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REVIEW

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T follicular helper cells in the humoral immune response to SARS-CoV-2 infection and vaccination

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Summary sentence: Review on the analysis of TFH cells during COVID-19 infection and vaccination and the role of these cells as biomarkers of neutralizing antibody development.

Abstract

Vaccination remains the most effective mechanism to reduce the impact of COVID-19. Induction of neutralizing antibodies is a strong correlate of protection from infection and severe disease. An understanding of the cellular events that underpin the generation of effective neutralizing antibodies is therefore key to the development of efficacious vaccines that target emerging variants of concern. Analysis of the immune response to Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infection and vaccination has identified circulating T follicular helper cells (cT_{FH}) as a robust correlate of the neutralizing antibody response. Here, we discuss the analysis of cT_{FH} cells and their lymphoid counterparts in human humoral immune responses during COVID-19, and in response to vaccination with SARS-CoV-2 spike. We discuss the phenotypic heterogeneity of cT_{FH} cells and the utility of cT_{FH} subsets as informative biomarkers for development of humoral immunity. We posit that the analysis of the most effective cT_{FH} will be critical to inducing durable immunity to new variants of SARS-CoV-2.

KEYWORDS
CD4+ T cells, COVID-19, SARS-CoV-2, T follicular helper cells

1 | INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused more than 200 million infections and at least 4.3 million deaths since it emerged in late 2019 (https://covid19.who.int). Concerted global efforts have mapped the immune response to SARS-CoV-2 infection in great detail and facilitated the development of highly effective vaccines to control the spread of SARS-CoV-2 and the COVID-19 pandemic.

SARS-CoV-2 is a single-stranded positive-sense RNA virus belonging to the Coronaviridae family.[1] This family encompasses six other coronaviruses known to infect humans: the endemic human coronaviruses (hCoV) 229E and NL63 (alphacoronaviruses) as well as HKU1 and OC43 (betacoronaviruses), and the highly pathogenic SARS-CoV and MERS-CoV (betacoronaviruses like SARS-CoV-2). Coronaviruses express 4 structural proteins: the surface glycoprotein spike (S), the membrane protein (M), the envelope protein (E), and the nucleoprotein (N).[1] The spike protein facilitates viral attachment and entry by engaging its cognate receptor, which in the case of SARS-CoV-2, SARS-CoV, and NL63 is angiotensin-converting enzyme 2 (ACE2). This interaction is specifically mediated by the Receptor Binding Domain (RBD) of S, located within the S1 subunit. The M and E proteins mediate virion assembly and structure, while N binds the genomic RNA to form a nucleocapsid. During infection, a plethora of additional nonstructural and accessory proteins are expressed that facilitate viral replication and immune evasion.[1]

Abbreviations: ACE2, angiotensin-converting enzyme 2; AIM, activation-induced marker; ASC, antibody-secreting cells; E, nucleoprotein; GC, germinal center; HCV, hepatitis C virus; HPV, human papillomavirus; M, membrane protein; MBC, memory B cell; N, envelope protein; RBD, Receptor Binding Domain; S, spike protein; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; T_{FH} cell, T follicular helper cell; VOC, variant of concern.

2 | OVERVIEW OF HUMORAL IMMUNITY TO SARS-COV-2 INFECTION

Neutralizing antibodies can block the entry of SARS-CoV-2 into host cells, primarily by preventing spike from engaging ACE2.[2] There is increasing evidence that neutralizing antibodies are a strong correlate of protection from acquiring SARS-CoV-2 infection, with studies showing that plasma neutralization titers correlate strongly with vaccine efficacy.[3,4] Numerous studies have also shown that passive transfer of neutralizing monoclonal antibodies into animal models provides sterilizing protection against experimental SARS-CoV-2 challenge.[5,6] Most neutralizing antibodies target the RBD,[7] though neutralizing antibodies against N terminal domain of S1, and the fusion machinery within S2 have been reported.[8,9]

The development of humoral immunity is underpinned by the activation of SARS-CoV-2-specific B cells, which enter a germinal center (GC) reaction and differentiate into long-lived, antibody-secreting plasma cells or memory B cells (MBCs). While the majority of this response is derived from de novo priming of naïve B cells, there is some evidence that cross-reactive MBCs that recognize epitopes conserved in endemic hCoV can be recalled upon infection with SARS-CoV-2.[10,11] However, such responses typically do not exhibit neutralizing activity.[10,11] In addition to neutralization, antibodies against SARS-CoV-2 can exert antiviral activity by clearing free virions or infected cells via Fc effector functions including antibody-dependent phagocytosis (ADP) and antibody-dependent cellular cytotoxicity (ADCC).[12,13] While most studies have focused on the Fc effector functions of S-specific antibodies, the M, N, and ORF3a proteins can all be detected on the surface of infected cells and be targeted by antibodies that mediate NK cell activation.[14] While it remains unclear the degree to which ADCC and ADP contribute to protection against SARS-CoV-2 in humans, several viral challenge studies in mice have shown the protective value of many RBD- and NTD-specific mAbs relies upon engagement with cellular Fc receptors in vivo.[15–17]

