PERSPECTIVE

The link between circulating follicular helper T cells and autoimmunity

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Abstract | Follicular helper T (T_{FH}) cells provide help to B cells, supporting the formation of germinal centres that allow affinity maturation of antibody responses. Although usually located in secondary lymphoid organs, T cells bearing features of T_{FH} cells can also be identified in human blood and their frequency and phenotype are often altered in people with autoimmune diseases. In this Perspective article, I discuss the increase in circulating T_{FH} cells seen in autoimmune settings and explore potential explanations for this phenomenon. I consider the multi-step regulation of T_{FH} cell differentiation by the CTLA4 and IL-2 pathways, as well as by regulatory T (T_{reg}) cells, and highlight that these same pathways are crucial for regulating autoimmune diseases. The propensity of infection to serve as a cue for T_{FH} cell differentiation and a potential trigger for autoimmune disease development is also discussed. Overall, I postulate that alterations in pathways that regulate autoimmunity may be coupled to alterations in T_{FH} cell homeostasis, suggesting that this population may serve as a core sentinel of dysregulated immunity.

Introduction

Coordinated interaction between different immune cells is crucial for the development of protective immunity. Nowhere is this more obvious than in the emergence of high affinity antibody responses, where carefully orchestrated contacts between dendritic cells (DCs), T cells and B cells culminate in a refined and long-lived antibody response. The critical go-between in this cellular trio is the follicular helper T (T_{FH}) cell that liaises first with the DC, before migrating to the B cell follicle for repeated interaction with B cells[1]. Such T_{FH} cells bear a characteristic phenotype including expression of markers such as CXC-chemokine receptor 5 (CXCR5), programmed cell death protein 1 (PD1), inducible T cell costimulator (ICOS) and the transcription factor BCL-6[2] (Box 1). T_{FH} cells are classically found in secondary lymphoid organs with a small population of similar cells present in the blood (referred to as circulating T_{FH} cells (cT_{FH} cells)). Curiously, multiple

studies have revealed that cT_{FH} cells are present at increased frequencies in many autoimmune diseases including systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Sjogren's syndrome (SS), autoimmune thyroid diseases (ATDs), myasthenia gravis (MG), type 1 diabetes (T1D) and multiple sclerosis (MS)[3]. Consistent with elevations in cT_{FH} cell numbers, autoantibodies are commonly associated with these conditions, and their presence often precedes symptomatic disease.

In this Perspective, I discuss core mechanisms controlling T_{FH} cell differentiation and highlight that these same pathways are linked to the regulation of autoimmune disease. I also discuss the potential for infection to serve as a link between T_{FH} cells and autoimmunity. Overall, I postulate that regulation of T_{FH} cells and regulation of autoimmunity are tightly coupled, perhaps explaining why increases in cT_{FH} cell numbers are evident across multiple autoimmune diseases.

Discovery of circulating T_{FH}-like cells

In 2005, a seminal publication from Vinuesa and colleagues showed that mutant mice with dysregulated T_{FH} cell differentiation exhibited systemic autoimmunity[4]. The causative mutation in these animals mapped to the *Roquin* (Rc3h1) gene, the product of which repressed ICOS expression and negatively regulated T_{FH} cell differentiation. These *sanroque* mice exhibited lupus-like pathology and robust development of T1D when crossed to a T cell receptor (TCR) transgenic mouse model. Impairing T_{FH} cell differentiation, by rendering *sanroque* mice heterozygous for BCL-6 or deficient for SLAM-associated protein (SAP, an adaptor protein required for T_{FH} cell–B cell interactions[5]), ameliorated the autoimmune phenotype, leading to reduced autoantibody production and decreased renal pathology[6]. These findings sparked interest in whether T_{FH} cell differentiation might be connected to lupus pathology in humans, leading to the demonstration that cells with a T_{FH} cell phenotype were elevated in the blood of patients with SLE[7]. Reports

documenting circulating T_{FH}-like cells in numerous other autoimmune diseases rapidly followed (reviewed in[8]).

The spotlight was then turned on the true identity of these blood-borne T_{FH}-like cells, and specifically their relationship to germinal centre (GC)-resident T_{FH} cells. Important work from the Ueno group revealed that circulating $CD4^+CXCR5^+$ T cells shared functional properties with T_{FH} cells from secondary lymphoid organs and could provide help to B cells via IL-21 production[9]. This led to the idea that blood-borne $CD4^+CXCR5^+$ T cells were a memory counterpart of lymphoid T_{FH} cells, and a substantial body of evidence has since emerged to support this view[10, 11, 12, 13, 14]. Consistent with this notion, individuals with defects in GC formation due to deficiency in ICOS or CD40L, or a developmental block in B cell development (BTK deficiency), have fewer circulating $CD4^+CXCR5^+$ cells, and they are absent altogether from the cord blood of newborns[15, 16]. Of note, many T_{FH} cell markers are downregulated in the memory phase[12, 17, 18, 19], perhaps explaining early controversies over the existence of a T_{FH} cell memory pool. Experiments involving the adoptive transfer of mouse T_{FH} cells into antigen-free hosts indicate that CXCR5 expression is least affected by the phenomenon of T_{FH} cell marker loss[20], suggesting it may be the most reliable marker for tracking cT_{FH} cells. Painstaking experiments in which cT_{FH} cells were purified from mouse blood revealed that these cells can home to secondary lymphoid tissues upon re-challenge and participate in GC reactions, providing direct evidence of their functional capacity upon antigen re-encounter[18]. Thus, the consensus view is that T_{FH} cells have a circulating memory compartment that retains expression of CXCR5, and to a variable extent other T_{FH} cell markers, and can be recalled to participate in the memory phase of humoral responses.

