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REVIEW

Human T follicular helper (Tfh) cells and disease

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The generation of protective antibodies by B cells following natural infection or vaccination requires 'help' from CD4$^+$ T cells. T follicular helper (Tfh) cells are the specialized CD4$^+$ T cell subset that has evolved the appropriate mechanisms to induce the activation and differentiation of B cells into immunoglobulin (Ig) secreting cells. As such, appropriate control of Tfh cell generation and function is essential to human health as overactivation is likely to result in autoimmunity, whereas underactivation is often associated with immunodeficiency. Furthermore, an understanding of the regulation of these cells may be invaluable to improved vaccine development strategies. Traditionally Tfh cells have been identified by their anatomical location in secondary lymphoid tissues, which has hindered the study of these cells in humans as access to these tissues is often not feasible. However, recent studies have identified the circulating counterparts to tissue Tfh cells and with this has come a wealth of knowledge gained from the study of these cells in human disease. Here we review some of the recent developments on the role of human Tfh cells in health and disease.
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CD4$^+$ T 'helper' cells are crucial for the functioning of an intact immune system owing to the diverse roles they play in both immune surveillance and protection against foreign pathogens. Underlying this flexibility is the capacity of naïve CD4$^+$ T cells to differentiate into different effector subsets that are equipped with specific functions to eradicate the immediate threat. Our immune systems are fine tuned in such a way that the antigen in question stimulates a particular cytokine environment, which in turn activates precise transcriptional networks that induce differentiation towards a specific T helper (Th) cell pathway. This was the basis of the original Th1-Th2 dichotomy, whereby interleukin (IL)-12 induces expression of the transcription factor T-bet (TBX21), which mediates the differentiation of IFNγ-secreting Th1 cells that are implicated in cell-mediated antiviral and antimicrobial immunity, whereas IL-4 induces the transcription factor Gata3 and the subsequent differentiation of IL-4-producing Th2 cells that function in immunity against extracellular pathogens. Since these pioneering discoveries, the number of effector Th subsets has grown to include Th9, Th17, Th22, regulatory T cells and T follicular helper (Tfh) cells.[1–3] Our focus will be on this latter subset of Tfh cells, which, of late, has received a lot of attention as a result of their implications in humoral immunity.

Tfh cells were first described over 10 years ago as CD4$^+$ T cells that resided in B cell areas of secondary lymphoid tissues in humans.[4,5] The ability of Tfh cells to leave the T cell area and localize to the B cell follicle is facilitated by their concurrent expression of the B cell zone homing chemokine receptor CXCR5 and downregulation of the T cell zone homing chemokine receptor CCR7. This close proximity to B cells allows Tfh cells to support their activation, expansion and differentiation.[6–8] This 'help' is provided in the form of expression of molecules such as CD40L and cytokines such as IL-21. Although the first definitions of Tfh cells were in a large part dependent on the anatomical location of these cells, more recent studies have revealed the existence of their circulating counterparts. Similar to Tfh cells found in human secondary lymphoid organs, blood CD4$^+$CXCR5$^+$ T cells express higher levels of IL-21 and are more efficient at inducing B-cell differentiation than CD4$^+$CXCR5$^-$ T cells.[9,10] However, a key difference between circulating Tfh cells and those found in secondary lymphoid tissues is the lack of expression of the transcription factor Bcl-6 by blood Tfh cells.[9–12] This is somewhat surprising given that Bcl-6 was shown to be required for the differentiation of Tfh cells.[13–15] One explanation would be Bcl-6 is no longer required for Tfh function once a CD4$^+$ T cell has committed to the Tfh cell pathway. Consistent with theory, Bcl-6 expression was only observed in 10–16% of CD4$^+$ T cells residing in germinal centers (GCs) of human lymph nodes (LNs) and tonsils.[16] Furthermore, studies that investigated the kinetics of Bcl-6 expression by murine Tfh cells revealed that some murine Tfh cells downregulated Bcl-6 expression.[17] Despite this, they did not observe many differences between Bcl-6$^+$ and Bcl-6$^-$ Tfh cells. Alternatively, this downregulation in Bcl-6 may simply reflect the slightly different differentiation stage of the Tfh-like cells in the circulation compared with Tfh cells in the tissues. Indeed it remains unclear, exactly how these Tfh-like cells in the blood are related to the bona fide tissue resident Tfh cells. For example, are they long-lived memory cells derived from tissue Tfh cells or more short-lived cells that are spilling out from ongoing GC reactions?