During primary SARS-CoV-2 infection, neutralizing antibodies are generally detectable between 7 and 14 days following symptom onset,[18] peak around 23 days, and start to decline thereafter.[19] S-specific antibodies that mediate Fc effector functions persist longer than neutralizing antibodies and could potentially contribute to protection upon reexposure.[12] The initial increase and subsequent decline of neutralizing antibodies have been linked to short-lived antibody-secreting cells (ASCs) that secrete high levels of antibodies in the early stages of infection and the rapidly decline afterward.[20] Despite this, S- and RBD-specific IgG antibodies are maintained for at least 11 months postinfection,[21,22] with serologic neutralizing activity similarly maintained for at least 12 months.[22,23] Critically, in individuals recovered from COVID-19, the bone marrow is populated by SARS-CoV-2 S-specific ASCs (plasma cells) that are stable for at least 11 months.[21]

Contrary to antibody titers, S-specific MBCs in peripheral blood increase in frequency during convalescence[22–27]), plateau at 8 months and are maintained for at least 12 months.[22] During that time, the MBC pool undergoes continued clonal evolution, with the emergence of new clones between 6 and 12 months and a gradual increase in VH gene mutational load.[22,26–28] Notably, monoclonal antibodies isolated from convalescent COVID-19 individuals can exhibit increased binding affinity for S or RBD, and increased neutralizing potency over time. This evolution of the MBC compartment is strongly indicative of persistent GC activity and ongoing affinity maturation.[22,26–28] Interestingly, N protein and SARS-CoV-2 viral RNA have been detected in intestinal biopsies of convalescent COVID-19 individuals taken ~4 months after initial diagnosis, which suggest residual viral replication in the gut could be a potential source of antigen to support ongoing GC activity. Alternatively, antigen may be retained in the form of immune complexes on follicular dendritic cells within classical lymphoid tissues for prolonged support of ongoing GC activity. Overall, primary SARS-CoV-2 infection elicits a humoral immune response with hallmarks of prolonged GC activity, the generation of long-lived plasma cells, an evolving population of MBCs and serologic maintenance of potent neutralizing antibodies.

3 | THE ROLE OF T_{FH} CELLS IN HUMORAL IMMUNITY TO SARS-COV-2

3.1 | Overview of lymph node and circulating TFH subsets

The generation of effective humoral immune responses requires a well-orchestrated series of cellular interactions between B cells and T follicular helper cell (T_{FH} cells). T_{FH} cells are a specialized subset of $CD4^+$ T cells that provide critical "help" signaling to B cells, promoting B cell survival and differentiation into MBCs and long-lived plasma cells. The repeated interactions of antigen-specific T_{FH} and B cells that take place in the GC of B cell follicles is a critical step in the generation of high-affinity and long-lived antibody responses following infection or vaccination. Phenotypically, human T_{FH} cells in lymphoid tissues are characterized by a lack of CCR7, coupled with expression of the chemokine receptor CXCR5, PD-1, markers of antigen experience (CD45RO),[29,30] and in some cases CD57.[31,32] Expression of the transcriptional repressor Bcl-6 is a defining feature of T_{FH} cells in lymphoid tissues.[31,33,34] T_{FH} cells provide B cell cognate help via costimulatory molecules such as CD40L and indirectly by ICOS, as well as local secretion of cytokines including IL-21. The importance of IL-21 is highlighted by correlations between serum IL-21 levels and antibody responses to influenza vaccination.[35]