Somewhat unexpectedly, the majority of cT_{FH} cells do not seem to derive from the GC environment itself. In fact imaging studies have shown that although GC T_{FH} cells frequently move between GCs, their egress to the circulation is rare[21]. Instead, it appears that circulating $CXCR5^+$ cells arise

mainly from T_{FH} cells that have not yet undergone sustained interaction with B cells. In line with this, the frequency of cT_{FH} cells appears undiminished in mice and humans that lack SAP expression and consequently exhibit impaired T cell–B cell interactions[22]. The link between lymphoid tissue T_{FH} cells and cT_{FH} cells has been compellingly demonstrated both by mass cytometric comparison of blood and tonsillar tissue with dimensionality reduction approaches[23] and by vaccination studies showing that blood $CD4^+CXCR5^+ICOS^+PD1^+$ cells[24] or $CD4^+CXCR5^+PD1^{hi}$ cells[25] are clonally related to lymph node GC T_{FH} cells. Overall this suggests a model in which T_{FH} cells at the T cell–B cell border can give rise to both GC-resident T_{FH} cells and cT_{FH} cells that travel through efferent lymph to the blood (**FIG. 1**). Consistent with this, T cells with T_{FH} cell features can be detected in thoracic duct lymph of mice and humans, and treatment with the drug FTY720, which prevents lymph node exit, dramatically decreases cT_{FH} cells in both species[18, 26, 27].

Common pathways regulate T_{FH} cell differentiation and autoimmunity

T_{FH} cell differentiation and provision of help to B cells is a tightly controlled process. B cell tolerance relies heavily on restricting T cell help, in part because the developmental regulation of T cells is more stringent; nascent T cells are screened against self-antigens during their thymic development, with transcription factors such as autoimmune regulator (AIRE) ensuring a broad representation of peripheral self-antigens. In contrast, peripheral self-antigens may be less available to B cells developing in the bone marrow and it has been reported that around 20% of mature naive B cells exhibit low levels of self-reactivity[28]. B cell antigen specificity is also prone to diversification within GCs, superseding any developmental constraints on self-reactivity. Thus, denying T cell help to self-reactive B cells is necessary to prevent the initiation of potentially damaging autoimmune humoral responses. Ensuring that T cell–B cell collaboration is tightly regulated is also important for optimal protective immunity (**Box 2**).

In considering the mechanisms controlling T_{FH} cell differentiation, three core and interconnected pathways emerge, involving CTLA4, regulatory T (T_{reg}) cells and IL-2. Curiously, these same pathways are well recognized for their ability to regulate autoimmunity. Below I explore the evidence that these pathways control T_{FH} cell differentiation and highlight their connection to autoimmune disease susceptibility.

CTLA4-mediated regulation of T_{FH} cells. CTLA4 controls T cell CD28 costimulation and is a crucial regulator of T cell responses (**BOX 3**). Multiple lines of evidence pinpoint the CTLA4–CD28 axis as a key modulator of T_{FH} cell responses (**BOX 3**). In mice, genetic deficiency or antibody-mediated blockade of CTLA4 triggers spontaneous T_{FH} cell differentiation and GC formation[29]. GCs that form following CTLA4 blockade are CD28-dependent[29], consistent with the established role of CTLA4 in regulating CD28 engagement, and the importance of the CD28 pathway for T_{FH} cells and GCs[30, 31]. Experiments with CD28 heterozygous mice revealed a relationship between the amount of CD28 engagement and propensity for T_{FH} cell differentiation[29], suggesting that CTLA4 may regulate this fate by policing access of CD28 to its ligands. CD28 likely promotes T_{FH} cell differentiation in multiple ways, including increasing ICOS expression which drives strong phosphoinositide 3-kinase (PI3K) activation important for T_{FH} cell formation[32], as well as regulating microRNAs implicated in T_{FH} cell fate[29, 33].

There is also evidence that the CTLA4 pathway regulates T_{FH} cells in humans. Individuals deficient in LRBA, that show defective CTLA4 trafficking and function, exhibit increases in circulating T cells expressing T_{FH} cell markers (CXCR5 and PD1)[34]. In addition, patients with cancer receiving immunotherapy with blocking anti-CTLA4 antibodies show an increase in circulating T cells with T_{FH} cell markers (E. Ntavli, N. M. Edner and L.S.K.Walker, unpublished work). Conversely, cT_{FH} cells are decreased following treatment with soluble CTLA4 molecules, such as the CTLA4–immunoglobulin fusion protein Abatacept, in multiple settings including in individuals with SS[35],

RA[36], MS[37] and T1D[38]. Thus, the CTLA4 pathway negatively regulates T_{FH} cell homeostasis in mice and humans, likely by restricting CD28 engagement.

CTLA4 and autoimmunity. The association between CTLA4 and autoimmunity is well documented. Genetic variation at the *CTLA4* locus is linked to numerous autoimmune diseases including T1D, RA, SLE, MG, ATD, celiac disease, alopecia areata and vitiligo (see Related links; GWAS Catalog). Mice genetically deficient for *Ctla4* develop lethal lymphoproliferation and multi-organ immune cell infiltration[39, 40], whereas heterozygous *CTLA4* mutations in humans are associated with an immune dysregulation syndrome with multiple autoimmune manifestations[41, 42]. Interfering with the CTLA4 pathway by immunotherapy in patients with cancer can also elicit autoimmune side-effects. CTLA4 function may be altered indirectly by mutations in genes encoding CTLA4 pathway regulators. For example, mutations in *LRBA* lead to reduced CTLA4 expression and autoimmune outcomes[43].

T_{reg} cell-mediated control of T_{FH} cells. T_{reg} cells express the transcription factor FOXP3 and play a crucial role in the maintenance of immune homeostasis. *Scurfy* mice, which lack functional T_{reg} cells due to a frameshift mutation disrupting *Foxp3*, exhibit a marked expansion of BCL-6$^+$CXCR5$^+$ T_{FH} cells in secondary lymphoid tissues[44]. Consistent with this, in mice expressing diphtheria toxin receptor under the control of the *Foxp3* promoter, short term depletion of T_{reg} cells enhances the generation of antigen-specific T_{FH} cells in response to immunization[45, 46]. Similar to mice, patients with IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) that have mutations in *FOXP3*, also exhibit an increased frequency of circulating CXCR5$^+$PD1$^+$ T_{FH} cells[47]. Thus, FOXP3$^+$ T_{reg} cells appear to control T_{FH} cell numbers in both mice and humans.

