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Long-lasting Response of Human Circulating T-follicular Helper Cells (cTfh) To Post SARS-CoV-2 mRNA Immunization

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The ongoing COVID-19 pandemic underscores the urgent need for effective vaccination strategies to mitigate disease burden. The development of neutralizing antibodies is a critical indicator of host defence mechanisms against life-threatening infectious diseases, such as those caused by SARS-CoV-2. Understanding the fundamental biological mechanisms and substantiality of these antibodies' production is essential for developing effective vaccines, particularly in the face of emerging variants. Circulating T follicular helper (cTfh) cells, which have emerged as significant predictors of neutralizing antibody levels, are of utmost importance in shaping long-term immunity following vaccination. We emphasize the pivotal role of cTfh cells in shaping long-term immunity, providing reassurance about the effectiveness of vaccines. In this study, we elucidate the functions of cTfh cells and their lymphoid counterparts during immune responses to SARS-CoV-2, particularly in the context of spike protein vaccination. We explore the phenotypic diversity of cTfh cells and their potential as biomarkers for development of SARS-CoV-2 vaccine efficacy with long-lasting immunity. The identification of specific cTfh subgroups may inform strategies for enhancing vaccine responses, especially concerning new SARS-CoV-2 variant-specific vaccines. Future research directions will focus on harnessing the predictive capabilities of cTfh cells to optimize vaccine development and improve immunological outcomes against evolving SARS-CoV-2 strains.

Keywords: T follicular helper cells; SARS-CoV-2; COVID-19; CD4+ T cells.

ABBREVIATIONS

PD-1 *: Programmed cell death protein-1*
CCR7 *: CC-chemokine receptor 7*
CXCR3 *: CXC-chemokine receptor 3*
CCR6 *: CXC-chemokine receptor 6*
CXCR5 *: CXC-chemokine receptor 5*
ICOS *: Inducible T cell co-stimulator*
Tfh *: Follicular helper T cell*
cTfh *: Circulating Tfh cell*

1. INTRODUCTION

The emergence of SARS-CoV-2, the virus responsible for the COVID-19 pandemic, has prompted an unprecedented global effort to develop effective vaccines. Among the most promising vaccine platforms are those based on mRNA technology, which offers several advantages, including rapid development, scalability, and the potential for modification [1]. As vaccination campaigns have progressed worldwide, understanding the immune responses elicited by these vaccines has become paramount. Investigating the dynamics of circulating T-follicular helper cells (cTfh), a subset of CD4+ T cells crucial for B cell activation and antibody production is essential for evaluating vaccine efficacy and durability [2]. The role of cTfh cells in orchestrating humoral immune responses has been extensively studied in the context of various infections and vaccinations. These specialized T cells live within secondary lymphoid organs, interacting with B cells to promote germinal center reactions, affinity maturation, and the generation of long-lived plasma cells and memory B cells. Importantly, cTfh cells play a central role in shaping the size and quality of antibody responses, which are critical for protection against viral infections [3].

In the context of SARS-CoV-2 mRNA vaccination, understanding the kinetics and durability of cTfh cell responses is particularly interesting. mRNA vaccines, such as those developed by Pfizer-BioNTech and Moderna, encode the viral spike (S) protein, which mediates viral entry into host cells. Upon vaccination, antigen-presenting cells process and present S protein fragments to T cells, leading to the activation and differentiation of cTfh cells. These cTfh cells then migrate to B cell follicles within lymphoid organs, where they provide help to B cells undergoing somatic hypermutation and class-switch recombination, ultimately promoting the production of high-affinity antibodies against SARS-CoV-2 [4,5]. Initial studies investigating cTfh responses to SARS-CoV-2 mRNA vaccination have offered valuable insights into the kinetics and size of these responses. For instance, several reports have shown robust expansion of cTfh cells following vaccination, peaking within weeks after the first dose and declining gradually thereafter. Significantly, the frequency and functionality of cTfh cells have been correlated with the size and persistence of antibody responses, suggesting their crucial role in vaccine-induced immunity. Furthermore,

emerging evidence suggests that cTfh cell responses to SARS-CoV-2 mRNA vaccination may show heterogeneity across individuals, influenced by factors such as age, sex, and immunological history. Understanding the determinants of cTfh cell dynamics and their impact on vaccine efficacy and durability is essential for optimizing vaccination strategies and informing public health policies [6,7].

