells in the PPs and consequently have impaired IgA affinity maturation. Such disruption ultimately leads to altered gut commensal microbiota and gut dysbiosis[73]. Moreover, evidence for the role of T_{FH} cells in driving gut IgA responses has been demonstrated through therapeutic approaches aimed to improve survival of PP T_{FH} cells. Indeed, such strategies were shown to enhance the production of high-affinity IgA and to reduce the bacterial load and ameliorate pathology in mice challenged with virulent *Escherichia coli*[74]. Collectively, T_{FH} cells are unparalleled mediators of mechanisms that drive, sustain, and regulate several aspects of immunity that ensure the proper resolution of infection.

The success of vaccine regimes fundamentally relies on protective functions afforded by T_{FH} cells, which in turn underpin the generation of long-lived B_{AS} and B_{Mem} cells (Fig. 2a). T_{FH} cell activation has been extensively investigated during immune responses against inactivated seasonal influenza vaccines[22,75,76] and, more recently, SARS-CoV-2 messenger-RNA vaccines[77,78]. Influenza vaccines induce active $cT_{FH}1$ ($CXCR3^+CCR7^-PD-1^+ICOS^+CD38^+$) cells, which correlate with vaccine-induced humoral immunity. $cT_{FH}1$ cells predominantly help B_{Mem} rather than naive B cells[75], which is concordant with the observation that CXCR3-expressing extrafollicular T_{FH} cells support B_{Mem} to B_{AS} differentiation in human tonsils[79]. Therefore, influenza vaccination may largely elicit short-lived extrafollicular responses[80]. By contrast, SARS-CoV-2 mRNA vaccines induce T_{FH} cell-supported GC responses that persist for >6 months[78,81]. Notably, such long-lasting GC responses enable the generation of B_{AS} cells that accumulate somatic hypermutations and produce high-avidity antibodies[82].

Interestingly, the same vaccine strategy can result in a spectrum of efficacy at the population level. While genetics and the environment are typically blamed for this phenomenon, it is tempting to speculate that variable T_{FH} cell function may partially explain diverse humoral immunity among individuals receiving the same vaccine. Older people are particularly at risk of not generating immunity following influenza vaccination, as clinical efficacy drops to 17–53% in this group, compared with 70–90% in young adults[83]. The generation of antigen-specific T_{FH} cells in older people is sup- pressed. Mechanistically, elevated inflammatory

pathways down-stream of TNF and IL-2 were the culprit for this defect[84,85], along with the possibility of decreased T$_{FH}$ function due to reduced leptin signaling[86]. Collectively, both the quantity and quality of T$_{FH}$ cells appear to be instrumental in determining robust vaccine responses at the population level.

Besides the canonical function of T$_{FH}$ cells in antibody responses, they can play a 'non-canonical' role in supporting anti-tumor immunity (Fig. 2b). T$_{FH}$ gene signatures, especially *CXCL13* and *IL21*, in tumor-infiltrating immune cells were strongly associated with patient survival[87]. CXCL13-producing T$_{FH}$ cells are enriched in cancer-associated tertiary lymphoid structures (TLSs) with GCs[88]. TLSs are ectopic cellular aggregates that resemble SLOs and represent a privileged area that recruits lymphocytes into tumors and sustains humoral and cytotoxic immunity[89]. Recent compelling evidence has demonstrated a role for T$_{FH}$ cells in promoting TLS formation. In a mouse model of colorectal cancer, introduction of *Helicobacter hepaticus* induced colonic TLSs, increased tumor immune infiltration and inhibited tumor growth. This depended on T$_{FH}$ cells because T$_{FH}$-deficient mice did not form TLSs and had deteriorated tumor control[90]. Besides actively orchestrating TLS formation, T$_{FH}$ cells can indirectly enhance CD8$^+$ T cell-mediated antitumor immunity through the secretion of IL-21. For instance, T$_{FH}$ cells promoted CD8$^+$ T cell function in tumor-draining lymph to control tumor growth, which was otherwise diminished in T$_{FH}$-deficient mice[91]. Further studies are required to investigate the differentiation and antigen specificity that regulate the development of CXCL13- and IL-21-producing T$_{FH}$ cells in anti-tumor immunity.

Pathogenic roles of T$_{FH}$ cells in autoimmunity, alloimmunity, allergy, and lymphoma, and as an HIV reservoir

Autoantibodies recognize self-antigens and initiate a range of pathogenic pathways, including interference with ligand–receptor interaction and signaling, cytolysis, and inflammation[92] (Fig. 3a). Although pathogenic autoantibodies with high affinities that carry somatic mutations are mainly considered products of GCs supported by GC-T$_{FH}$ cells[9,93,94], T$_{FH}$ cells also support the extrafollicular autoantibody pathway seen in lupus-like MRL/MpJ-*Fas*^{lpr} and DNASE1L3-deficient mice[95–97]. Excessive T$_{FH}$ cell numbers with aberrant function are reported in a long list of autoimmune diseases, including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome (SS), type 1 diabetes (T1D), and atherosclerosis[9,94]. Positive correlations between T$_{FH}$ cell activity, auto-antibody profiles, and disease biomarkers suggest an instrumental role of T$_{FH}$ cells in disease pathogenesis, which has been verified by mouse models whereby genetic ablation in CXCR5, ICOS, and SAP reduces autoantibody production and ameliorates disease[98–100].