T_{reg} cells constitutively express CTLA4. Interestingly, the enhanced T_{FH} cell differentiation associated with CTLA4 deficiency[29] can be recapitulated by loss of CTLA4 expression in T_{reg} cells

alone[45, 48]. To avoid widespread immune dysregulation, Sage et al.[48] used mice in which tamoxifen-inducible *Foxp3*-Cre was used to excise the floxed *Ctla4* gene in T_{reg} cells immediately prior to immunization, whereas Wing et al.[45] probed the impact of partial loss of CTLA4 expression using heterozygous *Ctla4*-flox/wt mice expressing *Foxp3*-Cre. In both settings, increases in T_{FH} cells were observed after immunization. Collectively, these findings illustrate that T_{reg} cells are a non-redundant population for mediating CTLA4-dependent regulation of T_{FH} cells.

The above observations could potentially reflect the role of CTLA4 in follicular regulatory T (T_{FR}) cells, the subset of T_{reg} cells that exhibit T_{FH} cell features and enter GCs[44, 46, 49]. For T_{reg} cells to acquire the T_{FR} cell programme they downregulate CD25 (IL-2Rα) expression, permitting them to express BCL-6 which would otherwise be antagonised by IL-2-driven BLIMP1 expression[50, 51]. T_{FR} cells are thought to optimize protective antibody responses while suppressing the generation of antibodies against self-antigens and allergens[44, 46, 49, 52, 53, 54] and, like T_{FH} cells, T_{FR} cells have a circulating counterpart that can be altered in autoimmunity[55, 56]. Interestingly, selective depletion of T_{FR} cells, either in mice with floxed *Bcl6* and *Foxp3*-driven Cre expression[54, 57, 58] or in mice with STOP-floxed *Cxcr5*-DTR and *Foxp3*-Cre[53], results in only minor or transient increases in T_{FH} cells, despite marked effects on B cell differentiation. As loss of CTLA4 expression in $FOXP3^+$ cells increases T_{FH} cells, but loss of T_{FR} cells does not, it is possible that substantial CTLA4 activity occurs outside of the B cell follicle during the initial encounter between T cells and DCs. At the T cell–B cell border and within the follicle, T_{FR} cells may employ additional suppressive mechanisms, such as expression of the neuropeptide neuritin[57] or targeting of B cell metabolic pathways[59].

In a fascinating twist, it appears that FOXP3-based regulation of T_{FH} cells can also operate in a cell-intrinsic manner. T_{FH} cells themselves upregulate FOXP3 expression in late stage GCs, and this is associated with loss of expression of the T cell help-associated genes *IL21* and *CD40L* and GC collapse[60]. These $FOXP3^+$ T_{FH} cells express high levels of CTLA4 and are reminiscent of the

$CD25^-$ T_{FR} cells described by Wing et al.[61], the transcriptional profiles of which place them equidistant between T_{FH} cells and activated T_{reg} cells. The division of labour between T_{reg} cells, T_{FR} cells and $FOXP3^+$ T_{FH} cells will need to be dissected by further experimentation. Taken together, T_{reg} cell populations play a key role in controlling T_{FH} cell numbers in both mice and humans, potentially via the CTLA4 pathway.

T_{reg} cells and autoimmunity. Many of the genes associated with susceptibility to autoimmunity are expressed in T_{reg} cells [62] and the pre-eminent role for T_{reg} cells in regulating autoimmunity is well recognized. Mice lacking T_{reg} cells develop lethal autoimmunity[63] and humans with an impaired T_{reg} cell compartment as a result of mutations in *FOXP3* develop the aggressive early-onset immune dysregulation syndrome IPEX[64]. Interestingly, deficits in T_{reg} cells can interfere with normal costimulatory control of T cell immunity — the unexpected exacerbation of disease in CD28-deficient non-obese diabetic mice was reconciled by the discovery of the role of CD28 in T_{reg} cell development[65], and recent findings suggest CD28 also contributes to T_{reg} cell homeostasis in humans[66]. A replete T_{reg} cell compartment is therefore key to the normal regulation of immune responses, and strategies aimed at augmenting T_{reg} cell numbers, by low dose IL-2 treatment or T_{reg} cell therapy, are being actively pursued in autoimmune settings.

IL-2-mediated regulation of T_{FH} cells. The IL-2 pathway is recognized as a major regulator of T_{FH} cell differentiation (**FIG. 2**). In mice, exogenous provision of IL-2 has been shown to suppress T_{FH} cell differentiation both in the context of viral infection[67] and autoimmunity[68]. In humans, IL-2 is also a known regulator of T_{FH} cell differentiation[69] and low dose IL-2 therapy can decrease numbers of cT_{FH} cells in individuals with autoimmune disease[70].

One potential mechanism by which IL-2 inhibits T_{FH} cells is to enhance T_{reg} cell homeostasis as IL-2 is an key cytokine for T_{reg} cell expansion and maintenance[71, 72, 73]. However, it is clear that IL-2

can also act directly on conventional T cells to inhibit T_{FH} cell differentiation. During T cell priming, IL-2 signalling can alter the balance between T_{FH} cell and non-T_{FH} cell effector differentiation[74, 75] via STAT5-mediated skewing of the BLIMP1:BCL-6 ratio[76, 77]. In an interesting twist, it turns out that the T cells induced to make IL-2 are not those that respond to it – instead the producers become T_{FH} cells while using IL-2 in a paracrine manner to preclude their neighbours from this fate[78]. This finding establishes a biological role for the synaptic-based delivery of IL-2 between adjacent T cells undergoing activation[79].