The immunoglobulin family consists of five classes: IgM, IgA, IgE, IgG, and IgD. Each class has its characteristics and function [8]. Primary immunoglobulin M (IgM) is expressed in the early stages of B cell maturation and is linked to primary immunological responses. IgA protects mucosal surfaces from bacteria, viruses, and toxins. IgE plays a role in allergic reactions, hypersensitivity reactions, and the defense against parasitic infections. IgG, which is functionally divided into four subclasses (IgG1, IgG2, IgG3, and IgG4), is essential for both the effectiveness of vaccinations and antiviral defences. IgD has a short serum half-life and is present at low concentrations, and its specific function is currently unknown [8,9] (Fig. 1).

This review aims to comprehensively evaluate the current literature on human cTfh cell responses to SARS-CoV-2 mRNA vaccination, focusing on the kinetics, size, durability, and correlates of protection. We will discuss key findings from clinical studies and preclinical models, highlighting gaps in knowledge and areas for future research. Additionally, we will explore the implications of cTfh cell responses for vaccine development, including strategies to enhance immunogenicity and durability. By elucidating the role of cTfh cells in vaccine-induced immunity against SARS-CoV-2, this review aims to contribute to the ongoing efforts to control the COVID-19 pandemic and prepare for future outbreaks.

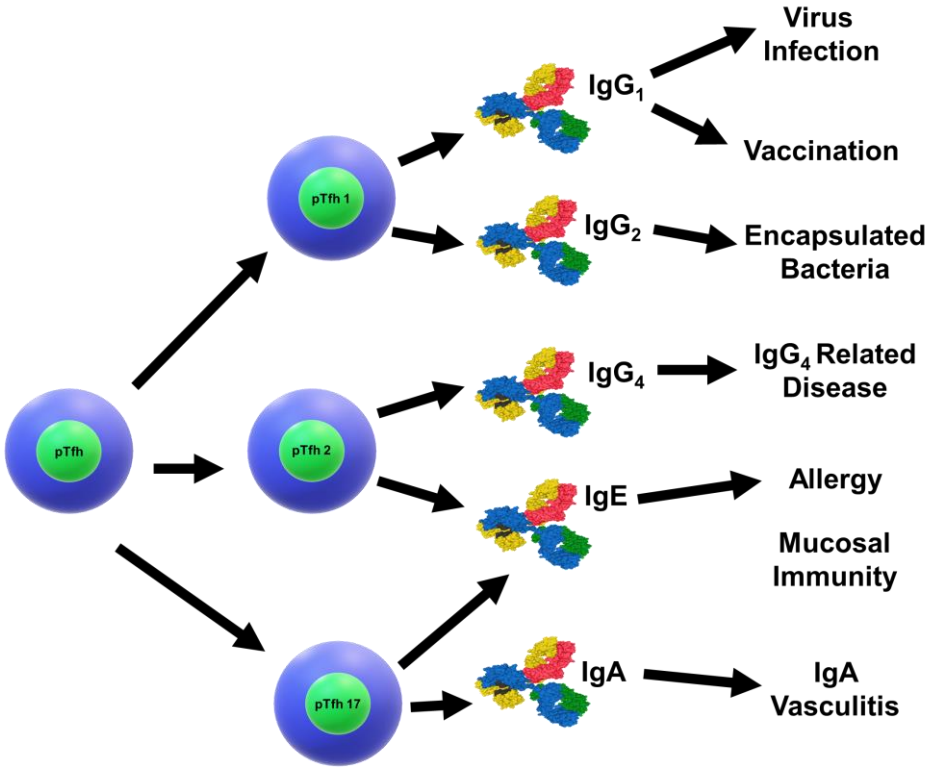

Fig. 1. Shows how several Tfh cell subsets contribute to the production of immunoglobulins with several functions. Pre-Tfh (pTfh) cells can differentiate into Tfh1, Tfh2, or Tfh17 phenotypes in response to different microenvironmental signals from different healthy or pathological conditions. Because each subgroup of Tfh cells has specific functional characteristics, they can coordinate B cell development towards producing various immunoglobulin types essential for various immunological responses. Reproduced/adapted with permission [9].