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The answer to this question probably varies depending on the study due to the fact that different studies define blood Tfh cells in different ways. Thus, although some studies have defined circulating human Tfh cells as total CXCR5$^+$ CD4$^+$ T cells, other studies have investigated a subset of CXCR5$^+$ CD4$^+$ T cells, such as CXCR5$^+$ ICOS$^+$, CXCR5$^+$ICOS^{hi}, CXCR5$^+$PD1$^+$, CXCR5$^+$PD1^{hi}, CXCR5$^+$ICOS$^+$PD1$^+$, CXCR5$^+$CD57$^+$ and CXCR5$^+$IL-21$^+$ subsets,[10,12,18–27] while other studies still have not used CXCR5$^+$ to define circulating Tfh cells, but focused on alternative markers of tissue Tfh cells, such as ICOS or IL-21 expression.[28–32] As there is yet to be a comprehensive study that isolates and compares characteristics of Tfh cells using all these different criteria for defining peripheral blood Tfh cells, it is unclear if these markers are defining a different population of Tfh cells or the same population that expresses all of these different markers. Regardless, it is likely that peripheral blood Tfh cells are a heterogeneous population, as the chemokine receptors CXCR3 and CCR6 have been used to define distinct Th1- (CXCR3$^+$CCR6$^-$), Th2- (CXCR3$^-$CCR6$^-$) and Th17-like (CXCR3$^-$CCR6$^+$) Tfh cells within the population of circulating CXCR5$^+$CD4$^+$ T cells. In addition to producing the Tfh cytokine IL-21, Th1-, Th2- and Th17-like Tfh cells also expressed their characteristic transcription factor(s) and cytokine(s), that is, *TBX21* and IFNγ, *GATA3*, IL-4, IL-5 and IL-13 and *RORC*, IL-17A and IL-22, respectively. Interestingly, using these criteria, Th2- and Th17-, but not Th1-like Tfh cells secreted IL-21 and were able to support B-cell differentiation in a IL-21- and ICOS-dependent manner.[9] Despite a degree of ongoing confusion, identification of a circulating correlate of tissue Tfh cells has provided an opportunity to more easily study these cells in human disease by the sampling of peripheral blood. This review will identify the different criteria used to study human Tfh cells and some of the recent developments of these cells in immunodeficiency, autoimmunity and cancer (Figure 1).

Tfh CELLS IN PRIMARY IMMUNODEFICIENCY

The study of Tfh cells in human primary immunodeficiencies has revealed some of the requirements for the generation and/or function of human Tfh cells (Figure 1). These primary immunodeficiencies are reviewed below.

X-linked lymphoproliferative disease

X-linked lymphoproliferative disease (XLP) is a primary immunodeficiency caused by mutations in *SH2D1A*, which encodes the small intracellular adaptor protein SAP.[33] XLP patients have a severe reduction in memory B cells and defects in GC formation, as well as hypogammaglobulinemia and impaired antigen-specific antibody responses indicating there is a significant defect in T-dependent B cell responses.[34,35] Consistent with this, XLP CD4$^+$ T cells were shown to be unable to support Ig production by B cells *in vitro*, suggesting that peripheral CXCR5$^+$ T cells from XLP patients are unable to function as bona fide Tfh cells.[34] Surprisingly, however, XLP patients have normal frequencies of circulating CXCR5$^+$, PD1$^+$ and CXCR5$^+$PD1$^+$ CD4$^+$ T cells.[19,36] Thus, the true nature of CXCR5$^+$ T cells in XLP patients and how they compare with circulating CXCR5$^+$ T cells in normal individuals requires further verification. A recent study found CD4$^+$ T cells from XLP patients could be induced to express ICOS, CD40L, IL-4, IL-10 and IL-21, but the kinetics of expression was different to that of normal CD4$^+$ T cells.[36] Specifically, ICOS and CD40L expression on normal CD4$^+$ T cells was highest after 2 days of *in vitro* activation and then gradually declined, but on XLP CD4$^+$ T cells ICOS and CD40L expression was often the lowest at day 2 and continued to increase such that they were still highly expressed at day 12. However, as total CD4$^+$ T cells rather than purified Tfh cells were examined, it is possible that these results reflect differences in the CD4 compartment of XLP patients compared with normal donors rather than genuine differences between CD4$^+$CXCR5$^+$ T cells in XLP patients and healthy donors. Regardless, it is clear from these studies in XLP patients that intact SAP signaling is required for the generation of functional Tfh cells in humans.

Hyper IgM syndrome

Hyper IgM syndrome is a primary immunodeficiency due to mutations in *CD40* or *CD40LG*. Hyper IgM patients have normal

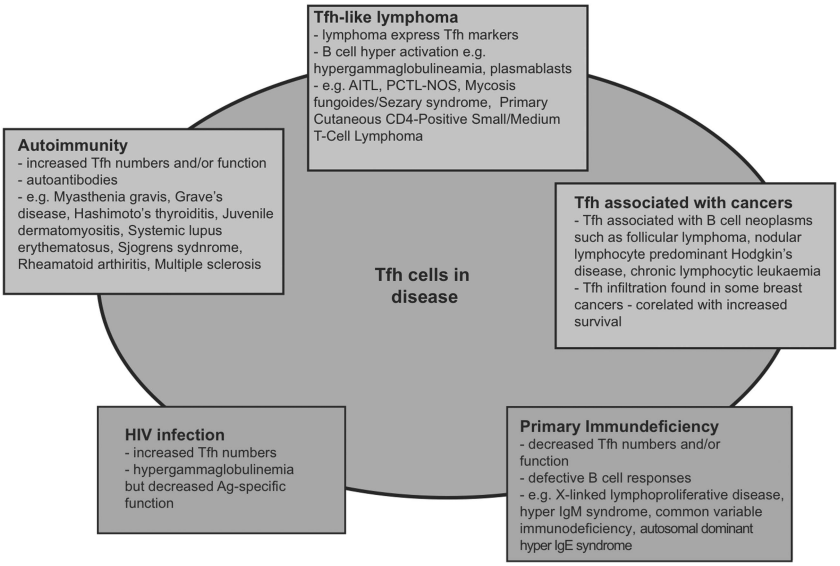

Figure 1 Human T follicular helper cells in disease. Human Tfh cells have been implicated in various diseases. Specifically, a decrease in Tfh cells has been observed in a range of primary immunodeficiencies, whereas an increase in Tfh cells has been associated with acquired immunodeficiency (i.e., HIV), autoimmunity, as well as various types of cancers. AITL (angioimmunoblastic T-cell lymphoma); PCTL-NOS (peripheral T-cell lymphoma-not otherwise specified).

to elevated serum IgM, but severely reduced serum IgG, IgA and IgE levels, reflecting the requirement for intact CD40-CD40L signaling for B cells to undergo Ig isotype switching. Furthermore, patients with hyper IgM syndrome have disrupted GC formation.[37,38] More recently, it was revealed that CD40L-deficient patients have a severe reduction in peripheral blood CXCR5$^+$ CD4$^+$ Tfh cells.[20] Thus, signals received via the CD40-CD40L axis are required not only for the activation and differentiation of B cells, but also for the differentiation of Tfh cells.