Such is the threat to T_{FH} cell differentiation posed by IL-2, a variety of mechanisms exist to subvert this inhibition. Transforming growth factor-β (TGFβ) insulates T cells from IL-2 signals by suppressing CD25 (IL-2Rα) expression[80], which may contribute to its ability to promote T_{FH} cell differentiation[81]. Activated DCs in the outer T cell zone upregulate CD25, allowing developing T_{FH} cells to approach the B cell follicle in an environment quenched of local IL-2[82]. Mature T_{FH} cells can be shielded from IL-2 in a different manner, becoming desensitized to IL-2 signalling via IL-6-mediated downregulation of the IL-2 receptor subunit CD122 (IL-2Rβ)[83]. The local availability of IL-2 and other cytokines is therefore an important factor in controlling T_{FH} cell numbers. Intriguingly, in conditions of limiting IL-2, T helper 1 cells can increase their BCL-6:T-bet ratio and assume a partial T_{FH} cell phenotype[84]. Thus, some cells bearing T_{FH} cell markers in autoimmune settings could conceivably have arisen via this route.

The intersection between IL-2-mediated and T_{reg} cell-mediated control of T_{FH} cell differentiation is a complex one. T_{reg} cells might be expected to promote T_{FH} cell differentiation by serving as an IL-2 sink[85, 86] but they can also limit T_{FH} cell formation by CTLA4-dependent regulation of costimulation[29, 45, 48]. One way to view this is that avoiding IL-2 signals may be necessary, but not sufficient, for T_{FH} cell differentiation and, as highlighted above, this can be achieved through a variety of means. In contrast, there seems to be little redundancy in control of CD28 costimulation,

with CTLA4-expressing T_{reg} cells being required to restrict T_{FH} cell numbers[45, 48]. Thus, in the absence of T_{reg} cells the effects of dysregulated CD28 costimulation may dominate over the lack of IL-2 consumption since other populations can compensate for the latter.

IL-2 and autoimmunity. The IL-2 pathway is strongly implicated in susceptibility to autoimmune disease. Single nucleotide polymorphisms (SNPs) in *IL2RA* are associated with multiple autoimmune diseases including T1D, RA, IBD, MS, Crohn's disease, alopecia areata and vitiligo (see GWAS catalog). In addition to *Il2RA* SNPs[87], autoimmune variants have been identified in *IL2*, *IL2RB* and *PTPN2*, a phosphatase involved in many signalling pathways including IL-2 receptor signalling. T cell-specific deficiency in *Ptpn2* increased frequencies of T_{FH} cells and GC B cells in non-obese diabetic mice and was associated with exacerbated diabetes[88]. It is likely that multiple pathways control IL-2 receptor signalling, since defects are evident in T1D and MS even after controlling for *IL2RA* and *PTPN2* genotypes[89]. Mice deficient in IL-2, or the IL-2 receptor subunits CD122 (IL-2Rβ) or CD25 (IL-2Rα) develop lethal autoimmunity[90, 91, 92] and mutations in CD122 cause life-threatening immune dysregulation in humans[93]. Importantly, although IL-2 pathway genes can clearly modulate autoimmunity via effects on T_{reg} cell homeostasis, they can also act in conventional T cells: for example, a SNP at the *Il2RA* locus associated with protection from T1D and MS was linked to higher levels of CD25 expression on conventional memory $CD4^+$ T cells[94]. Consistent with the role of the IL-2 pathway in the regulation of autoimmunity, low dose IL-2 can be used therapeutically across a wide range of autoimmune diseases[95].

Collectively, these studies highlight the connection between control of T_{FH} cell differentiation and the genetic regulation of autoimmunity. Notably, genes in the CTLA4 and IL-2 pathways are consistently highlighted in genetic analyses across a broad range of common autoimmune conditions[96, 97] and SNPs associated with autoimmunity are enriched in CpG demethylated regions specifically found in T_{reg} cells[62]. This places CTLA4, IL-2 and T_{reg} cells at the heart of the shared

genetic susceptibility to autoimmune disease that underpins heritability. Importantly, any defects in the CTLA4 or IL-2 pathways or deficits in T_{reg} cell homeostasis or function would lead to a dysregulated T_{FH} cell compartment, as well as propensity to autoimmune disease (**FIG. 3**). This could be one reason that increases in cT_{FH} cells are seen across multiple autoimmune disease settings.

Infection as a link between T_{FH} cell differentiation and autoimmunity

The principal biological role for T_{FH} cells lies in protection from infectious disease. T_{FH} cell differentiation in response to viral, bacterial, parasitic or fungal antigens is key for the generation of protective antibody responses, in particular affinity-matured neutralizing antibodies[98]. Mimicking infection by vaccination also induces T_{FH} cells, with transient increases in cT_{FH} cells being detectable in the blood[99].

Notably, infectious triggers have been postulated for many autoimmune diseases[100, 101, 102, 103, 104]. Lyme disease is the quintessential example whereby immune responses to tick-borne *Borrelia burgdorferi* can give rise to Lyme arthritis with autoimmune T and B cell responses[105]. In most of the common autoimmune diseases, a clear link to an individual pathogen is lacking, however circumstantial evidence is often strong. For example, there is a known association between enteroviral infections and T1D[106], and enteroviral capsid protein[107] and an antiviral signature[108] have been detected in the pancreatic islets of people with T1D. Furthermore, recent data show a clear link between EBV infection and the development of MS[109, 110].

Persistent T_{FH} cells in chronic infection: helpers for autoreactive B cells? Although T_{FH} cell increases are short-lived in acute infection, chronic viral infections elicit persistent T_{FH} cell responses[111, 112]. Such increases in T_{FH} cells could interfere with competitive selection within the GC, potentially allowing the survival of self-reactive B cells. Indeed, persistent viral infection can

elicit polyclonal B cell activation and production of autoantibodies[113]. In light of this, it is interesting that prolonged enteroviral infection, rather than multiple short-duration infections, is associated with islet autoimmunity in T1D[114].

The ability of self-reactive B cells to take up viral antigens, via pinocytosis, Fc receptors or complement receptors, may allow them to present viral peptides and solicit help from virus-specific T_{FH} cells[113]. In experimental systems, B cells specific for the central nervous system self-antigen myelin oligodendrocyte glycoprotein (MOG) can co-capture influenza virus haemagglutinin and MOG from cell membranes and obtain help from haemagglutinin-specific T cells to produce anti-MOG antibodies[115]. Interestingly, the ability of T_{FH} cells to provide help to bystander B cells and elicit autoantibody production appears to be enhanced by lymphopenia[20], a state long associated with autoimmunity. Conceivably, lymphopenia in the context of SARS-CoV-2 infection could contribute to the generation of autoantibodies documented in patients with COVID-19[116].