2. EMERGING ROLE OF Tfh CELLS FOR NOVEL SARS-CoV-2 VACCINE DEVELOPMENT

According to the findings, CD4 T-cell responses to SARS-CoV-2 were 80–100% on antigen-specific T-cell responses in patients treated after SARS-CoV-2 infection, with most research mainly concentrating on spike (S) protein [10-13]. According to other investigations, T-cells have also been found to target the nucleocapsid (N) and membrane (M) proteins [14]. Developing effective SARS-CoV-2 vaccines has been recognized as a significant step toward preventing the COVID-19 pandemic. Neutralizing antibodies (nAb) are a reliable indicator of immunity against infection and immunization in rhesus macaques [15,16]. Neutralizing antibodies have been identified as the critical factor of vaccination effectiveness in phase I/II trials of the Pfizer and Moderna vaccines and many other candidate vaccines [17-20]. Whereas the Pfizer vaccine was showcased to induce CD4 and CD8 T-cell responses in trial participants, and vaccine-induced CD4 T-cell responses correlated with antibody levels [21], with our knowledge of both the precise immune factors that are associated with protection as well as the durability of this immune function is still lacking. Furthermore, the factors that trigger the production of nAb in the presence of spontaneous SARS-CoV-2 infection are crucial to investigate, especially in the situation of new viral variations that may be different mechanisms of pathogenic infections developing [22,23]. Several studies have found a significant increase in activation and exhaustion markers on T cells in moderate and severe SARS-CoV-2 illnesses [24-27]. Whereas studying lymphoid tissues directly within patients is challenging, circulating T follicular cells (cTfh), also known as T follicular helper cells (Tfh), act as a helpful alternative for investigating Tfh responses in germinal centers. However, significant debate was over the optimum method to recognize these cells. It was widely agreed that they express CXCR5, a lymph node homing receptor, but several researchers also utilized PD1 expression in combination with CXCR5 to identify cTfh [28-30]. Even though the number of CXCR5+ PD1+ CD4 T cells in the bloodstream is generally low, these cells are closely related to Tfh in lymphoid tissue [31] and enhance adaptive immunity defences [32,33]. In the circumstances of infection and Vaccination against numerous diseases, antigen-specific cTfh is associated significantly with neutralizing antibodies [29,34-38]. Even if cTfh responses were not discussed in the specific situation of SARS-CoV or MERS-CoV infection, CD4 T-cell responses were demonstrated to be crucial in regulating SARS-CoV throughout mouse models [39], as well as Tfh frequencies in draining lymph nodes associated with neutralizing antibodies throughout the latest research on the MERS-CoV vaccine in mice [40].

Much research about SARS-CoV-2-specific T follicular helper cellular transformation by different signaling pathways exists. Thevarajan et al. were among the first to publish SARS-CoV-2 cTfh frequencies, discovering that overall cTfh frequencies are increased during acute infection [41]. Several studies have discovered a link between total CD4 T-cell frequencies and antibody levels [42,43]. Another research report enhanced gene expression of CXCR5 and ICOS, two Tfh markers, among SARS-CoV-2-specific CD4 T-cells but did not confirm cTfh effects [44]. However, Kaneko et al. discovered that BCL6-expression throughout germinal centre Tfh has been lost within thoracic lymph nodes of deceased donors with COVID-19, implying that Tfh response initiation may be inhibited in serious SARS-CoV-2 infection [45]. The initiation of antigen-specific Tfh responses, especially in asymptomatic patients with COVID-19, is still a mystery. In their study, Juno et al. investigated circulating Tfh, recognized as CD45RA CXCR5+ CD4 T cells, in the blood of SARS-CoV-2 infected people. They discovered a link between S protein-specific cTfh and nAb, indicating that effective Tfh responses were produced in moderate SARS-CoV-2 infection [46]. Unfortunately, these findings leave other aspects unresolved, particularly once those responses change throughout the recovery period. Even though this research work provided a preliminary look at antigen specific Tfh responses, which included PD1, a conventional Tfh marker, within the research frame, PD1 expression needed more utilized to characterize the Tfh population and was not published. Furthermore, Ox40 and CD25 were utilized as initiation markers to detect antigen-specific responses that have already been reported and demonstrated to contain a significant fraction of regulating T-cells [47]. A current study has the durability of S protein-specific CD4 T-cell responses after recovery [48]. The authors have considered only the incidence of circulating Tfh (ICOS+ CXCR5+ CD4 T cells) each month and three months after the symptoms started. While they found responses higher than backgrounds after three months, there was no change throughout the duration.