The anatomic localization of GC T_{FH} within lymphoid tissues hinders their sampling in many human cohort studies. However, a subset of circulating $CD4^+$ T cells exists in blood with significant similarities to lymph-tissue T_{FH}. These cells, known as circulating T_{FH} (cT_{FH}), share surface expression of CXCR5 with lymphoid T_{FH} and constitute ~10% of the $CD4^+$ T cell compartment in peripheral blood.[36] Despite their shared expression of CXCR5 and ability to support B cell activation, there are several key transcriptional and phenotypic differences between circulating and GC T_{FH}. While cT_{FH} development depends on the key T_{FH} lineage-defining transcription factor Bcl-6,[37] cT_{FH} cells exhibit low or no expression of Bcl-6, in stark contrast to GC T_{FH}

cells.[36,38] Circulating T_{FH} cells do, however, express c-Maf,[39] which can drive expression of CXCR5 and IL-21 in the absence of Bcl-6.[40] Additionally, cT_{FH} typically have a quiescent phenotype and lack expression of activation markers such as PD-1, ICO,S or CD38, while retaining expression of CCR7. Nonetheless, transcriptomic, epigenetic, and TCR repertoire analyses have provided evidence to support a clonal and developmental overlap between GC T_{FH} and cT_{FH}.[41] Indeed, analysis of $CXCR5^{bright}PD\text{-}1^{bright}$ $CD4^+$ T cells from human thoracic duct lymph suggests this population is a trafficking intermediate between GC T_{FH} and cT_{FH} cells, which continually emigrate from lymphoid tissues.[41]

Functionally, cT_{FH} exhibit greater capacity for IL-21 and IL-10 secretion compared with $CXCR5^-$ $CD4^+$ T cells, thereby allowing them to provide superior B cell help in vitro compared with conventional memory $CD4^+$ T cells, as determined by B cell survival and differentiation in ASCs.[36] Within the cT_{FH} population, however, studies have described significant phenotypic and functional heterogeneity. Similar to conventional memory $CD4^+$ T cells, cT_{FH} cells express chemokine receptors associated with distinct T helper subsets (CXCR3 and CCR6), lineage-defining transcription factors (T-bet, GATA3, ROR-γt), and low levels of cytokines (IFNγ, IL-4, IL-17) upon activation. Consequently, cT_{FH} can be defined as $CXCR3^+CCR6^-$ T-bet^+ IFNγ-producing $cT_{FH}1$ cells (group 1 cT_{FH} cells based on a recently proposed unifying nomenclature,[42]), $CXCR3^-CCR6^-$ $GATA3^+$ IL-4-producing $cT_{FH}2$ cells (group 2) and $CXCR3^-CCR6^+$ ROR-γt^+ IL-17A-producing $cT_{FH}17$ cells (group 3). While the developmental pathways and functional implications of this phenotypic polarization within the T_{FH} lineage are not fully understood, these distinct subsets are reported to exhibit differential capacity to provide B cell help in vitro, with $CXCR3^-$ $cT_{FH}2/17$ cells exhibiting superior capacity to secrete IL-21 and provide help to naïve B cells, as determined by B cell survival and differentiation in ASCs.[36,43] Although $CXCR3^+$ $cT_{FH}1$ cells do not provide significant help to naïve B cells, they can support the activation of MBCs and their differentiation into ASCs in vitro.[36,43]

3.2 | Overview of human cT_{FH} responses following infection and vaccination

Consistent with linkage between lymph node resident T_{FH} and cT_{FH} populations, an activated subpopulation of $PD\text{-}1^+ICOS^+CD38^+Ki67^+$ cT_{FH} transiently emerges into the blood following viral infection or vaccination.[33,38,43–46] Both peptide/MHC-II tetramers and antigen restimulation assays have demonstrated that this population contains $CD4^+$ T cells specific for vaccine or infection-associated antigens.[38,43,46] The appearance of this cT_{FH} population is temporally linked to the emergence of ASCs and correlates with the magnitude and qualitative aspects of the serologic response. This phenomenon has been widely observed following vaccination of human subjects with the inactivated influenza vaccine,[38,43,44,47] Human Papillomavirus vaccine,[48] YF-17D vaccine,[46] rVSV-ZEBOV Ebola vaccine,[49] hepatitis B vaccine,[50] experimental malaria vaccination,[51,52] oral-inactivated *Escherichia coli* vaccine,[53] as well as during acute infection with influenza virus,[45] human immunodeficiency virus,[39,54] hepatitis C virus,[55] Epstein–Barr virus,[56] and malaria.[57,58] It is clear that the analysis of cT_{FH} cells in peripheral blood following vaccination or infection can provide important insights into humoral immunity.