Common variable immunodeficiency due to ICOS deficiency

Common variable immunodeficiency describes a heterogeneous group of individuals with hypogammaglobulinemia, defects in the generation of antigen-specific antibodies, and increased susceptibility to bacterial infections. In the majority of cases the genetic lesion underlying this disease is unknown. However, in 2003 one group reported mutations in *ICOS* accounted for some cases of common variable immunodeficiency.[39] ICOS deficient patients have a reduction in B cell numbers, defects in the generation of memory B cells and low levels of serum Igs.[39] Furthermore, a deficit in germinal centers was observed in the LNs of one ICOS-deficient patient.[20] When peripheral blood Tfh cells were examined, a reduction in circulating CXCR5$^+$ CD4$^+$ T cells was observed with an almost complete absence of the CXCR5$^+$CD57$^+$ fraction.[20] Following *in vitro* stimulation, T cells from ICOS-deficient patients showed defects in the upregulation and maintenance of CXCR5 expression, suggesting that ICOS signaling is required for the maintenance and/or survival of human CXCR5$^+$ Tfh cells.[20]

Autosomal dominant Hyper IgE syndrome

Autosomal dominant hyper IgE syndrome (AD-HIES) is an immunodeficiency characterized by a clinical triad of recurrent *Staphylococcal aureus* and *Candida albicans* infections, recurrent cyst-forming pneumonia and as the name suggests, elevated levels of serum IgE.[40] Importantly, although AD-HIES patients do not exhibit hypogammaglobulinemia *per se*, they fail to generate normal titers of antigen-specific antibodies and have a severe reduction in memory B cells.[41] AD-HIES was revealed to be due to mutations in *STAT3*,[42,43] a transcription factor, that is downstream of numerous cytokine signaling pathways, including those involved in the differentiation of human Tfh cells, specifically IL-6, IL-12, IL-21 and IL-27. Consistent with this, we recently reported a decrease in peripheral blood CXCR5$^+$CD4$^+$ T cells in AD-HIES patients.[18] Although the contribution of different STAT3-signaling cytokines to the defect in circulating CXCR5$^+$ T cells in AD-HIES is currently not known, IL-12 was found to be the main inducer of naive CD4$^+$ T cells towards the Tfh cell differentiation pathway *in vitro*.[44,45] Interestingly, in the absence of STAT3, there was a defect in IL-12 driven IL-21 expression *in vitro*, but ICOS, CXCR5 and Bcl-6 induction were all intact.[18] Hence, the IL-12-STAT3 signaling pathway contributes to IL-21 expression but not other aspects of Tfh cell differentiation. It is likely that other cytokines also contribute to Tfh cell generation in humans, as recently it was reported that IL-6 and IL-21 could induce ICOS expression in cord blood CD4$^+$ T cells via a STAT3 dependent pathway.[46,47]

Mendelian susceptibility to mycobacterial disease due to mutations in *IL12RB1*

Mendelian susceptibility to mycobacterial disease is a rare primary immunodeficiency where individuals present with extreme susceptibility to poorly virulent *Mycobacteria* and *Salmonella* strains, but not to other stains of bacteria, viruses, fungi or parasites, and are otherwise relatively healthy.[48] Furthermore, humoral immune responses are believed to be intact in these individuals as antigen-specific antibodies are generated following natural infection or vaccination.[49,50] The most common cause of Mendelian susceptibility to mycobacterial disease is mutations in the beta 1 chain of the IL-12 receptor (IL-12Rb1), which is part of the receptors for both IL-12 and IL-23.[48] As IL-12 was revealed to be the main inducer of human Tfh cell differentiation *in vitro*,[44,45] the presence of peripheral blood CXCR5$^+$ Tfh cells was recently addressed, albeit with conflicting results.[18,51] Consistent with normal antibody responses in IL-12Rb1-deficient patients, we found a normal frequency of circulating CXCR5$^+$ Tfh cells in these individuals and concluded that in the absence of the IL-12 pathway, other cytokines such as IL-6, IL-21, IL-23 and IL-27 could still give rise to normal Tfh cell differentiation.[18] In contrast, a decrease in peripheral blood CXCR5$^+$ Tfh cells and memory B cells and impaired GC formation was recently been reported in IL-12Rb1-deficient patients.[51] However, despite this, IL-12Rb1-deficient patients displayed normal levels of serum IgM and IgG, as well as tetanus toxoid-, rubella-, EBV-, CMV- and varicella virus-specific IgG. Plasma cells and Ig-expressing B cells were also detected in the LNs of these patients.[51] Thus, it is likely that in the absence of IL-12 signaling, compensatory mechanisms exist such that the humoral immune response is largely unaffected.