Additional analysis of CD4 T-cell and cTfh responses during the recovery period might help to determine how much these responses change and evolve. Several recent studies [49,50] have investigated cTfh groups up to 6 months after the onset of symptoms. (Investigations used too much). Unfortunately, this ongoing research failed due to the failure to examine connections among antigen-specific cTfh and SARS-CoV-2-specific antibodies. In addition, these types of research were predominantly concerned with S protein-specific effects. For example, Infrastructural assistance emerges in HIV infection during Vaccination, when CD4 T-cell responses towards internal structural proteins correspond with neutralizing antibodies against the outer envelope protein [51,52], emphasizing the necessity of studying cTfh responses from across the SARS-CoV-2 proteome. Boppana et al. report on SARS-CoV-2-specific CD4 T-cell activation to membrane (M), nucleocapsid (N), and spike (S) proteins in 26 recovered patients who were studied in real-time. They studied antigen-specific cTfh (CXCR5+ PD1+ CD4 T cells) and found connections among antigen-specific cTfh responses across all protein specificities with antibody neutralization during the first convalescent visit. They observed that the M protein-specific cTfh responses increase during Check Up 1 towards Check Up 2 (>5% activation of the total cTfh population). These responses did not correspond to antibody neutralization at only the second recovered visit and more than thirty days after illness onset. Such findings are always the earliest to investigate the speed of cTfh responses following SARS-CoV-2 infection and the link between neutralizing antibodies and cTfh sensitivities to SARS-CoV-2 M and N proteins. This research also suggests that cTfh development may be postponed in SARS-CoV-2 infections [53].

3. PHENOTYPES AND FUNCTIONS OF FOLLICULAR HELPER T CELLS (Tfh) OF SARS-CoV-2 INFECTION

Follicular Helper T Cells (Tfh) cells are used as a functional indicator; they can promote B cells by increasing antibody production, long-lived plasma cells, and memory B cells [54,55]. Tfh cell markers, which typically include chemokine receptor CXCR5, transcription factor Bcl-6, PD-1, CD40 ligand (CD40L), and ICOS in humans and mice, are essential for identifying Tfh cells with their various subgroups not only in lymphoid tissue but also in circulation [56-59]. Additionally, the phenotypes of Tfh cells were linked to distinct phases of immunological responses [60,61]. Within secondary lymphoid organs, naïve $CD4^+$ T cells develop into Tfh cells through CXCR5 overexpression and CCR7 reduced expression, which is controlled by antigen-specific conventional dendritic cells (DCs) and monocyte-derived DCs [58,62,63]. Tfh cells migrate into CXC-chemokine ligand 13 (CXCL13)-enriched B lymphoid follicles inside the germinal centre (GC) due to enhanced CXCR5 and reduced CCR7 [58,64]. In Human and mouse GCs, the unique transcription factor Bcl-6 was exclusively expressed among Tfh cells and was highly expressed within CXCR5hiCCR7low/-Tfh cells [64-67]. A cytokine, Interleukin-21 (IL-21), is firmly and selectively released only Tfh cells, which increases Tfh cell proliferation and enhances B cell differentiation and antibody production, which is typical for Tfh cells [68-72]. In both mice as well as humans, ICOS reduction greatly lowers GC reactions but also Tfh cells, indicating that ICOS expression in Tfh cells is required during Tfh cell differentiation and maintenance, GC production, and B cell differentiation, including antibody responses [73-75]–66]. As an essential effector molecule, ICOS could also promote IL-21 release within Tfh cells [75-77]. Tfh cell development and activation could be considerably enhanced by increased PD-1 expression [78-80]. Tfh cells were usually classified as having three phenotypes: precursor-Tfh (Pre-Tfh) cells, canonical GC Tfh cells with $PD\text{-}1^{++}$ and $ICOS^{++}Bcl\text{-}6^+$ $CCR7^-$ $CXCR5^{++}CD4^+$ T cells characterized as $PD\text{-}1^+$ $ICOS^+$ $Bcl\text{-}6^{low}CCR7^{low}CXCR5^+$ $CD4^+$ T cells, and memory Tfh cells similar to Pre-Tfh cells in lymphoid tissue [33,81,82]. Tfh cells regulate B cell differentiation, becoming memory B cells as well as plasma cells in GC, and even select for high-affinity antibody production to form long-term innate immunity [83-86].