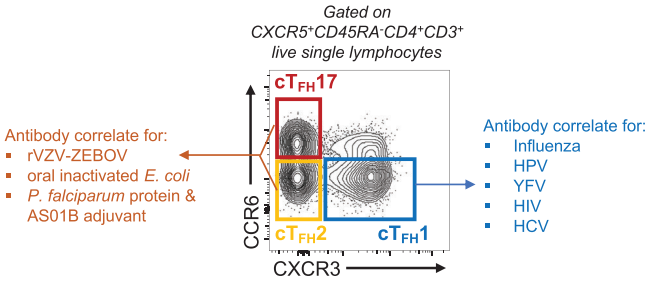

FIGURE 1 **Circulating T_{FH} subsets as correlates of antibody responses.** Flow cytometry plot of cT_{FH} subsets based on CXCR3 and CCR6 expression and their association with antibody responses in different contexts

While activation of the overall cT_{FH} population is a consistent biomarker of robust serologic responses to infection or vaccination, the relationship between the 3 distinct cT_{FH} subsets and antibody production appears to be context dependent (Figure 1). Activation of $cT_{FH}1$ cells has been associated with multiple aspects of humoral immunity (antibody titers and/or ASCs and/or antigen-specific B cells) for a number of viral infections and vaccines, including influenza,[43–45] HPV,[48] Yellow fever virus,[46] HIV,[54] and HCV.[55] On the other hand, vaccination with rVZV-ZEBOV, oral-inactivated *E. coli*,[53] or *Plasmodium falciparum* parasite protein antigens with AS01B adjuvant[52] results in activation of $CXCR3^-$ $cT_{FH}2/17$ cells, which positively correlate with antibody responses to cognate antigens. Of note, although acute malaria infection drives activation of $cT_{FH}1$, $cT_{FH}2$, and $cT_{FH}17$ cells, this does not correlate with antibody responses in children[57] and higher activation of $cT_{FH}1$ cells is associated with an increased likelihood of symptomatic *Plasmodium vivax* infection.[58] Overall, the population of cT_{FH} cells that emerges in peripheral blood after vaccination or infection is strongly associated with the development of protective humoral immunity. It has become evident that dividing this population into CXCR3/CCR6 subsets provides additional information and that these subsets maybe more reliable biomarkers than the total population. However, their ontogeny, their functional differences, and the context-specific correlations between cT_{FH} subsets and humoral immunity warrant further investigation. Comparative analysis of the TCR repertoire analysis as well as singe-cell transcriptomic and epigenomic analyses could provide novel insights into cT_{FH} polarization and help resolve discrepancies surrounding cT_{FH} subsets.

3.3 | Human cTFH responses in COVID-19

Many recent studies have analyzed the magnitude and phenotype of cT_{FH} cell responses during the acute and convalescent phases of SARS-CoV-2 infection (Table 1).[59] In the blood, activated cT_{FH} ($PD\text{-}1^+ICOS^+$)[60–64] with increased expression of CD38[60] and reduced expression of CCR7[63] are evident during acute infection. These

TABLE 1 Key studies of human cTFH responses following SARS-CoV-2 infection and vaccination

Cohort	Detection method	Dominant phenotype/cytokine profile of cTFH cells	Key cTFH findings	Reference
Acute and convalescent cohorts				
Acute and convalescent COVID-19	Bulk ICOS+PD-1+ cTFH cells	Activation of CXCR3+CCR6−cTFH cells	• CXCR3+ cTFH cells positively correlated with neutralizing antibody titers	60
Acute and convalescent COVID-19	Antigen-specific cTFH cells by AIM	Greater frequency of CXCR3+CCR6− than CXCR3−CCR6+ S-specific cTFH cells in acute samples Greater frequency of CXCR3−CCR6+ than CXCR3+CCR6− S-specific cTFH cells in convalescent samples IFNγ producing cTFH cells IL-17A assessed but not detected	• Spike-specific cTFH cells detected in acute and convalescent COVID-19 individuals • Spike-specific CXCR3−CCR6+ cTFH cells correlated with lower disease severity	65
Acute and convalescent COVID-19	Bulk ICOS+PD-1+ cTFH cells	CXCR3+ cTFH cells	• ICOS+PD-1+ CXCR3+ cTFH cells increased acute mild and severe COVID-19 compared to healthy controls • ICOS+PD-1+ CXCR3− cTFH cells modestly increased in severe COVID-19 compared with healthy controls • ICOS+PD-1+ CXCR3+ and CXCR3− cTFH cells positively correlated with ASC frequencies and CXCL13 levels in plasma	64
Convalescent cohorts				
Convalescent COVID-19	Antigen-specific cTFH cells by AIM	Not reported	• Spike-, membrane protein-, and nucleocapsid-specific cTFH cells detected • Spike-, membrane protein-, and nucleocapsid-specific cTFH cells positively correlated with neutralizing antibodies and N-specific IgG antibodies	69
Convalescent COVID-19 (up to 8 months)	Antigen-specific cTFH cells by AIM	Increase in CCR6+ spike-specific cTFH cells over time	• Spike- and membrane-specific cTFH cells persist up to at least 6 months postsymptom onset • Reduction in frequency of PD-1hi spike-specific cTFH cells over time	23
Convalescent COVID-19	Bulk cTFH cells	CXCR3+ or CXCR3−	• CCR7loPD-1+ cTFH cells increased in convalescent COVID19 individuals compared with healthy controls • Frequency of CXCR3+, but not CXCR3−, cTFH cells positively correlated with IgG, IgM and IgA SARS-CoV-2-specific antibodies	63