Potential for infection to unleash self-reactive T_{FH} cells. Chronic infections may alter T cell activation thresholds via the upregulation of costimulatory ligands in response to Toll-like receptor engagement or pro-inflammatory cytokines. This costimulatory ligand upregulation may outpace CTLA4-dependent ligand downregulation, permitting the activation of self-reactive T cells normally censored by insufficient CD28 engagement[117]. Prolonged increases in T_{FH} cells could exacerbate this by increasing levels of IL-21, the archetypal T_{FH} cell-associated cytokine, which can counteract T_{reg} cell suppression[118, 119] and may reinforce T_{FH} cell differentiation[120]. IL-21 is overexpressed in several autoimmune diseases including SLE, RA, SS and T1D[121], and although it can also derive from other cells, significant correlations between IL-21 production and T_{FH} cell frequencies have been noted[122, 123]. Thus, chronic infectious settings associated with elevated IL-21, blunted T_{reg} cell function and increased costimulation could permit self-reactive T cells to acquire a T_{FH} cell phenotype.

Infection may play a role in epitope spreading, which is a feature of many autoimmune diseases and is frequently associated with disease progression. One mechanism that may contribute to this phenomenon is the invasion and reuse of existing GCs by B cells bearing a different specificity, particularly in the context of shared T cell help (for example when B cells specific for distinct regions of a protein interact with the same T_{FH} cell due to presentation of a common peptide)[124]. Cross-reactive recognition of bacterial antigens by self-reactive T cells could conceivably form the basis of shared T cell help in autoimmune settings[125]. Notably the presence of adjuvants, including the bacterial molecule lipopolysaccharide, can enhance GC invasion and reuse[124], suggesting that in addition to unleashing new cohorts of T_{FH} cells, infection may also enhance the sharing of T cell help between B cells of different specificities.

The type 1 interferon connection. Infection and autoimmunity may also intersect mechanistically at involvement of the type 1 interferon (IFN) pathway. Type 1 IFNs provide the key "early warning" signal of viral infection and play complex roles in protection or pathology following infection with viruses, bacteria, parasites and fungi[126]. The type 1 IFN pathway has been widely associated with autoimmune diseases, most notably in the case of SLE, but also in T1D, MS, RA and others[127, 128, 129, 130]. There are conflicting reports on the impact of type 1 IFNs on T_{FH} cell differentiation. There is evidence that type 1 IFNs promote acquisition of at least some aspects of the T_{FH} cell phenotype[131], although other data suggest that they support T helper 1 cells at the expense of T_{FH} cells[132]. Studies focusing on DCs as the target cells for type 1 IFNs point to a positive role in T_{FH} cell differentiation following immunization or vaccination[133, 134] but a negative role in the setting of Plasmodium infection[135]. As might be expected for the type 1 IFN pathway, timing, location and context are likely to be key in determining outcome[136].

Future directions

Different autoimmune diseases are highly distinct in their presentation, with unique tissue-specific features: for example, bone erosion in RA, renal pathology in SLE and metabolic abnormalities in T1D. Given the striking differences in disease processes and clinical presentation, it is remarkable that increases in cT_{FH} cells appear to be a unifying theme across a large number of autoimmune diseases. In this article I highlight that CTLA4, IL-2 and T_{reg} cells are key modulators of T_{FH} cell differentiation and are also major players in the regulation of autoimmunity. This fundamental connection may go some way to explaining why dysregulated T_{FH} cell homeostasis is a feature of so many different autoimmune diseases. Reinforcing this connection, several other T_{FH} cell-associated genes (including *CXCR5*, *CCR7*, *ICOSL*, *PD1*, *IL4R*, *IL21R* and *CD40*) are linked to autoimmune disease susceptibility. The notion that genetic predisposition to autoimmunity may be coupled to genetic predisposition to T_{FH} cell formation provides a new framework for considering the increased cT_{FH} cells widely reported in autoimmune settings.

Since infection can lead to T_{FH} cell differentiation, it is conceivable that infectious triggers occurring on autoimmune-susceptible backgrounds may cause discernible changes to T_{FH} cell populations that precede autoimmunity. Thus, T_{FH} cells may represent the consummate biomarker in autoimmunity, neatly integrating genetic and environmental risk. Accordingly, T_{FH} cell profiling may be increasingly important in the prediction of autoimmune disease development and for monitoring and predicting clinical response to immunotherapies[38].

The precise contribution of T_{FH} cells, and their circulating counterparts, to pathology likely differs between autoimmune diseases. Dysregulated T_{FH} cell homeostasis may allow the production of autoantibodies that mediate diverse effector functions — for example, enhancing cross-presentation of islet antigens in T1D[137], driving renal pathology in SLE[138] and promoting osteoclastogenesis and bone loss in RA[139, 140]. As well as acting on B cells, the capacity for T_{FH} cells to produce IL-21 and CXCL13[8] may influence $CD8^+$ T cell activation and ectopic lymphoid structure formation

respectively whereas dysregulated IFNγ production by T_{FH} cells[141] has been shown to drive autoimmune GCs and systemic autoimmune disease[142].

Altogether, closer study of T_{FH} cell populations in blood, lymphoid organs and tissues promises to yield new insight into autoimmune pathogenesis, as well as allowing us to harness the full biomarker potential of this population.

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Competing interests

The author is an inventor on a patent relating to TFH cell profiles and predicting response to costimulation blockade in autoimmunity.