T$_{FH}$ cells also drive alloimmunity upon tissue transplant. In chronic graft-versus-host disease (cGvHD), increased ICOS$^+$PD-1$^+$ cT$_{FH}$ cells were found in individuals with cGvHD after allogeneic hematopoietic stem cell transplantation (HSCT) and were correlated with B cell activation and B$_{AS}$ differentiation. In line with this, T$_{FH}$ signatures declined during remission[101]. Intriguingly, a T$_{FH}$ cell-supported extrafollicular (rather than GC) response is required for HSCT-induced cGvHD in mice[102]. The generation of donor-specific antibodies represents a significant risk after organ transplantation and is estimated to be responsible for 30–50% of renal graft failures, which are associated with the expansion of CCR7$^-$PD-1$^+$ cT$_{FH}$ cells[103]. Suppressing T$_{FH}$ function by CXCR5 deficiency in T cells reduces donor-specific-antibody responses and protects against allogeneic kidney transplant rejection in mice[104]. Together, T$_{FH}$ cells support both extrafollicular and GC pathways to promote autoimmunity and alloimmunity (Fig. 3a).

In allergic rhinitis, atopic dermatitis, food allergy, and asthma, immune hypersensitivity to innocuous environmental antigens induces skewed type 2 immune responses and excessive IgE pro- duction. Cross-linking of allergen-IgE to the high-affinity receptor FcεRI induces an inflammatory cascade in mast cells and basophils that results in the release of potent biologically active mediators, such as histamine, and causes a cluster of allergic symptoms[105] (Fig. 3b). IL-4-producing CXCR5$^+$ T$_{FH}$2 cells, rather than CXCR5$^-$ T$_H$2 cells, are fundamental for allergenic IgE production[106], shown by diminished allergen-specific IgE production in T$_{FH}$-deficient mice[107]. In individuals with allergic rhinitis or asthma, CXCR3$^-$CCR6$^-$ cT$_{FH}$2 cells expressing IL-4 are expanded and correlate with allergen-specific IgE[106,108,109]. Interestingly, a minority of IL-4$^+$ T$_{FH}$2 cells also express IL-13. During allergic responses in mice, ablation of IL-13$^+$IL-4$^+$ T$_{FH}$2 cells (denominated as T$_{FH}$13) drastically reduced IgE$^+$ B$_{GC}$ cells without affecting total GCs, resulting in more than a tenfold decrease in high-affinity IgE titers. This suggests T$_{FH}$13 cells are specifically responsible for high-affinity IgE production[39]. Questions arise as to how targeting this T$_{FH}$ subset may aid in diagnosis and therapy.

T$_{FH}$ cells can have pathogenic roles in the development of lymphomas. B$_{GC}$ cells undergo somatic hypermutations that cause genomic instability. They account for the origin of ~80% of B cell non-Hodgkin lymphomas (B-NHLs), including follicular lymphoma (FL)[110]. T$_{FH}$ cells are particularly abundant in the FL microenvironment and are associated with worsened prognosis, likely caused by T$_{FH}$ cell-derived IL-4 and CD40L in supporting FL survival and proliferation[13,111] (Fig. 3c). Apart from supporting B-NHLs, T$_{FH}$ cells can themselves grow into lymphomas, particularly angioimmunoblastic T cell lymphoma (AITL) (Fig. 3d). AITL mouse models suggest a T$_{FH}$ origin[112,113], but the exact etiology of human AITL requires further study[114].

Finally, T$_{FH}$ cells are vulnerable to HIV infection and represent a significant viral reservoir. In HIV-infected human lymph-node biopsies, BCL6$^+$ GC-T$_{FH}$ cells contain the highest percentage of HIV DNA and are most efficient in supporting productive infection[115]. Therefore, the HIV reservoir persisting in T$_{FH}$ cells constitutes a significant obstacle to HIV cure[116] (Fig. 3e).

Modulating the quantity and quality of T$_{FH}$ cells in health and disease

T$_{FH}$ cells are an important therapeutic target because of their diverse functions in human health and disease. Such function is shaped by their interactions with DCs, B cells, follicular regulatory T (T$_{FR}$) cells, and follicular cytotoxic T (T$_{FC}$) cells and is controlled by a sophisticated molecular network including transcription factors (for example BCL6, BLIMP1, TCF1, LEF1, c-MAF, ASCL2, IRF4, BATF, STAT3, STAT5, TOX, TOX2, and FOXO1), co-receptors and signaling molecules (for example ICOS, CD28, CTLA-4, PD-1, CD40L, OX40, SLAM, SAP, and mammalian target of rapamycin (mTOR)), cytokines (for example IL-2, IL-6, IL-12, IL-21, and leptin) and their receptors, chemokine and migratory receptors (for example CXCR5, CCR7, EBI2, and S1PR2), microRNAs, and epigenetic modifiers (for example Roquin, EZH2, miR-17~92, miR-155, and miR-146a) (reviewed in refs. [10],[28],[49]) (Fig. 4a). We will emphasize how the knowledge of T$_{FH}$ regulation can help to design T$_{FH}$-targeting therapeutic approaches, with promising results shown in clinical trials (Fig. 4b).