Circulating Tfh (cTfh) cells in the blood typically have two different phenotypes: central memory Tfh cells (PD-1- $ICOS^-CCR7^{high}BCL\text{-}6^-$ $CXCR5^+$ $CD4^+$ T cells) and effector memory Tfh cells ($PD\text{-}1^+ICOS^+CCR7^{low}BCL\text{-}6^-$ $CXCR5^+$ $CD4^+$ T cells) [61,87,88]. Similarly, cTfh cells were categorized under three subgroups depending on the expression of either CXCR3 as well as CCR6: Tfh1 ($CXCR3^+$ $CCR6^-$), Tfh2 ($CXCR3^-$ $CCR6^-$), Tfh17 ($CXCR3^-$ $CCR6^+$), and Tfh1/17 ($CXCR3^+$ $CCR6^+$) cells that contain the hallmark gene transcription factors and cytokines comprising Th1 (T-bet as well as IFN-g), Th2 (GATA3, IL-4, IL-5, and IL-13), with Th17 (RORgt, IL-17, and

IL-22) cells [61,87,89]. cTfh2 with cTfh17 cells potentially stimulates B cell differentiation with antibody production and regulates (Ig-antibodies) isotype changing. Although cTfh1 cells are not commonly considered B cell helpers, ICOS+ PD-1highCCR7lowcTfh1 cells control B cell differentiation and trigger antibody responses [89-95]. Depending on the expression of ICOS, PD-1, and CCR7, as well as CXCR3 and CCR6, these findings show functionally different cTfh cell subgroups. Furthermore, emerging unique subgroups distinguish among Th1, Th2, and Th17 cells while sharing some fundamental properties. Tfh-like cells were also recognized in non-lymphoid tissues, such as arthritis synovium and skin, but also salivary glands of patients, that also widely express low expression of CXCR5 and Bcl-6 and high PD-1, ICOS, OX40, and IL-21, especially in comparison to Tfh cells in secondary lymphoid organs, that also express tissue-specific chemokine receptors, such as CCR2, CCR5, CX3C-chemokine receptor 1 (CX3CR1) and CXCR4 [82,96-100]. Tfh13 cells, a unique Tfh cell subgroup that produces and secretes IL-4 with IL-13, have been newly found to be essential for IgE production in both human and mouse allergies, and they exhibit the signaling molecules Bcl-6 and GATA3 [101-103].

According to current research, specific phenotypes for Tfh cells are required during B cell differentiation and high-affinity antibody production (Table 1). More importantly, follicular regulatory T (Tfr) cells are a subpopulation of Foxp3+ Treg cells inside the GC, launched through Foxp3+/-precursors and not just from Tfh cells [104-107]. Tfr cells express Tfh cell markers such as CXCR5, Bcl-6, PD-1, and ICOS and Treg cell molecules such as CD25, Foxp3, Blimp-1, and CTLA-4 [108-111]. Tfr cells, like Treg cells, play a significant role in immunosuppression that is greater than that of Tfh cells. Tfh cells can restrict GC responses and reduce Tfh and B cell activation inside GCs via inhibitory molecules such as CTLA-4, PD-1, and IL-10, as well as TGF-b production. Tfh/Tfr cell equilibrium was required to regulate immunological homeostasis and modulate innate immunity [92,96,111-114].

4. MECHANISM OF SARS-CoV-2 mRNA

“Presently, the emerging SARS-CoV-2 infectious outbreak is causing a significant challenge for global healthcare across the globe. Innate immunity is required for neutralizing antibodies and is essential in vaccination reactions against pathogenic virus infections, such as SARS-CoV-2, which have been linked to Tfh cell differentiation and function” [55,115-120] (Fig. 2). Tfh cells have been studied for their role in regulating the eradication of SARS-CoV-2 infections and developing novel vaccines.