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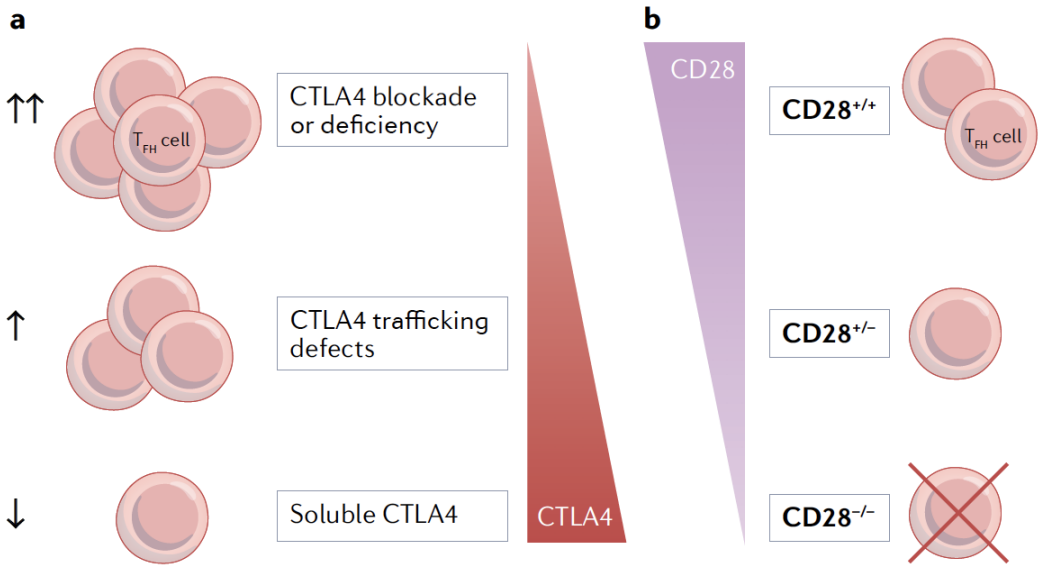
Box 1 | Transcription factors controlling follicular helper T cell development

BCL-6 is an essential transcription factor for follicular helper T (T_{FH}) cell differentiation[143, 144, 145] and its repression of BLIMP1 is necessary[144], but not sufficient[146], for the T_{FH} cell fate. Other key transcription factors include MAF, induced by ICOS ligation[147], and BATF, which controls expression of BCL-6 and MAF[148, 149]. ASCL2 promotes early T_{FH} cell differentiation by upregulating expression of genes such as *CXCR5*[150], whereas repression of KLF2 expression is required to maintain the T_{FH} cell phenotype[151]. STAT proteins play a major role in influencing T_{FH} cell differentiation in response to cytokines: STAT5 inhibits T_{FH} cell differentiation following IL-2 exposure and conversely STAT3, STAT1 and STAT4 promote T_{FH} cell differentiation in response to cytokines such as IL-6, IL-21 and IL-12[81, 152, 153].

Box 2 | Optimal germinal centre B cell selection relies on restricting T_{FH} cell numbers

Upon antigen engagement, B cells migrate to the T cell–B cell border in search of T cell help[154]. Restricting the number of T cells available limits how many B cells can seed germinal centres (GCs)[155], reducing the number of clones exposed to hypermutation. T cell–B cell interactions at the T cell–B cell border support the extrafollicular response[156] and dysregulation of extrafollicular T cells may lead to autoimmunity[157]. In the follicular response, B cells compete again for T cell help in the GC light zone, with those expressing the highest affinity receptors able to capture more antigen and present higher peptide concentrations. Immunoglobulin gene mutations resulting in enhanced antigen affinity trigger GC B cells to upregulate CCL22 and CCL17 expression, allowing them to preferentially attract T cell help[158]. Limiting the number of T cells available, and ensuring that their cytokine output remains "stingy"[159], are crucial features of the competitive environment required for affinity maturation to occur.

Box 3 | CTLA4 function



CTLA4 acts as a competitive inhibitor for the T cell costimulator CD28 as both receptors bind to the same ligands, CD80 and CD86, but CTLA4 binds with higher affinity. Furthermore, CTLA4 and CD80 form high avidity dimer–dimer interactions[160]. CTLA4 can function in a cell-extrinsic manner to remove CD80 and CD86 from antigen-presenting cells by a process known as transendocytosis[161]. Expression of CTLA4 by regulatory T (T_{reg}) cells is required to prevent fatal autoimmune disease in mice[162], and T_{reg} cells can use CTLA4 to control the levels of CD80 and CD86 on dendritic cells trafficking from peripheral tissues to lymph nodes[117]. As the biological role of CTLA4 is to regulate the CD28 pathway, phenotypes associated with CTLA4 deficiency are abrogated in the absence of their shared ligands[163].

Follicular helper T (T_{FH}) cells are reciprocally regulated by CTLA4 and CD28. Mice lacking CTLA4 systemically or in T_{reg} cells exhibit exaggerated T_{FH} cell differentiation and this is recapitulated by injection of blocking anti-CTLA4 antibodies [29, 45, 48] (see figure, part a). Humans with CTLA4 trafficking defects also exhibit increases in T_{FH} cell numbers [34]. Conversely, soluble CTLA4 fusion proteins decrease T_{FH} cell numbers in mice and humans[35, 36 38]. Mice with wild-type CD28 expression have intact T_{FH} cell development, those with lower CD28 expression due to CD28 gene heterozygosity show a reduced propensity for T_{FH} cell development whereas complete CD28 deficiency abrogates T_{FH} cell development[29, 31] (see figure, part b).