(Continues)

TABLE 1 (Continued)

Cohort	Detection method	Dominant phenotype/cytokine profile of cTFH cells	Key cTFH findings	Reference
Convalescent COVID-19	Antigen-specific cTFH cells by AIM	CXCR3+ or CXCR3−	• Spike-specific cTFH cells detected in convalescent individuals • Spike-specific cTFH cells in convalescent were mostly CXCR3−CCR6+CXCR3−CCR6+ spike-specific cTFH cells negatively correlated with neutralizing antibodies, while CXCR3+CCR6− spike-specific cTFH cells positively correlated with neutralizing antibodies	66
Convalescent COVID-19	Antigen-specific cTFH cells by AIM	IFNγ producing cTFH cells	• Spike-specific cTFH cells detected in convalescent individuals • Spike-specific cTFH cells persist up to at least 3 months postsymptom onset	67
Convalescent COVID-19	Antigen-specific cTFH cells by AIM	Spike-specific cTFH 1 and cTFH 2 Membrane-specific cTFH 1 and cTFH 1/17 Nucleocapsid-specific cTFH 1	• Spike-, membrane protein- and nucleocapsid-specific cTFH cells detected • Spike-specific CD4+ T cells more polarized toward cTFH than Th1, while nucleocapsid-specific CD4+ T cells more polarized toward Th1 than cTFH	68
Convalescent COVID-19 (up to 150 days)	Antigen-specific cTFH cells by AIM	Not reported	• Spike-specific cTFH cells detected in convalescent individuals • Decay of spike-specific cTFH cells, with a t1/2 of 128 days	24
Convalescent COVID-19	Bulk PD-1+CXCR5+ cTFH cells & antigen-specific cTFH cells by AIM	CXCR3+ or CXCR3− IFNγ and IL-21 producing cTFH cells other cytokines not reported	• CXCR3+ cTFH cells positively correlated with neutralizing antibody titers • CXCR3− cTFH cells negatively correlated with neutralizing antibody titers	62

(Continues)

TABLE 1 (Continued)

Cohort	Detection method	Dominant phenotype/cytokine profile of cTFH cells	Key cTFH findings	Reference
Vaccination cohorts				
Healthy and MS patients on a-CD20 therapy receiving 2 doses of mRNA vaccine	Antigen-specific cTFH cells by AIM	CXCR3+	• Poor maintenance of cTFH cells postvaccination in MS-aCD20 patients • Spike-specific cTFH cells positively correlated with spike-specific IgG and memory B cell responses to vaccination	75
Naïve and COVID-19 recovered individuals receiving 2 doses of BNT162b or mRNA-1273 vaccine	Antigen-specific cTFH cells by AIM	Not reported	• Vaccination induced spike-specific cTFH cells in both groups • Pre-vaccination spike-specific cTFH frequencies in recovered individuals, positively corelated with postvaccination neutralizing antibodies to D164G and B.1.351 (beta) variant	76
Naïve and COVID-19 recovered individuals receiving 1 dose of BNT162b vaccine	Antigen-specific cTFH cells by AIM	IFNγ producing cTFH cells IL-17A assessed but not detected	• Vaccination induced spike-specific cTFH cells in both groups • Previously infected individuals had higher frequencies of spike-specific cTFH cells at ~3 weeks postvaccination (1 dose) • Pre-vaccination spike-specific cTFH frequencies in recovered individuals, positively corelated with postvaccination IgG and IgA, but not IgM, antibodies to spike	78
Naïve and COVID-19 recovered individuals receiving 2 doses of BNT162b or mRNA-1273 vaccine	Antigen-specific cTFH cells by AIM	Not reported	• Vaccination induced spike-specific cTFH cells in both groups • Spike-specific cTFH cells peak at ~1 month and then wane, contrary to spike-specific TH1 cells that are stable for at least 6 months • Spike-specific cTFH cells at 2 weeks postvaccination in naive individuals correlates with postvaccination neutralization titers and spike-specific MBCs	77

activated PD-1$^+$ICOS$^+$ cT$_{FH}$ cells emerge transiently during the acute phase of infection and generally wane after approximately 14 days postsymptom onset. Consequently, the use of antigen-specific T cell assays (either activation-induced marker (AIM) or intracellular cytokine staining) are critical for studying SARS-CoV-2-specific cT$_{FH}$ responses during convalescence. Such assays have demonstrated that S-specific cT$_{FH}$ cells that emerge during acute infection[65] persist in convalescent individuals for at least 6 months,[23] with a half-life of ~129 days.[24]