Targeting the cytokine milieu has demonstrated the efficacy of inhibiting pathogenic T$_{FH}$ cells

The cytokine milieu governs T$_{FH}$ cell differentiation. IL-6, IL-21, and leptin activate the STAT3 pathway and thus promote mouse and human T$_{FH}$ differentiation, while STAT4-activating cytokines, such as IL-12, TGF, and Activin A more selectively induce human T$_{FH}$ cells[10]. These T$_{FH}$-promoting cytokines function in specific synergistic or sometimes redundant manners. In autoimmune diseases such as RA, excessive IL-6 drives STAT3 hyperactivation and aberrant T$_{FH}$ activation[117], so IL-6 inhibition by tocilizumab (a monoclonal antibody that binds to IL-6R) reduced T$_{FH}$ activity in people with RA[118]. Ustekinumab (an IL-12 and IL-23 antagonist) also suppressed T$_{FH}$ cell function in Crohn's disease[119]. Furthermore, Janus kinase (JAK) inhibitors (tofacitinib, baricitinib, and filgotinib) can broadly inhibit signals of T$_{FH}$-inducing cytokines and likely inhibit T$_{FH}$ differentiation. In contrast to T$_{FH}$-promoting cytokines, the IL-2–STAT5 axis inhibits T$_{FH}$ differentiation[120–122]. Therefore, low-dose IL-2 therapy has been found to suppress T$_{FH}$ differentiation in people with SLE[123],[124]. Intriguingly, IL-2 has been shown to enhance the conversion of T$_{FH}$ to T$_{FR}$ in vitro[125], which should be formally tested in vivo. Targeting the cytokine milieu has been clinically proven to be effective in suppressing T$_{FH}$ differentiation.

Cytokines do not only control T$_{FH}$ differentiation; they also underlie T$_{FH}$ functional heterogeneity. Therefore, targeting specific cytokines to modulate particular, but not all, T$_{FH}$ functions is becoming increasingly attractive. Dupilumab (a monoclonal anti- body that binds to IL-4Rα, required by IL-4 and IL-13) is approved for treating allergic diseases[126]. Notably, dupilumab treatment in IgG4-related dacryoadenitis and sialadenitis selectively reduce T$_{FH}$2 cells[127]. Furthermore, IL-21 blockade can suppress T$_{FH}$ cell-mediated B$_{AS}$ differentiation and ameliorates autoantibody production in pre-clinical models[128]. Anti-IL-21 monoclonal anti-bodies (NNC0114-0006, NNC0114-0005, and BOS161721) have been tested in several autoimmune diseases, including a recent randomized phase 2 trial showing their promise in preserving β-cell function in recent-onset T1D[129]. Another T$_{FH}$ cytokine, CXCL13, supports the formation of T$_{FH}$-dependent GCs and TLSs in inflammatory tissues[130]. Anti-CXCL13 can ameliorate collagen-induced arthritis and experimental autoimmune encephalomyelitis (EAE)[131].

Targeting cytokines also holds the potential to promote T$_{FH}$ function in vaccination and infection. For instance, the formula of lipid nanoparticles in SARS-CoV-2 mRNA vaccines strongly induces IL-6 and supercharges T$_{FH}$ differentiation[132]. In infectious diseases, including AIDS, malaria, and COVID-19, pro-inflammatory cytokines suppress T$_{FH}$ cell function and the GC reaction[65,133,134]. Mouse models of infections with the parasite *Plasmodium berghei* ANKA or the bacterium *Ehrlichia muris* revealed that high TNF production disrupts SLO architectures and prevents GC-T$_{FH}$ maturation. Therefore, blocking TNF largely restored GC-T$_{FH}$ maturation and GC function[135,136]. This suggests that TNF inhibitors (etanercept, infliximab, and adalimumab) might impede inflammation and support GC-T$_{FH}$ generation in infections. Altogether, cytokines, as primary therapeutic targets, can control the quantity and quality of T$_{FH}$ cells.

Targeting co-receptor signaling to inhibit T cell activation and suppress T$_{FH}$ cell differentiation

Targeting the CTLA-4 pathway has led to clinical success in thwarting pathogenic T$_{FH}$ cells. CTLA-4 is a natural inhibitor of T cell costimulation and binds to the costimulatory ligands CD80 and CD86 with higher affinity than CD28 to interrupt CD28– CD80 or CD28–CD86 interactions. Cell-expressed CTLA-4 undergoes constitutive endocytosis and re-cycling to the cell sur- face and can remove costimulatory ligands from the surface of antigen-presenting cells (APCs) through transendocytosis[137,138]. By regulating CD28 signal strength, CTLA-4 controls T$_{FH}$ cell differentiation[139]. The CTLA-4–immunoglobulin fusion proteins abatacept and belatacept are approved for use in RA and kidney transplantation. Abatacept is effective at suppressing T cell activation, including pathogenic T$_{FH}$ cell function, indicated by the

reduction in cT$_{FH}$ cells in a range of disease settings, including RA, SS, multiple sclerosis, and T1D[12].