Figures

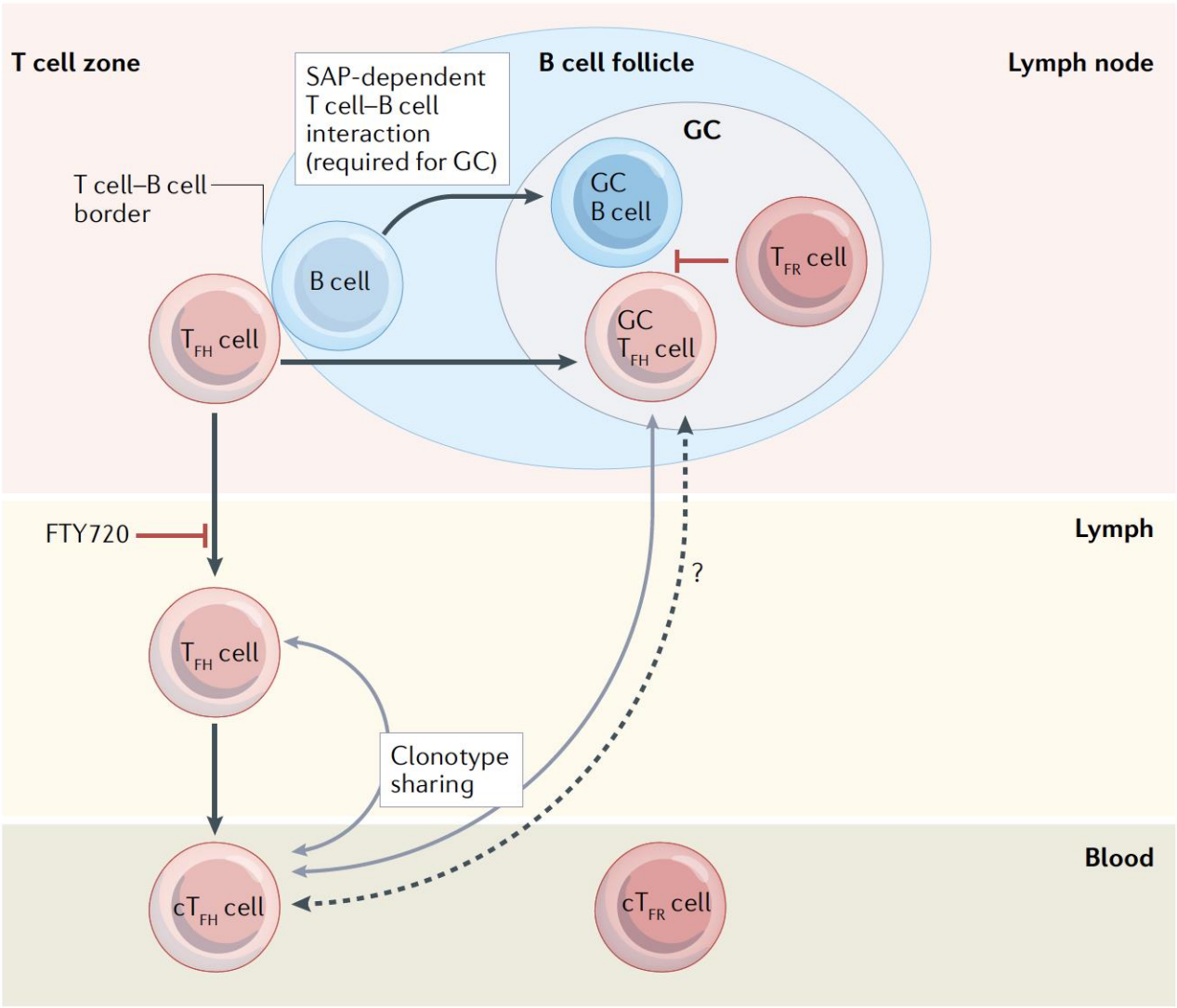


Figure 1 | Blood-borne circulating follicular helper T (cT$_{FH}$) cells are clonally related to their lymphoid counterparts. Circulating T$_{FH}$ cells do not depend on germinal centres (GCs) as they can arise in the context of SLAM-associated protein (SAP) deficiency where sustained T cell–B cell interactions are abrogated[22]. Blocking lymph node exit, by treatment with FTY720, dramatically decreases cT$_{FH}$ cells in mice and humans[18, 26, 27]. Clonotype sharing reveals a developmental relationship between T$_{FH}$ cells found in the GC and the blood [24 25] and those found in the lymph and the blood [26] . It is possible that some GC T$_{FH}$ cells enter the circulation from GCs but this is likely to be rare[21] .