Tfh CELLS IN HIV

There has been significant interest in Tfh cells, not only in PID, but also in acquired immunodeficiency caused by human immunodeficiency virus (HIV) infection. HIV infects CD4$^+$ T cells resulting in their progressive decline and patients becoming susceptible to opportunistic infections. The virus has evolved numerous mechanisms to evade the host immune response, including alterations to humoral immunity, such that B cells are unable to make broadly neutralizing antibodies to eliminate the virus. However, somewhat inexplicably, HIV infected individuals develop hypergammaglobulinemia but equivalently fail to generate effective antibodies against HIV or protein antigens following vaccination.[52] This conundrum has been recently investigated by analyzing Tfh cells during HIV infection. Despite the progressive decline in total CD4$^+$ T cells during HIV infection, an increase in Tfh cells (defined as either CD45RA$^-$CXCR5^{hi}, CXCR5$^+$PD1$^+$ Bcl-6$^+$ or CXCR5$^+$PD1$^+$ CD4$^+$ T cells in the LNs or CD57$^+$CD4$^+$ T cells in the peripheral blood) was observed in HIV infected individuals.[53–56] This population of expanded T cells resemble bona fide Tfh cells as they express Bcl-6, ICOS, CD40L and PD1, and secrete IL-4, IL-10 and IL-21. Tfh cells from the LNs of HIV-infected patients often have elevated IL-21 expression.[54–56] It is not clear if this expansion is driven by HIV. Some studies observed a positive correlation between Tfh cells and viremia[55,56] and found that LN Tfh cells declined following combination antiretroviral therapy,[54,56] however, others found no such correlation.[54] Nevertheless, the Tfh cell population in the GC was found to be the major compartment for infection and replication of HIV.[56,57]

The expansion in LN Tfh cells in HIV-infected patients correlated with an increase in GC B cells and plasma cells and a decrease in memory B cells.[54–56] Similar to Tfh cells, the expansion in GC B cells and plasma cells in viremic HIV patients decreased following antiretroviral therapy.[56] Furthermore, there was a positive correlation between Tfh cells and serum IgG, suggesting that the Tfh cell expansion is responsible for the hypergammaglobulinemia seen in these patients.[54] However, the quality of the antibodies generated is

likely to be ineffective, as the response to GAG proteins was five times higher than that of antibodies directed against envelope proteins such as gp120[54] and broadly neutralizing antibody responses have been found to primarily target epitopes within and around the CD4-binding site of gp120.[58] Recently, Tfh cells from HIV-infected patients were found to be poor supporters of B cell survival and defective in inducing IgG production *in vitro*.[55] This was due to interactions between PD1 on Tfh cells and PDL1, which was highly expressed on GC B cells from HIV-infected patients. Ligation of PD1 by PDL1 decreased the survival of Tfh cells as well as their expression of ICOS, IL-21, IL-10 and IL-4, and blocking PDL1 and PDL2 increased IgG production in a coculture assay of Tfh cells and B cells.[55] Thus, although Tfh cells are expanded in HIV-infected individuals, they are unlikely to be able to induce intact antigen-specific immune responses owing to an aberrant immunoregulatory role of PDL1-expressing B cells, rather than an intrinsic Tfh cell defect in providing help for B-cell differentiation.

Further confirmation that Tfh cells in HIV-infected individuals are ineffective B-cell helpers has come from studies of HIV patients following administration of the influenza vaccine. Although the majority of healthy individuals are able to generate protective antibodies following vaccination with the 2009 H1N1 vaccine, this only occurred in ~50% of HIV-infected patients.[59] Healthy controls and HIV-infected responders to the vaccine had an increase in memory B cells and short-lived plasmablasts but non-responders did not. The expansion in B cells correlated with an increase in H1N1 antibodies, and an increase in serum IL-21 and IL-21 receptor expression on B cells was observed in healthy donors and HIV responders but not non-responders.[59] In a follow up study, an increase in peripheral blood $CXCR5^+$ Tfh cells was found in healthy donors and HIV-infected responders but not non-responders following immunization with the 2009 H1N1 vaccine.[23] This increase in Tfh cells correlated with an increase in memory B cells and the appearance of anti-H1N1 antibodies.[23] Thus, the ability to generate an antigen-specific response to the 2009 H1N1 vaccine relied on the generation of Tfh cells. More recently, Licco *et al.*[60] have identified a resting peripheral blood memory population of $CXCR5^+PD1^+CXCR3^-$ $CD4^+$ T cells that is increased in rare HIV individuals that are capable of generating broadly neutralizing antibodies against HIV. Furthermore, these $CXCR5^+PD1^+CXCR3^-$ $CD4^+$ T cells also expressed CCR7 and contained tetanus specific cells. This population is likely to represent central memory T cells that are the counterparts to GC Tfh cells as they can induce memory B-cell differentiation into $CD20^{low}CD38^+$ plasmablasts and IgG producing cells and display the cytokine and transcriptional phenotype of bona fide Tfh cells.[60] Interestingly, this population was observed to display superior Tfh cell function over $CXCR5^-$, $CXCR5^+PD1^-CXCR3^+$, $CXCR5^+PD1^-CXCR3^-$ or $CXCR5^+PD1^+CXCR3^+$ $CD4^+$ T cells. Thus in addition to acting as an important biomarker for HIV patients that can produce broadly neutralizing antibodies, the potential targeting of this population may improve vaccine development to HIV and other similar pathogens.