The relationship between cT$_{FH}$ frequency, phenotypic and functional polarization, and serologic responses to SARS-CoV-2 has also been assessed. The bulk PD1$^+$ICOS$^+$ cT$_{FH}$ population that emerges in the acute phase of COVID-19 is predominantly composed of CXCR3$^+$ cT$_{FH}$1 cells,[60,64] similar to influenza infection.[45] However, analysis of S-specific cT$_{FH}$ by AIM has demonstrated a dominant population of CXCR3$^-$CCR6$^+$ cells.[23,65,66] Interestingly, the proportion of CXCR3$^-$CCR6$^+$ S-specific cT$_{FH}$ cells is higher in late convalescence (6 months) than in early convalescence (1–2 months) or during the acute phase.[23,65] Despite the detection of CXCR3$^-$CCR6$^+$ cT$_{FH}$17 cells, antigen-specific cT$_{FH}$ cells in recovered COVID-19 patients consistently produce IFNγ and IL-21, but not IL-17, among multiple independent studies.[62,65–67]

Phenotypic polarization of cT$_{FH}$ has been linked to the development of robust neutralizing antibody responses. Indeed, CXCR3$^+$ cT$_{FH}$1 cells are consistently associated with high titers of spike binding or neutralizing antibodies. This is observed for both the total ICOS$^+$PD-1$^+$ cT$_{FH}$1 population (which also correlates with the ASC response and plasma CXCL13),[60,62–64] as well as S-specific cT$_{FH}$1.[66] Activation of cT$_{FH}$1 cells in acute COVID-19 also positively correlates with the titers and avidity of RBD-specific IgM antibodies.[60] Conversely, the relationship between cT$_{FH}$2, cT$_{FH}$17, and antibody responses is more variable across cohorts and assays. Identification of S-specific cT$_{FH}$ has suggested that the frequency of cT$_{FH}$2 responses positively correlate with neutralizing antibody titers, while S-specific cT$_{FH}$17 were a strong negative correlate of neutralizing activity.[66] In contrast, total ICOS$^+$PD-1$^+$ cT$_{FH}$2/17 cells have been variably correlated with the antibody response within different cohorts.[60,62–64] Altogether, the current data indicate that CXCR3$^+$ cT$_{FH}$1 cells are a strong correlate of neutralizing and total antibodies against SARS-CoV-2, while the role of CXCR3$^-$ cT$_{FH}$2 and cT$_{FH}$17 cells requires further investigation, as do the differences between total ICOS$^+$PD-1$^+$ and AIM$^+$ cT$_{FH}$ cells.

Although most studies have focused on S-specific cT$_{FH}$ cells, cT$_{FH}$ specific for N and M have also been studied.[65,68,69] Frequencies of cT$_{FH}$ cells specific for S, N, and M positively correlated with plasma neutralization activity as well as N-specific IgG antibodies.[69] Interestingly, polarization between cT$_{FH}$1, cT$_{FH}$2, and cT$_{FH}$17 subsets has been reported to differ for cT$_{FH}$ cells specific for different SARS-CoV-2 antigens,[68] although the significance of this observation is currently unclear.

A potential impairment of T$_{FH}$ cells in some cases of severe COVID-19 has also been reported. Specifically, GC B cells and T$_{FH}$ cells were diminished in lymphoid tissues in a subset of deceased COVID-19 patients.[70] Additionally, a population of cT$_{FH}$ cells expressing cytotoxicity-associated transcripts like PRF1 and GZMB (encoding perforin and granzyme B respectively) and termed cytotoxic cT$_{FH}$ cells was increased in hospitalized versus nonhospitalized individuals and was correlated with lower antibody titers to S.[71] These observations are however contrary to the higher antibody titers observed in severe COVID-19,[60] since impaired T$_{FH}$ activity would be expected to result in lower antibody titers. These observations therefore warrant further investigation to understand the role and function of T$_{FH}$ cells in severe COVID-19 and whether they relate a specific subset of patients.