CD28 costimulation is broadly required for T cell activation, whereas ICOS costimulation specifically supports pre-T$_{FH}$ differentiation[19,21] and subsequent T–B engagement for GC-T$_{FH}$ maturation[140]. Given that ICOS blockade inhibits T$_{FH}$ function and ameliorates disease in pre-clinical models of autoimmunity, alloimmunity, and allergy[141], monoclonal antibodies that block ICOS (MEDI-570) or ICOSL (AMG 557/prezalumab) have been developed. In non-human primates, MEDI-570 selectively depleted ICOS$^+$ T cells and attenuated the T-dependent antibody response[142]. The depletion effect of MEDI-570 may explain the benefit in a phase 1 trial for refractory AITL[143]. MEDI-570 and prezalumab have also been trialed in small cohorts of individuals with SLE, lupus arthritis, or SS, with promising results that support further clinical evaluation[141]. Another costimulator molecule, OX40, cooperates with ICOS to amplify T$_{FH}$ function[144,145]. RNA-containing immune complexes induce OX40L expression on APCs in individuals with SLE, which promotes aberrant T$_{FH}$ differentiation[146]. Therefore, dually targeting both ICOS and OX40 can be conceived as a way to repress pathogenic T$_{FH}$ cells in autoimmune diseases.

Another co-receptor signaling axis, the CD40–CD40L axis, plays a crucial role in mediating T$_{FH}$ cell help to B cells[10]. Within CD40– CD40L antagonists under development, the second-generation inhibitors (VIB4920, a CD40L-binding protein lacking a Fc domain; BI 655064, humanized monoclonal with a mutant Fc) spare the first-generation inhibitors' adverse effects, which cross-link CD40L on the surface of platelets, leading to thromboembolism. Both VIB4920 and BI 655064 showed results in reducing B cell activation, autoantibody production, and inflammation in RA in phase Ib/IIa trials[147,148]. Blocking CD40–CD40L interactions thus represents an attractive therapeutic target to inhibit T$_{FH}$ function.

Targeting TCr recognition and antigen bioavailability to improve T$_{FH}$ function in vaccination

There are currently no successful vaccine strategies against some of the most notorious infectious diseases, such as HIV and malaria. New studies have highlighted the benefits of increasing the quantity of T$_{FH}$ cells in improving vaccine efficacy. Protective antibody responses in HIV largely hinge on the production of broadly neutralizing anti- bodies that can neutralize multiple strains. These responses, how- ever, are hindered by the very low frequency of naive B cells that can progressively evolve into broadly-neutralizing-antibody-producing cells through the GC reaction[149]. These rare precursor B cells fail to be recruited into GCs, given the fierce competition with immuno- dominant B cell clones that do not produce broadly neutralizing antibodies. One solution is to enhance T$_{FH}$ function, which results in increased recruitment of rare broadly neutralizing antibody pre- cursor B cells in GCs[150].

Improving antigen bioavailability is a vital consideration to optimize GC-T$_{FH}$ cells, whose differentiation and maintenance require persistent antigen presentation[17]. In comparing two dosing strategies, exponentially increasing dosing significantly exceeds bolus dosing in inducing T$_{FH}$ and GC responses, resulting in more than a tenfold increase in antibody production in mice[151]. Similarly, slowly delivering HIV envelope protein in rhesus macaques (*Macaca mulatta*) induced long-lasting GCs (6 months) and produced high-titer neutralizing antibodies after a single booster[152]. Sustained antigen bioavailability also contributes to the success of mRNA vac- cine technology[153]. As a result, SARS-CoV-2 mRNA vaccines induce antigen-specific T$_{FH}$ and B$_{GC}$ cells in draining lymph nodes for at least 6 months[78,81].

Targeting metabolic pathways in T$_{FH}$ differentiation and survival

Recent studies have highlighted metabolic pathways in controlling T$_{FH}$ function that can serve as therapeutic targets. The mTOR (mammalian target of rapamycin) pathway integrates TCR, co-receptor, and cytokine signals and senses environmental cues and nutrients to support T cell activation, metabolism, and differentiation[154]. mTOR signaling is required for T$_{FH}$ differentiation: mTOR complex 1 (mTORC1) upregulates glycolysis for cell activation and proliferation; mTORC2 more specifically induces T$_{FH}$ differentiation[155,156]. The magnitude of mTOR activation correlates with TCR signal strength[154], suggesting the induction of stronger TCR–mTOR signals by higher antigen doses. Indeed, high-dose influenza vaccines are superior to standard-dose vaccines in inducing otherwise compromised active T$_{FH}$ cell differentiation in adults older than 65 (ref. [157]). Conversely, mTOR inhibition can reduce T$_{FH}$ cell function, shown by reduced T$_{FH}$ differentiation and alloimmunity in kidney transplant recipients following treatment with the mTOR inhibitor sirolimus[158].

Metabolites can also specifically regulate T$_{FH}$ differentiation and survival. For example, phosphatidylethanolamine locates at the plasma membrane outer layer to prevent CXCR5 internalization and degradation, and is thus essential for T$_{FH}$ differentiation[159]. Once generated, T$_{FH}$ cells are dependent on selenoenzyme glutathione peroxidase 4 to mitigate ferroptosis and survival. As such, selenium supplementation has been shown to increase GPX4 expression, thus promoting T$_{FH}$ quantity and improving vaccine responses[24]. Therefore, tuning pathways for phosphatidylethanolamine synthesis or the selenium–ferroptosis axis holds promising applications in regulating the fitness of T$_{FH}$ cells in health and disease.