Tfh CELLS IN AUTOIMMUNE CONDITIONS

Numerous groups have studied the presence of circulating Tfh cells as a potential biomarker of disease in various autoimmune conditions. Specifically, the frequency of these cells in the peripheral blood and whether they correlate with disease scores and/or serum autoantibodies has been examined (Figure 1). The implication of circulating Tfh cells in human autoimmune conditions is reviewed below.

Autoimmune myasthenia gravis

Myasthenia gravis (MG) is an autoimmune condition characterized by muscle weakness due to the production of autoantibodies, such as those against the acetylcholine receptor and muscle-specific tyrosine kinsase, which block neuromuscular transmission.[61] It is believed to be a $CD4^+$ T-cell mediated disorder and thymectomy has been used as an effective form of treatment.[61] As a result of the implication that $CD4^+$ T cells mediate disease, there has been growing interest in the role of Tfh cells in the pathogenesis of MG. Indeed, circulating total $CXCR5^+$,[24,62] $CXCR5^+CD57^+$,[25] $CXCR5^+ICOS^{hi}$ and $CXCR5^+PD1^{hi}$ [62] $CD4^+$ T cells have been reported to be expanded in patients with MG. Furthermore, these studies revealed blood Tfh cells were associated with disease scores, suggesting that the Tfh cells are involved in disease progression in MG. Specifically, there was a positive correlation between the frequency of $CD4^+CXCR5^+$ T cells and disease severity,[24] and the frequency of $CD4^+CXCR5^+ICOS^{hi}$ or $CD4^+CXCR5^+PD1^{hi}$ and autoantibodies.[62] Furthermore, $CXCR5^+$ $CD57^+$ $CD4^+$ T cells decreased with clinical improvement.[25] MG patients have an increase in serum CXCL13,[62–64] which is made by human Tfh cells and is the ligand for the chemokine receptor CXCR5. Not surprisingly, there was a positive correlation between serum CXCL13 levels and $CXCR5^+ICOS^{hi}$ $CD4^+$ T cells in MG patients.[62] IL-21 expression was also increased in PBMCs from MG patients and there was a positive correlation between IL-21 expression and $CXCR5^+ICOS^{hi}$ $CD4^+$ T cells in these patients.[62]

Autoimmune thyroid disease

Graves' disease and Hashimoto's thyroiditis are two types of autoimmune thyroid diseases where an immune response is mistakenly mounted against the thyroid. This response is mediated by antigen-specific $CD4^+$ T cells that induce the production of autoantibodies. In Graves' disease production of antibodies directed against thyroid stimulating hormone receptor results in hyperthyroidism. In contrast, in Hashimoto's thyroiditis autoantibodies directed against the thyroid lead to its destruction and subsequent hypothyroidism.[65] $CXCR5^+$ T cells expressing high levels of ICOS, IL-21 and Bcl-6 have been observed in the thyroid of patients with Hashimoto's thyroiditis.[66] Interestingly, an increase in circulating $ICOS^{hi}CXCR5^+$ and $PD1^{hi}CXCR5^+$ $CD4^+$ T cells have been detected in patients with Graves' disease and Hashimoto's thyroiditis.[66] Furthermore, there was a positive correlation between $ICOS^{hi}CXCR5^+$ $CD4^+$ T cells and autoantibodies in patients with these autoimmune thyroid diseases. In addition to this, a decrease in $ICOS^{hi}CXCR5^+$ $CD4^+$ T cells was detected in some, but not all patients with Graves' disease 6 months posttreatment with antithyroid drugs.[66] Compared with $CD4^+$ T cells from normal donors, $CD4^+$ T cells from patients with Graves' disease or Hashimoto's thyroiditis displayed increased IL-21 secretion and expression following *in vitro* culture. However, Bcl-6 expression was comparable with that of normal healthy donors. These results support the observations that circulating $CXCR5^+$ $CD4^+$ T cells are likely to express the Tfh cytokine IL-21, but not the transcription factor Bcl-6. Furthermore, although there was an increase in $ICOS^{hi}CXCR5^+$ $CD4^+$ T cells, there was no difference in total $CXCR5^+$ or $ICOS^{hi}$ $CD4^+$ T cells. Taken together, these results indicate circulating Tfh cells are increased in autoimmune thyroid diseases. Further, it suggests the $ICOS^{hi}CXCR5^+$ $CD4^+$ T-cell population may be different to the $CXCR5^+$ or $ICOS^{hi}$ $CD4^+$ T-cell populations and highlights the problems with identification of blood Tfh cells.

Juvenile dermatomyositis

Juvenile dermatomyositis is a rare disease that is classified as an idiopathic inflammatory myopathy and has autoimmune origins.[67] It is a systemic autoimmune condition that mainly affects the muscles and the skin, although other organs can also be involved. Up to 70% of patients with juvenile dermatomyositis have serum autoantibodies, but it is not know how these autoantibodies are associated with disease in these patients. Recently, an increase in $CXCR5^+CCR6^+$ Th17-like and $CXCR5^+CXCR3^-CCR6^-$ Th2-like, but not $CXCR5^+CXCR3^+$ Th1-like Tfh cells was detected in patients with juvenile dermatomyositis.[9] Interestingly, although total circulating $CXCR5^+$ Tfh cells were found to secrete high amounts of IL-21, IL-10 and CXCL13, express ICOS and induce B-cell differentiation, further subdivision revealed these characteristics of bona fide Tfh cells were specific to $CXCR5^+CCR6^+$ Th17-like and $CXCR5^+CXCR3^-CCR6^-$ Th2-like, but not $CXCR5^+CXCR3^+$ Th1-like Tfh cells. Thus it is only the Th2 and Th17-like Tfh cells, which have B-cell helper activity that is increased in patients with juvenile dermatomyositis. Consistent with this, disease score and numbers of plasmablasts ($CD19^+CD20^-CD27^+CD38^{hi}$) correlated positively with Th2 and Th17-like Tfh cells and negatively with Th1-like Tfh cells.[9] Interestingly, plasmablast numbers did not correlate with either total $CXCR5^+$ T cells or $ICOS^+CXCR5^+$ T cells, further suggesting that not all $CXCR5^+$ T cells are useful indicators of disease inducing Tfh cell activity.