In summary, the overall activation of cT$_{FH}$ cells during COVID-19 as well as their phenotypic polarization are correlates of neutralizing activity and B cell responses (Figure 2). What remains unclear, however, is the ontogeny of distinct cT$_{FH}$ subsets and their relationship to GC T$_{FH}$ activity. It is pertinent to further understand the potential of cT$_{FH}$ cells as biomarkers of the establishment and recall of humoral immunity to SARS-CoV-2, especially in the context of emerging variants of concern (VOCs) with increased potential for escape from humoral immunity.

3.4 | Human cT$_{FH}$ cell responses after SARS-CoV-2 vaccination

Vaccination with approved COVID-19 vaccines induces neutralizing antibodies that have been associated with protection from infection. Analysis of axillary draining lymph nodes after mRNA vaccination demonstrated potent GC reactions that persist for at least 12 weeks after booster immunisation.[72] Importantly, S-specific T$_{FH}$ cells are induced at those sites and correlate with S-specific GC B cells.[73,74] Analysis of paired lymph node and blood samples has demonstrated that while S-specific cT$_{FH}$ cells with an activated phenotype (CD38$^+$HLA-DR$^+$ICOS$^+$) peak within the first month before returning to a resting phenotype and declining in frequency, S-specific T$_{FH}$ cells in lymph nodes exhibit relatively constant frequencies for at least 60 days.[73] Although limited to a small number of donors, these analyses suggest that mRNA vaccines induce robust GC reactions that may underscore the strong immunogenicity profile of this vaccine.

Vaccination of naïve (previously uninfected) individuals induces S-specific cT$_{FH}$ cells[75–77] with a CXCR3$^+$ phenotype[75] and the ability to produce IFNγ but not IL-17A.[78] The frequency of S-specific cT$_{FH}$ cells peaks within ~1 month after vaccination and then wanes, contrary to S-specific T$_H$1 cells, the frequency of which is stable for at least 6 months.[77] The frequency of S-specific cT$_{FH}$ cells, as well as S-specific conventional CD4$^+$ T$_H$1 cells, at 2 weeks postvaccination correlates with neutralizing antibodies to spike as well as VOCs and with S and RBD-specific MBC responses at 1 month after vaccination.[77] This indicates a role of cT$_{FH}$ cells as biomarkers for the development of neutralizing antibodies and MBCs following vaccination with spike. Consistently, vaccination of individuals after recovery from COVID-19 induces greater S-specific cT$_{FH}$ responses than in naive individuals.[78] Importantly, the pre-vaccination frequency of S-specific cT$_{FH}$ cells in recovered individuals positively correlates with postvaccination neutralizing titers to ancestral viruses as well as VOCs.[76,78] Overall, it is