Targeting the interactions between T$_{FH}$ cells and other cell types

T$_{FH}$ differentiation and survival are dependent on cognate interactions with DCs and B cells. Most DC subsets can present antigens and prime CD4$^+$ T cells for T$_{FH}$ differentiation, with a

preferential role by type 2 conventional DCs (cDC2s)[160]. Poor T_{FH} cell responses to influenza vaccination in aged mice and older people are associated with impaired cDC2 activation, which can be boosted using the TLR7 agonist imiquimod[161,162]. Following priming by DCs, GC-T_{FH} maturation and maintenance are mediated by cognate interactions with B cells, especially B rituximab (monoclonal antibody binding to CD20) effectively abolishes T_{FH} cells in spleen[163]. Therefore, therapeutically targeting DCs and B cells can modulate T_{FH} function.

T_{FR} cells are another important immune cell type that closely inter- acts with T_{FH} cells. They counteract T_{FH} cells and suppress antibody responses[94,106,164]. By expressing CTLA-4, IL-10, and neuritin, T_{FR} cells suppress B_{AS} differentiation, excessive IgE production, and autoantibody development[165–167]. T_{FR} cells differ from T_{FH} cells in TCR repertoire and differentiation kinetics. They carry a repertoire that overlaps with that of T_{reg} cells and is biased to self-antigens[168,169], and are induced later than T_{FH} cells in GC responses[50,170]. Therefore, specific antigen-stimulation schemes may preferentially modulate T_{FR} over T_{FH} cells. As a proof-of-concept, allergen immunotherapy in people with allergic rhinitis expands T_{FR} cells with improved functions[171].

Last but not least, CXCR5$^+$ T cells are essential in suppressing efforts to recapitulate T_{FC}-mediated viral clearance included engineering T cells expressing both chimeric antigen receptor (CAR) specific to the virus and CXCR5. In simian immunodeficiency virus (SIV)-infected rhesus monkeys, CXCR5-expressing CAR-T cells accumulated in lymphoid follicles, contacted with SIV$^+$ cells, and lowered viral loads, representing a new strategy for long-term remission of HIV[173]. Therefore, the induction of T_{FC} cells or engineering CXCR5-expressing CAR-T cells can help to eradicate T_{FH} infection or treat AITL.

Conclusion and future perspectives

Despite many important studies not being included owing to space constraints, this Review presents the rapidly growing evidence describing the broad role of T_{FH} cells in the immune regulation of human diseases and vaccination. Mechanistic investigations and clinical studies have demonstrated that therapeutic approaches can control T_{FH} cell quantity and quality. Innovative formulas and adjuvants enable potent T_{FH} differentiation after vaccination; conversely, drugs blocking costimulatory or cytokine signals inhibit T_{FH} cell function in individuals with autoimmune diseases. Future studies are required to understand T_{FH} cell subsets (heterogeneity) associated with specific characteristics of specificity, plasticity, and survival. Cutting-edge technologies that integrate single-cell and spatial omics for intercellular tissue dynamics[174] and new analytic methods that dissect individual differences[175] will help achieve ground-breaking discoveries. We are looking forward to the fine-tuning of T_{FH} cell function, such as inhibiting pathogenic T_{FH} cells in autoimmune, alloimmune, and allergic

diseases with- out compromising protection against infection, or promoting T_{FH} cell-mediated selection of B_{GC} cells to induce production of broadly neutralizing antibodies after HIV vaccination.

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Competing interests

The authors declare no competing interests.