Systemic lupus erythematosus (SLE)

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease of the connective tissues that is thought to be both B- and T-cell mediated. It is often characterized by the presence of autoimmune antinuclear antibodies, such as those directed against double stranded DNA.[68] Thus there has been much interest in the role of Tfh cells in mediating disease in SLE. Accordingly, an increase in total $ICOS^+$,[28–30] $CXCR5^+ICOS^{hi}$,[12] $CXCR5^+PD1^+$,[69] $CXCR5^+PD1^{hi}$[12] or $CXCR5^+CD57^+$[12] $CD4^+$ have been detected in SLE patients. Interestingly, as discussed above, although peripheral blood Tfh cells are not known to express Bcl-6, one study found an increase in circulating $CXCR5^+$ $Bcl\text{-}6^+$ in patients with SLE.[26]

The value of each of these different staining strategies to identify blood 'Tfh' cells as a potential biomarker of disease varied. In the case of $ICOS^+$ $CD4^+$ T cells, an increase was only detected in SLE patients with active disease,[30] although there was no correlation between $ICOS^+CD4^+$ T cells and disease score[28–30] Although at least in one study the frequency of $ICOS^+$ $CD4^+$ T cells decreased following treatment with prednisolone.[30] Interestingly, SLE B-cells activated via ICOS-ICOSL interactions *in vitro*, enhanced the production of anti-double stranded DNA antibodies.[29]

Consistent with an increase in Tfh cells in SLE patients, an increase in $IL\text{-}21^+$ $CD4^+$,[26,31] and serum IL-21 and CXCL13 have been detected in patients with SLE.[12,70] Furthermore, although the increase in serum CXCL13 levels correlated with disease scores in SLE patients,[70] $IL\text{-}21^+$ $CD4^+$ T cells did not.[31] Interestingly, the increase in $IL\text{-}21^+$ $CD4^+$ T cells in SLE were detected in both the $CXCR5^+$ and $CXCR5^-$ $CD4^+$ T cell subsets.[26] However, $CXCR5^+IL\text{-}21^+$ $CD4^+$ T cells correlated positively with memory B cells and negatively with naïve B cells, whereas $CXCR5^-IL\text{-}21^+$ T cells did not. Instead, $CXCR5^-IL\text{-}21^+$ $CD4^+$ T cells correlated with the presence of Th17 cells.[26] These results suggest that although IL-21 is involved in the differentiation of both memory B cells and Th17 cells, these effects of IL-21 are mediated by $CXCR5^+$ and $CXCR5^-$ $CD4^+$ T cells, respectively.

In regard to the presence of circulating Tfh cells and associations with disease in SLE, it was found that $CXCR5^+$ $Bcl\text{-}6^+CD4^+$ Tfh cells did not correlate with disease scores or anti-double stranded DNA antibodies. However, the increase in $CXCR5^+$ $Bcl\text{-}6^+$ $CD4^+$ Tfh cells did positively correlate with an expansion in $CXCR5^+$ $Bcl\text{-}6^+$ B cells.[26] With respect to $CXCR5^+$ $CD4^+$ T cells, the presence of $CXCR5^+PD1^+$ $CD4^+$ T cells positively correlated with disease score, antinuclear antibodies and $CD138^+$ $CD19^+$ plasma cells.[69] Furthermore, following treatment with corticosteroids there was a clinical improvement and a corresponding decrease in the frequency of $CXCR5^+PD1^+$ Tfh cells.[69] The $CXCR5^+ICOS^{hi}$ population did not correlate with disease score or change following immunosuppression, but was associated with end organ damage and the presence of high titers of autoantibodies.[12] Nevertheless, not all studies observed an increase in circulating $CXCR5^+$ T cells in SLE patients, and a decrease in peripheral blood $CD4^+CXCR5^+$ T cells has been reported although it was argued this was due to a migration of these cells into the tissues.[70]

Overall these studies support the use of Tfh cell markers as a potential biomarker of SLE disease, however, more work is needed to determine exactly which markers will be the most informative.