Much research showed a high frequency of cTfh cells with the $CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ phenotype. In a patient with especially non-recovering COVID-19 patient, specific plasma SARS-CoV-2-binding IgM and IgG antibodies increased exponentially approximately 20 days after infection, with a combination of enhanced specific plasma SARS-CoV-2-binding IgM as well as IgG antibodies [41]. Single-cell investigation showed that individuals with active COVID-19 infection had higher frequencies of cTfh cells and a large percentage with specific anti-SARS-CoV-2 antibodies, such as IgA and IgG [121]. “In recovered COVID-19 patients, the percentages of Spike (S)-specific cTfh cells ($CD3^{+}CD4^{+}CD45RA^{-}CXCR5^{+}$) are continuously produced after S-peptide activation and show a significant phenotypic inclination toward the aCCR6+CXCR3-cTfh17 cell phenotype. Another study discovered that significantly enlarged CXCR3+cTfh1 cells were associated with such a solid neutralizing immunological response to influenza vaccination and live attenuated yellow fever immunization” [37,122].

The latest research found that increasing numbers of CCR7lowPD-1+ cTfh-effector memory (em), cTfh1 but instead cTfh2 cells, and also high IL-1b and TNF-a, are found in $CXCR5^{+}CD45RACD25^{-}CD4^{+}$T cells, and therefore, cTfh1 cells were related to increased SARS-CoV-2-specific IgG/IgM antibodies. While CCR7highPD-1-cTfh-central memory (cm) and cTfh17 cells within CXCR5+CD45RACD25-CD4+T cells were reduced in recovered patients compared to healthy patients, cTfr cells within Treg cells were increased. The frequency of high cTfh-em, low cTfh-cm, and cTfr cells was also associated with disease severity [43]. These findings suggest that cTfh cell morphological characteristics can produce significant neutralized antibodies toward SARS-CoV-2 in COVID-19-recovered patients, which will help develop antibody-based treatments and vaccines for COVID-19.

Table 1. Phenotypes of Tfh cell subsets in blood and lymphoid tissue

Location	Cell subsets	Phenotypic markers	Ref.
Lymphoid tissues	Pre-Tfh cells	PD-1^{+} ICOS^{+} CCR7^{low}Bcl-6^{low}Blimp-1^{-} CXCR5^{+}	[62,87,88]
	GC Tfh cells	PD-1^{++}ICOS^{++}CCR7- Bcl-6^{+} Blimp-1^{-} CXCR5^{++}	
	Memory Tfh cells	PD-1^{+} ICOS^{+} CCR7^{low}Bcl-6^{low}Blimp-1^{-} CXCR5^{+}	
Blood	cTfh1/17 cells	IFN-g $^{+}$ IL-17A^{+} Bcl-6- Blimp-1^{-} CXCR5^{+} (or) CXCR3^{+} CCR6^{+} Bcl-6^{-} Blimp-1^{-} CXCR5^{+}	
	cTfh17 cells	IL-17A^{+} Bcl-6^{-} Blimp-1^{-} CXCR5^{+} (or) CXCR3^{-} CCR6^{+} Bcl-6^{-} Blimp-1^{-} CXCR5^{+}	
	cTfh2 cells	IL-4^{+} Bcl-6^{-} Blimp-1^{-} CXCR5^{+} (or) CXCR3^{-} CCR6^{-} Bcl-6^{-} Blimp-1^{-} CXCR5^{+}	
	cTfh1 cells	IFN-g $^{+}$ Bcl-6^{-} Blimp-1^{-} CXCR5^{+} (or) PD-1^{+} ICOS^{+} CCR7^{low}CXCR3^{+} CCR6^{-} Bcl-6^{-} Blimp-1^{-} CXCR5^{+}	
	Effector memory Tfh cells	CD40L+ /PD-1+ /ICOS+ CCR7^{low}Bcl-6- Blimp-1- CXCR5+	
	Central memory Tfh cells	PD-1- ICOS-CCR7^{high}Bcl-6^{-} Blimp-1^{-} CXCR5^{+}	
	cTfh13 cells	IL-13^{hi}IL-4^{hi}IL-5^{hi}IL-21^{low}Bcl-6^{+} GATA3^{+} CXCR5^{+}	[101-103]