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Figures:
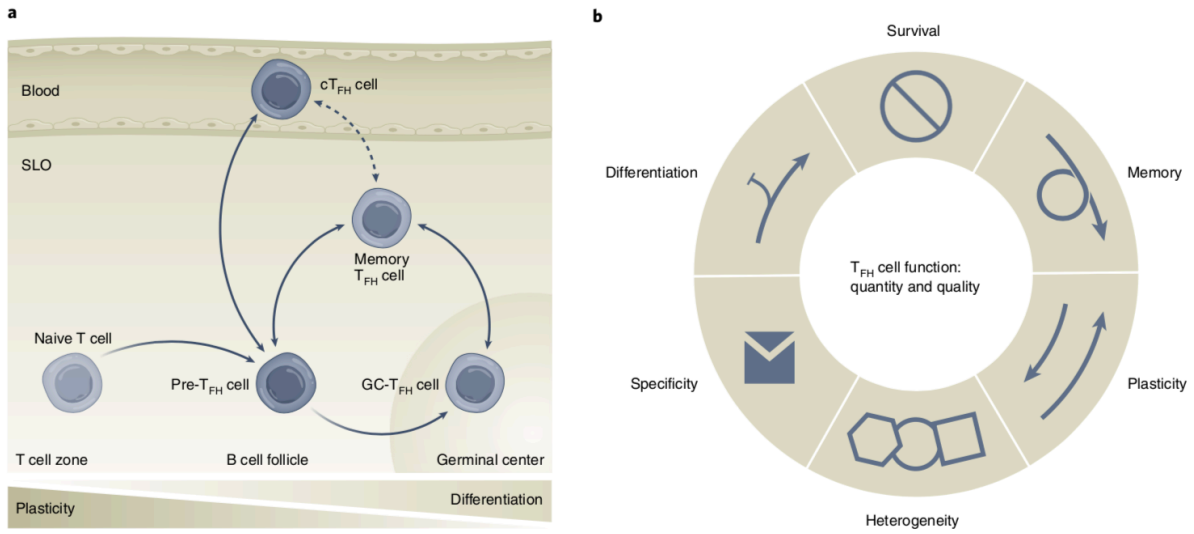
Fig. 1 | elements of T_FH function. a, Stepwise differentiation of T_FH cells, with different levels of plasticity associated with heterogeneous T_FH cell subsets. **b**, Six essential elements to assess T_FH cell quantity and quality.
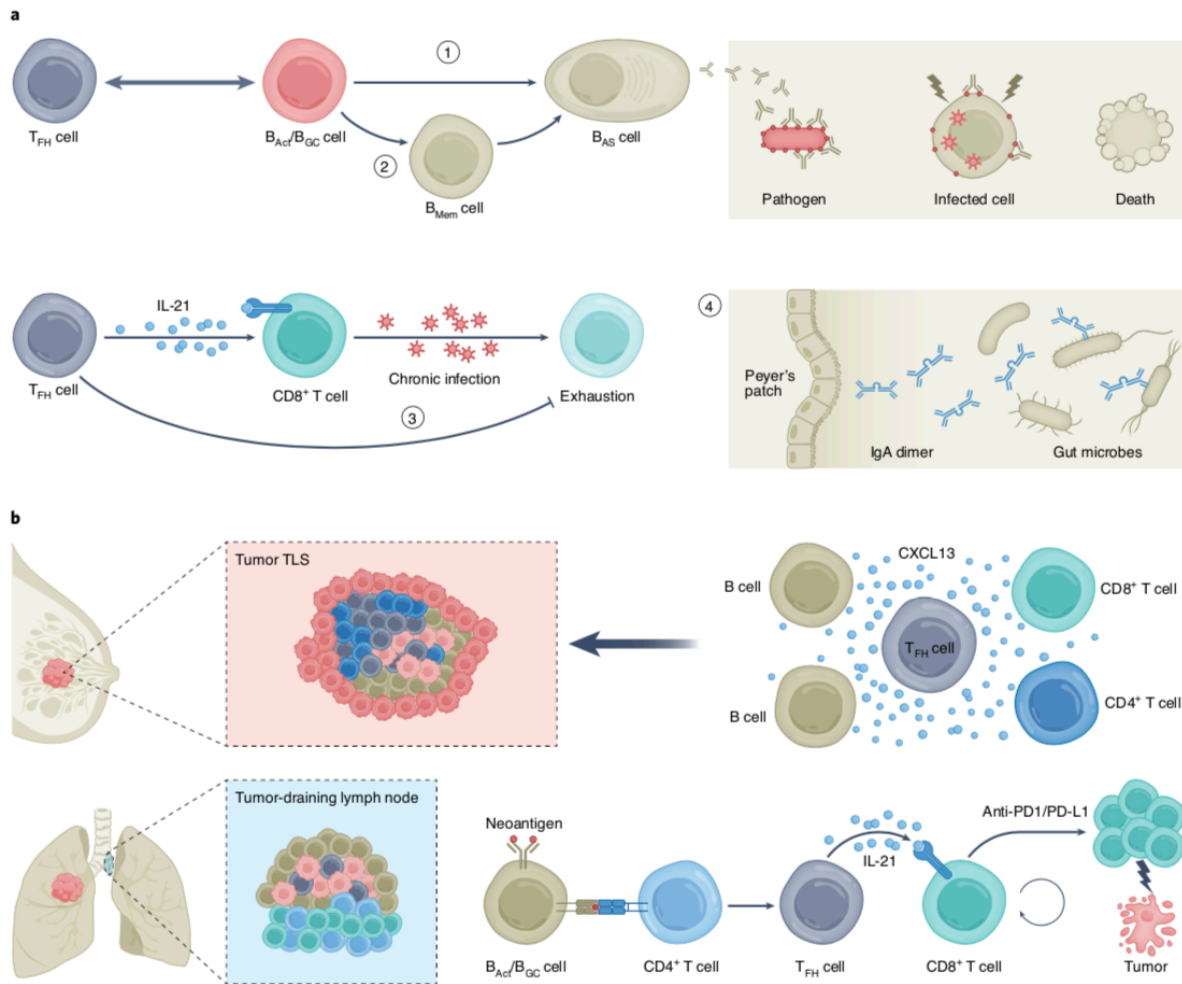
Fig. 2 | Protective functions of T$_{FH}$ cells in infection, vaccination, and cancer. a, In infection and following vaccination, T$_{FH}$ cells support the production of protective antibodies that inhibit pathogen replication and promote clearance. T$_{FH}$ cells also support the generation of B$_{Mem}$ cells that act upon reinfection and the function of CD8$^+$ T cells in chronic infection. T$_{FH}$ cells play an essential role in maintaining gut immune homeostasis and microbial symbiosis. B$_{Act}$, activated B cells. **b**, In the tumor microenvironment, T$_{FH}$ cells produce CXCL13 to recruit lymphocytes and promote the formation of tertiary lymphoid structures (TLSs). In tumor-draining lymph nodes, neoantigen-primed B cells induce the differentiation of T$_{FH}$ cells, which sustain CD8$^+$ T cells for anti-tumor immunity.
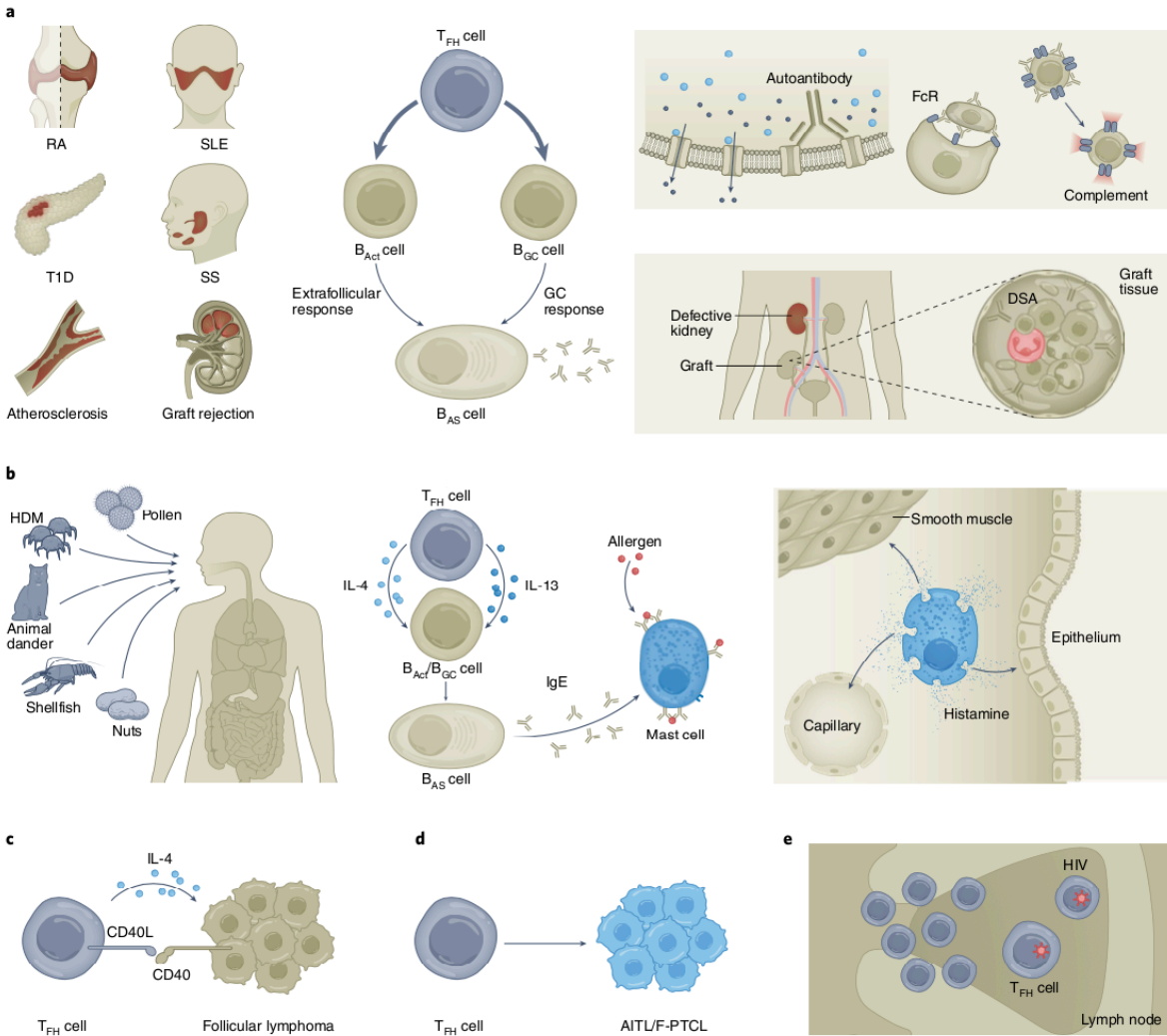
Fig. 3 | Pathogenic roles of T$_{FH}$ cells in human diseases. a, In autoimmune and alloimmune diseases, T$_{FH}$ cells support both extrafollicular and gC pathways to promote humoral immunity that disrupts normal cellular function and injures self or graft tissues. FcR, Fc receptor; DSA, donor-specific antibodies. **b**, In allergic diseases, allergen-primed T$_{FH}$ cells, especially the IL-4- and IL-13-producing T$_{FH}$2 subset, drive excessive IgE production, which sensitizes effector cells, such as mast cells, to release biologically active mediators, causing a cluster of allergic symptoms. HDM, house dust