Sjogrens syndrome

Sjogrens syndrome (SS) is a systemic autoimmune disease characterized by destruction of the exocrine glands, such as the salivary and lacrimal glands. In fact, the chemokine CXCL13 and $CXCR5^+$ expressing $CD4^+$ T cells have been detected in the salivary gland of SS patients.[71] Consistently, an increase in total $CXCR5^+$, $CXCR5^+ICOS^{hi}$ or $CXCR5^+PD1^{hi}$ Tfh cells has been observed in the peripheral blood of SS patients.[12,72] Further division of $CD4^+CXCR5^+$ T cells into Th1- ($CXCR3^+CCR6^-$), Th2- ($CXCR3^-CCR6^-$) and Th17-like ($CXCR3^-CCR6^+$) Tfh cells revealed an increase in Th17-like, but not Th1- or Th2-like Tfh cells.[72] Compared with Th1- and Th2-like Tfh cells, Th17-like Tfh cells expressed higher levels of ICOS, PD1, CD40L, IL-21 and Bcl-6 and lower amounts of CCR7. Furthermore, this increase in Th17-like Tfh cells positively correlated with the presence of autoantibodies. However, these $CCR6^+$ Th17-like Tfh cells were not enriched for IL-17, suggesting that they no longer represent circulating Th17 cells and have instead adapted to a more Tfh cell phenotype.[72] These results are in contrast to those of Morita *et al.*,[9] who found $CCR6^+CXCR5^+$ Th17-like Tfh cells were still enriched for IL-17 production. Thus, although both studies support peripheral blood $CXCR5^+$ $CD4^+$ T cells being the circulating counterparts to tissue Tfh cells, the subdivision of these cells into Th1-, Th2- and Th17-like Tfh cells may require further validation. More recently, an increase in $CXCR5^+ICOS^+PD1^+$ Tfh cells was detected in SS patients with glandular involvement but not in those with only sicca symptoms, such as dry eyes and dry mouth.[73] Furthermore, $CXCR5^+ICOS^+PD1^+$ Tfh cells had a positive correlation with serum autoantibodies and IL-21 levels. Interestingly, SS patients that were positive for IL-12 had increased frequencies of Tfh cells,[73] which is consistent with the role of IL-12 in the generation of human Tfh cells.

Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease that primarily affects the synovial joints of the hand and feet. As the presence of autoantibodies such as rheumatoid factor and anti-cyclic citrullinated peptide (anti-CCP) antibody is characteristic to RA,[74] numerous groups have investigated the role of Tfh cells in the

pathogenesis of this disorder. Subsequently increases in circulating $CXCR5^+$, $ICOS^+$, $ICOS^{hi}$, $CXCR5^+ICOS^{hi}$, $CXCR5^+PD1^+$ and $CXCR5^+PD1^{hi}$ $CD4^+$ T cells have been reported in patients with RA,[21,22,27] suggesting Tfh cells are involved in the disease-process of RA. Consistent with this increase in Tfh cells, RA patients have an increase in serum IL-21 levels.[21,22,27,32] Increases in $CXCR5^+ICOS^+$, $CXCR5^+ICOS^{hi}$ and $CXCR5^+PD1^{hi}$ Tfh cells and IL-21 have been positively associated with disease scores and/or the presence of anti-CCP antibodies.[21,22,27,32] Furthermore, a recent study revealed a positive correlation between circulating $CXCR5^+$ $CD4^+$ Tfh cells and $CD19^+$ B cells.[27] Intriguingly, this study also found clear differences between $CXCR5^+ICOS^+$ and $CXCR5^+PD1^+$ Tfh cells. Specifically, their cohort of RA patients displayed a positive correlation between $CXCR5^+PD1^+$ and $CD95^+$ (i.e., activated) B cells, but a negative correlation between $CXCR5^+ICOS^+$ and $CD95^+$ B cells. Furthermore, there was a positive correlation between $CXCR5^+ICOS^+$ and both anti-CCP antibodies and disease score, but a negative correlation between $CXCR5^+PD1^+$ and the presence of rheumatoid factor. Although there was a decrease in $CXCR5^+PD1^+$ Tfh cells and serum IL-21 in RA patients that responded to treatments, the frequency of $CXCR5^+ICOS^+$ Tfh cells remained unchanged.[27] Taken together, these results indicate $CXCR5^+ICOS^+$ and $CXCR5^+PD1^+$ cells are likely to represent two different populations of Tfh cells. The functional differences between these populations of Tfh cells will require further investigations.

It is worth noting that not all studies have revealed an increase in circulating Tfh cells in RA. In fact, even after identifying circulating Tfh cells using all known criteria for defining Tfh cells, that is, $CD4^+CXCR5^+$, $CD4^+CD45RO^+CXCR5^+$, $CD4^+CXCR5^+ICOS^{hi}$, $CD4^+CXCR5^+PD1^{hi}$, $CD4^+CXCR5^+CXCR3^+$, $CD4^+CXCR5^+CCR6^+$, $CD4^+CXCR5^+CXCR3^-CCR6^-$, Chakera *et al.*[75] found there was a comparable frequency of Tfh cells in their cohort of RA patients and normal donors. Nevertheless, they argued these differences were due to the fact that the average time from diagnosis of their RA cohort was 56 months, whereas most of the other studies consisted of newly diagnosed RA patients. The reason being that the longer disease period had allowed circulating Tfh cells to migrate from the blood to sites of ectopic GC formation. Consistent with this hypothesis, a more recent study revealed that although early stage RA patients had elevated levels of serum IL-21, serum IL-21 was lower in patients with chronic RA such that it was no longer statistically different to that of normal healthy donors.[32] Theses studies highlight the interpretation of the presence of circulating Tfh cells in disease should be performed with caution as it can vary significantly with disease progression.

MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is a neurodegenerative disorder caused by damage to the myelin and axons of the brain and spinal cord. It is a progressive disorder with no known cures. Although the precise cause of this damage is not known, it is likely to be immune mediated with involvement of both humoral and cellular responses.[76] Consistent with this hypothesis, intrathecal antibodies and ectopic lymphoid follicle-like structures containing germinal centers have been observed in the meninges of some MS patients. Furthermore, the presence of these ectopic GCs has been associated with more rapid disease progression and generally a poorer prognosis.[77] More recently, a study has found an increase in peripheral blood $ICOS^+CXCR5^+$ Tfh cells in MS patients, irrespective of whether they were at the relapsing-remitting or progressive phase of disease, compared with normal healthy controls.[78] Furthermore, this increase in $ICOS^+CXCR5^+$ Tfh cells positively correlated with the presence of peripheral blood plasmablasts and disease scores in these patients. Subdivision of Tfh cells into $CXCR3^+$ Th1-, $CXCR3^-CCR6^-$ Th2- and $CCR6^+$ Th17-like Tfh cells revealed a decrease in Th1-like Tfh cells in relapsing-remitting and progressive MS patients and an increase in Th17-like Tfh cells in progressive MS patients compared with normal donors.[78] Consistent with a role for Tfh cells in the pathogenesis of MS, $IL\text{-}21^+$ $CD4^+$ T cells have been detected in active lesions in MS patients.[79] Taken together these results suggest that Tfh cells may drive ectopic GC formation and subsequent autoantibody-mediated demyelination and axon damage in MS. As such, peripheral blood $CXCR5^+$ Tfh cells may be an indicator of disease progression in MS.

Tfh CELLS IN CANCER

Increasing evidence now shows a link between Tfh and multiple cancers. These Tfh associated cancers can be divided into two categories: those where the neoplastic cells themselves display a Tfh-like phenotype and those where the tumor cells do not have a Tfh phenotype but in which non-neoplastic Tfh cells seem to have a role (Figure 1).

The best described of the former is angioimmunoblastic T-cell lymphoma (AITL), which is a subset of peripheral T-cell lymphoma (PCTL). The neoplastic cells in AITL are typically $CD4^+$ and, in the early stages of disease, the cells are often found in the follicular regions of the LN, which lead to the suggestion that AITL was derived from Tfh cells. Subsequent histological analysis demonstrated that the malignant T cells in AITL express many markers of Tfh cells such as CXCL13, CXCR5, CD40L, OX40, SAP, ICOS, c-maf and Bcl-6[80–87] and that their gene expression profile resembles that of Tfh cells.[80] However, it should be noted that AITL are relatively heterogeneous and in many cases the lymphoma cells are not found to express all of the above markers. AITL is also often associated with symptoms of B-cell dysregulation including expansion of B cells, hypergammaglobulineamia and autoantibodies[88,89] indicating that by driving B-cell expansion and activation these malignant Tfh cells in AITL are also functionally acting like normal Tfh cells.

Tfh cell like properties, however, are not exclusive to AITL. Numerous reports have shown that a proportion of PCTL not otherwise specified (PCTL-NOS), particularly the follicular variant, also express varying levels of Tfh markers such as Bcl-6, PD1, CXCL13, SAP and ICOS. Similarly, several cutaneous T-cell lymphomas, namely Mycosis fungoides/Sezary syndrome[87,90] and Primary Cutaneous CD4-Positive Small/Medium T-Cell Lymphoma[91] can also express Tfh markers. Once again the Tfh-like phenotype of these lymphomas is often associated with B cell features such as the presence of B-cell blasts, plasma cells or hypergammaglobulinemia.[85,89,91,92]

Infiltrates of non-neoplastic T cells expressing Tfh cell markers have also been observed in cases of GC derived B-cell lymphomas such as follicular lymphoma and nodular lymphocyte predominant Hodgkin's disease.[93] Ahearne *et al.*[93,94] showed that patients with chronic lymphocytic leukemia also display increased $CD4^+CXCR5^+$ T cells in the blood. Evidence suggests that the Tfh cells present in these neoplasms may have a role in promoting survival or proliferation of the neoplastic cells through their expression of CD40L, IL-4 and IL-21.

In contrast, a recent paper suggests that there may also be a role for Tfh cells in anti-tumor responses. Gu-Trantien *et al.*[95] reported that some breast cancers had a high levels of lymphocyte infiltrates and that this included Tfh cells. Further, tumors that had a higher level of Tfh infiltration were associated with increased survival. Whether the Tfh cells have a functional role in inhibiting tumor growth or if their

presence simply reflects the presence of tumor associated lymphoid structures and a greater overall immune response remains to be seen.

CONCLUDING REMARKS

Tfh cells are required to provide 'help' to B cells for the generation of antibodies following natural infection and vaccination. However, due to the location of Tfh cells in secondary lymphoid organs, the study of these cells in humans has been difficult. Improved access to tissues, the identification of circulating Tfh cells and an increased appreciation into the importance of examining Tfh cells in humans has resulted in an enhanced understanding of these cells. In particular the identification of Tfh cells in the peripheral blood has been instrumental to the study of these cells in human disease, which has subsequently revealed mechanisms in the differentiation and function of human Tfh cells. Some important work still remains to be done, for example, the field lacks a comprehensive study to reveal the level of heterogeneity within the CXCR5$^+$ population and in many cases more work needs to be done to determine whether the alterations in Tfh cells that are observed in these conditions are causative or merely a consequence of other immune dysregulation. Irrespective of this, though numerous studies have indicated enumeration of Tfh cells using various criteria has the potential to act as a biomarker of disease or vaccination outcomes. Furthermore, as we move into the bioinformatics era, improved availability and affordability of techniques such as whole-genome sequencing to reveal the genetic lesions in rare immunodeficient patients, has opened new avenues for the study of Tfh cells in the immune response. These are all steps towards the goal of being able to manipulate Tfh cells for the purposes of generating new and improved therapeutics and vaccines to combat disease.

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