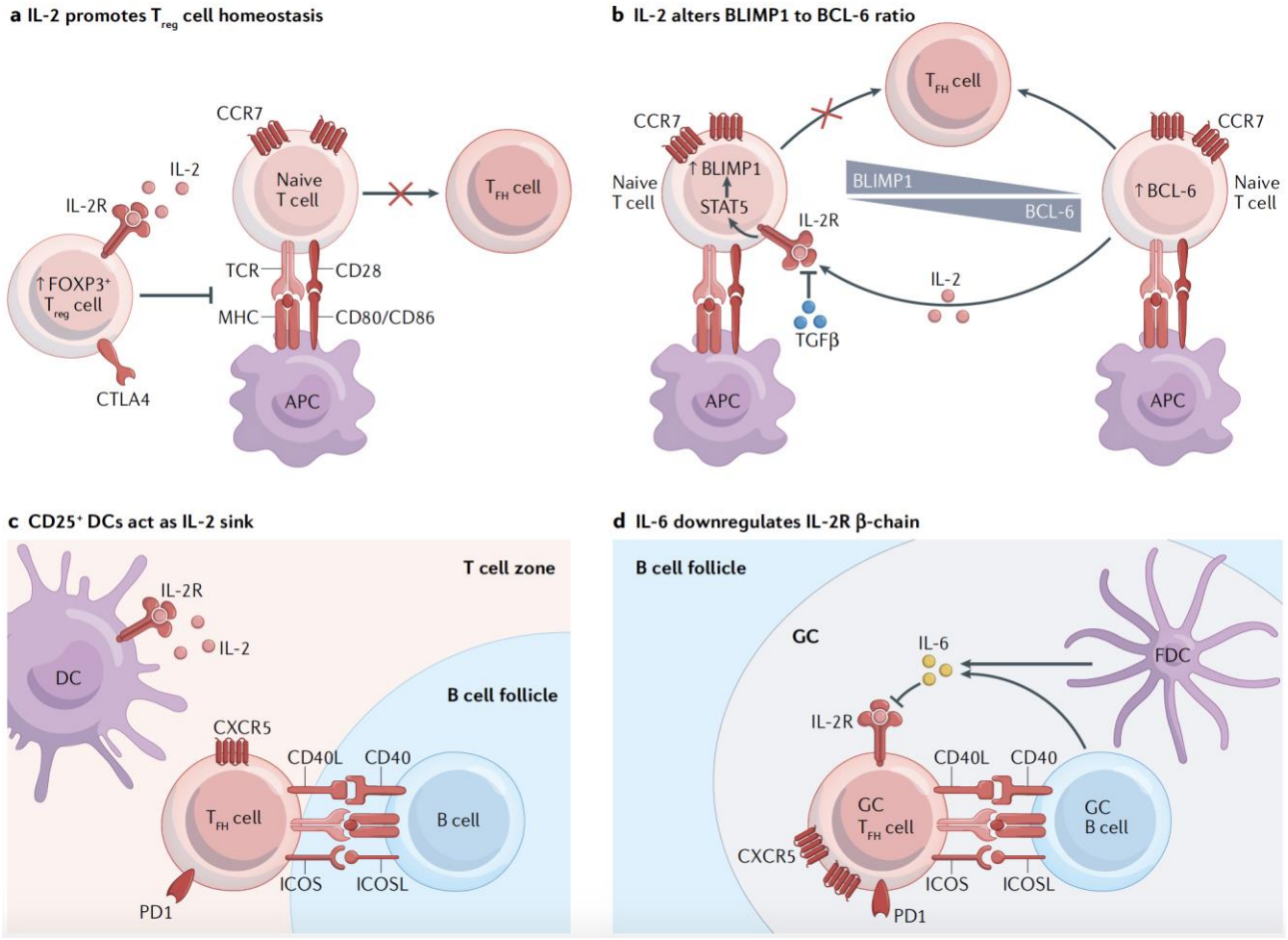


Figure 2 | IL-2-based inhibition of follicular helper T (T_{FH}) cells and adaptations to subvert this. IL-2 plays an important role in promoting regulatory T (T_{reg}) cell homeostasis; T_{reg} cell-deficient mice[44] and humans[47] exhibit increased T_{FH} cell numbers. During T cell priming, IL-2 upregulates BLIMP1, skewing the BLIMP1:BCL-6 ratio and inhibiting T_{FH} cell differentiation[74, 75, 76, 77]. Transforming growth factor-β (TGFβ) decreases IL-2 signalling by suppressing CD25 (IL-2Rα) expression[80]. CD25-expressing dendritic cells (DCs) in the outer T cell zone can capture local IL-2, favouring T_{FH} cell differentiation[82]. Within the germinal centre (GC), IL-6 can desensitise IL-2 signalling in mature T_{FH} cells by downregulating the IL-2 receptor subunit CD122 (IL-2Rβ)[83].

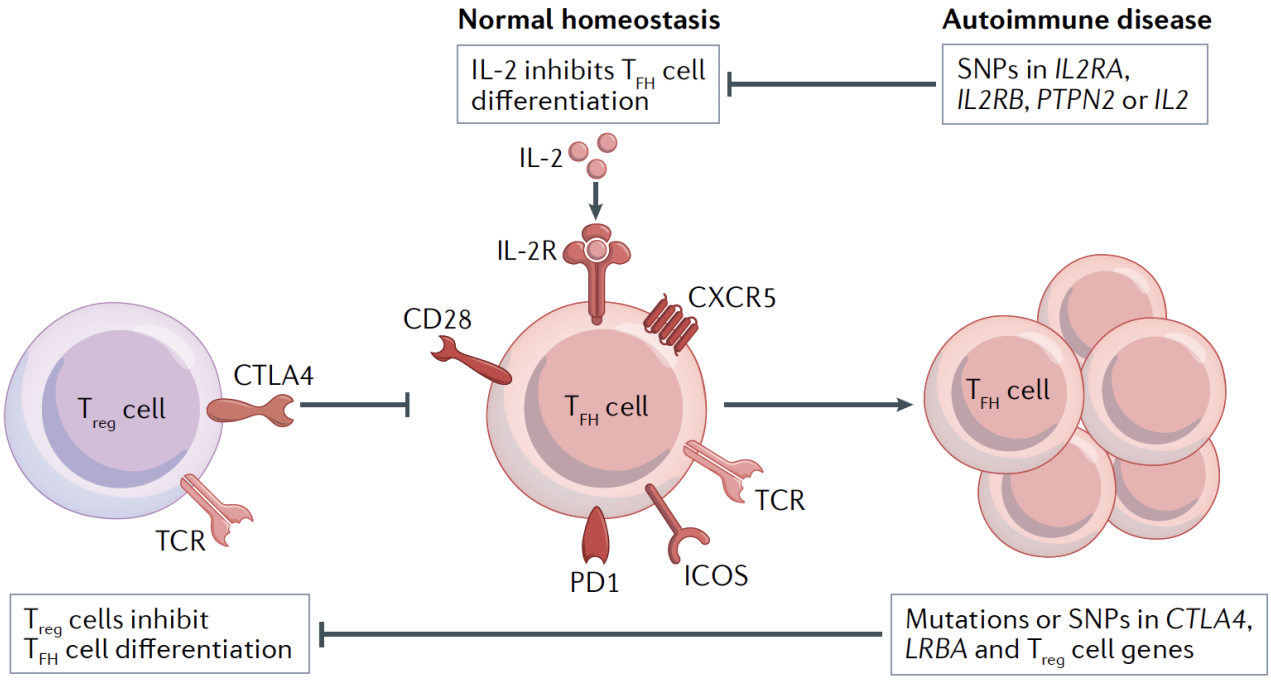


Figure 3 | Common pathways control autoimmune susceptibility and T_{FH} cell homeostasis. In healthy individuals, T_{FH} cell homeostasis is maintained by the CTLA4 pathway and regulatory T (T_{Reg}) cells as well as by IL-2. Defects in the IL-2 pathway can increase T_{FH} cell numbers and single nucleotide polymorphisms in the IL-2 pathway are associated with autoimmune disease. Altered CTLA4 expression or trafficking (for example owing to *LRBA* mutations) or defects in T_{reg} cells can increase T_{FH} cell numbers and are also associated with autoimmune disease susceptibility.