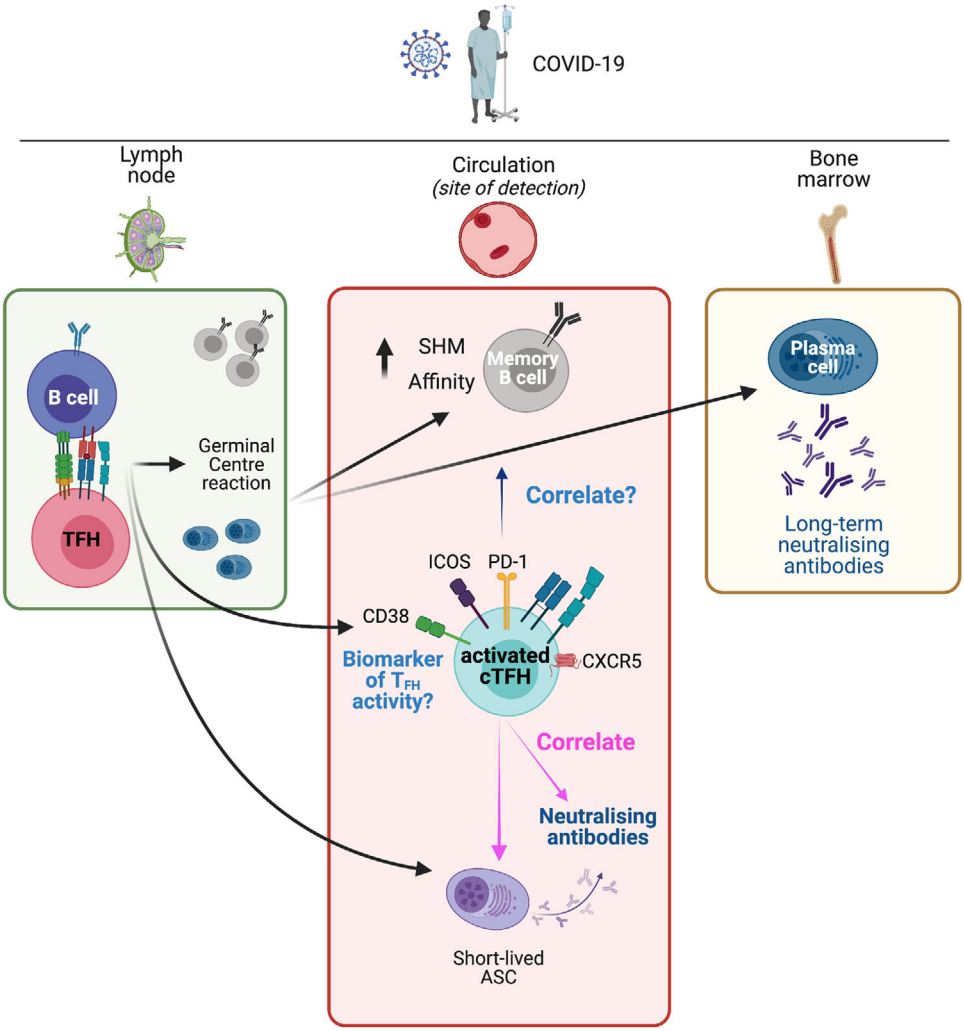

FIGURE 2 **Lymphoid and circulating T_{FH} responses in COVID-19**. SARS-CoV-2 antigen in the lymph nodes results in activation of antigen-specific B cells and T_{FH} cells. Their interaction leads to the initiation of the germinal center reaction. This results in the development of memory B cells with increased somatic hypermutation (SHM) and increased affinity, as well as long-lived plasma cells that traffic to the bone marrow and provide a long-term source of neutralizing antibodies. A population of short-lived antibody-secreting cells (ASCs) appears in the circulation and provides a raid source of neutralizing antibodies. Concurrently, a population of activated ($CD38^+$, $PD\text{-}1^+$, $ICOS^+$) cT_{FH} cells appears in the circulation. This population contains antigen-specific cT_{FH} cells (not depicted). Although memory B cells and ASCs are primarily located in lymphoid tissues, they are typically measured in blood samples, where they correlate with activated cT_{FH} cells. Activated cT_{FH} cells also correlate with the development of neutralizing antibodies. These cT_{FH} cells are a potential biomarker of T_{FH} activity in lymphoid tissues but it remains to be determined if this population of cT_{FH} cells are predictive of long-term neutralizing antibodies, or of the development of long-lived plasma cells and the prolonged evolution of the MBC pool. The figure was created with BioRender.com

becoming evident that cT_{FH} cells have an important role as biomarkers of humoral immunity following SARS-CoV-2 vaccination. It will be important to further characterize and understand lymphoid and circulating T_{FH} cell responses following the administration of different recently developed vaccine platforms and if/how they differentially induce such responses, especially in the context of heterologous prime-boost vaccination.

4 | CONCLUSIONS

There is increasing evidence that analysis of cT_{FH} responses can provide critical insights into magnitude and qualitative aspects of the humoral immune response to SARS-CoV-2 infection and vaccination (Figure 1). The current data strongly support the notion that cT_{FH} cells, and particularly the $cT_{FH}1$ subset, are informative biomarkers of the development of neutralizing antibodies and MBCs targeting the wild-type Spike as well as VOCs. Nonetheless, important questions remain regarding the differential role of CXCR3/CCR6 cT_{FH} subsets in the development of neutralizing antibodies and the persistence of spike-specific cT_{FH} memory. In order to effectively harness the capacity of T_{FH} to drive strong neutralizing antibody responses to vaccination, it will be critical to understand why only some cT_{FH} subsets positively correlate with antibody titers, and how T_{FH} quality can be manipulated through novel vaccine platforms. Additionally, while cT_{FH} activation and the frequency of antigen-specific cT_{FH} cells are biomarkers of neutralizing antibodies in the acute and early convalescent

phase of COVID-19 infection and vaccination, it is not known whether cT_{FH} cells are predictive of long-term neutralizing antibodies, or of the development of long-lived plasma cells. As studies seek to understand the immunologic mechanisms underlying the prolonged evolution of the MBC pool, we will gain more information about the duration of GC T_{FH} responses and their relationship to cT_{FH} frequency and phenotype. Addressing these questions would be pivotal in harnessing their potential for the development of effective vaccination strategies.

AUTHORSHIPS

M. K., W. S. L., and J. A. J. prepared the manuscript. A. K. W. and S. J. K. revised the manuscript.

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DISCLOSURES

The authors declare no conflicts of interest.

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