mite. **c,d**, T$_{FH}$ cells support the development and progression of B cell non-Hodgkin lymphomas, such as follicular lymphoma, through IL-4 and CD40L. They are with the potential to derive peripheral T cell lymphoma, including angioimmunoblastic T cell lymphoma (AITL) and follicular peripheral T cell lymphoma (F-PTCL). **e**, In an HIV infection, the HIV reservoir in T$_{FH}$ cells constitutes a significant obstacle that prevents a cure.
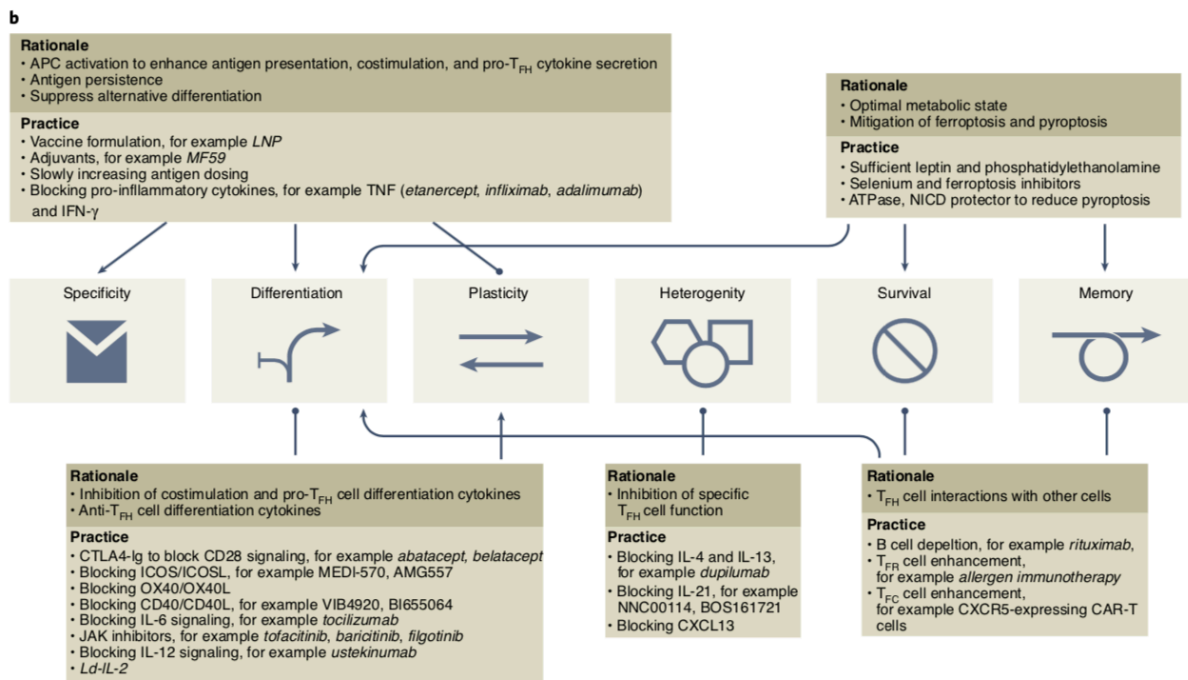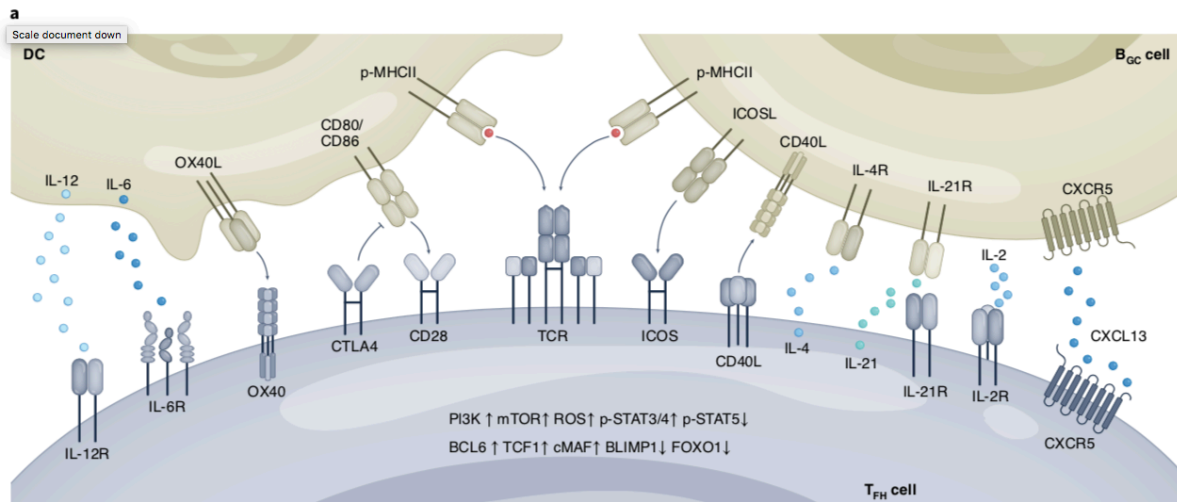
Fig. 4 | Targeting T$_{FH}$ cells' critical regulatory pathways in disease therapy and vaccination.
a, A summary of critical extracellular regulators and intracellular pathways for T$_{FH}$ functions.
b, The rationale and practice of targeting six essential elements to either enhance (upper section) or inhibit (lower section) T$_{FH}$ function. Approved therapies are marked in italic. LNP, lipid nanoparticle.