***† PD-1**, programmed cell death protein-1; **CCR7**, CC-chemokine receptor 7; **CXCR3**, CXC-chemokine receptor 3; **CCR6**, CXC-chemokine receptor 6; **CXCR5**, CXC-chemokine receptor 5; **ICOS**, inducible T cell co-stimulator*

Table 2. Tfh cell responses in various vaccine candidates of SARS-CoV-2

Vaccine candidates	Phenotypes	Function	Antibody isotypes	Ref.
	mRNA vaccines			
BNT162b2 mRNA vaccine	AIM$^+$ CXCR5$^+$ CD45RA-CD3$^+$ cTfh cells expansion, AIM cells include CD69$^+$ OX40$^+$ or CD69$^+$ CD40L$^+$ (or) CD69$^+$ 4-1BB$^+$ (or) OX40$^+$ 4-1BB$^+$ (or) CD40L$^+$ 4-1BB$^+$ (or) CD40L$^+$ OX40$^+$	Positively correlate with anti-spike-specific IgA and IgG titers.	IgA, IgG	
RBD mRNA (receptor binding domain, RBD)	B220-CD4$^+$ CD44hiCD62L-CXCR5$^+$ Bcl-6$^+$ Tfh cells, B220-CD4$^+$ CD44hiCXCR5$^+$ PD-1hi IL-21$^+$ Tfh cells, B220- CD4$^+$ CD44hiCXCR5$^+$ Bcl-6$^+$ ICOS$^+$ Tfh cells, B220- CD4$^+$ CD44hiCXCR5$^+$ PD-1hi IFN-g $^+$ Tfh cells notable expansion	Elicit potent SARS-CoV-2-specific GC B responses and induce robust and specific antibody responses, including neutralizing antibodies.	gG1, IgG2a, IgG2b,	[124]
full SΔ furin mRNA	B220- CD4$^+$ CD44hiCD62L-CXCR5$^+$ Bcl-6$^+$ Tfh cells, B220-CD4$^+$ CD44hiCXCR5$^+$ PD-1hi IL-21$^+$ Tfh cells, B220-CD4$^+$ CD44hiCXCR5$^+$ Bcl-6$^+$ ICOS$^+$ Tfh cells B220-CD4$^+$ CD44^{hi}CXCR5$^+$ PD-1^{hi}	Elicit potent SARS-CoV-2-specific GC B responses and induce robust and specific antibody responses, including neutralizing antibodies.	IgG1, IgG2a, IgG2b,	[130]
mRNA-1273	IL-21$^+$ CXCR5$^+$ PD−1$^+$ ICOS$^+$ Tfh cells expansion.	Induce robust and specific antibody responses, including neutralizing antibodies.	IgA, IgG	[123]
	Protein vaccines			
StriFK-FH002C	PD-1$^+$ CXCR5$^+$ CD4$^+$ Tfh cells expansion	Induce specific antibody responses, including neutralizing antibodies.	IgG, IgG1, IgG2a, IgG2b	[129]
Spike (S) and receptor binding domain (RBD) protein subunit vaccine	CXCR5^{++}BCL-6$^+$ CD4$^+$ CD3$^+$ B220-Tfh cells expansion	Induce specific antibody responses, including neutralizing antibodies.	IgG	[125]
NVX-CoV2373	CXCR5$^+$ PD-1$^+$ CD4$^+$ Tfh cells expansion	Induce specific antibody responses, including neutralizing antibodies.	IgG	[4]
rRBD-AddaVax	B220-CD4$^+$ CD44^{hi}CD62L-CXCR5$^+$ Bcl-6$^+$ Tfh cells, B220-CD4$^+$ CD44hiCXCR5$^+$ PD-1hi IL-21$^+$ Tfh cells B220-CD4$^+$ CD44hiCXCR5$^+$ PD-1hi IL-4$^+$ Tfh cells slight expansion	Delay in eliciting potent SARS-CoV-2-specific GC B responses induces robust and specific antibody responses, including neutralizing antibodies.	IgG1	[130]

*† **Tfh**, follicular helper T cell; **cTfh**, circulating Tfh cell*

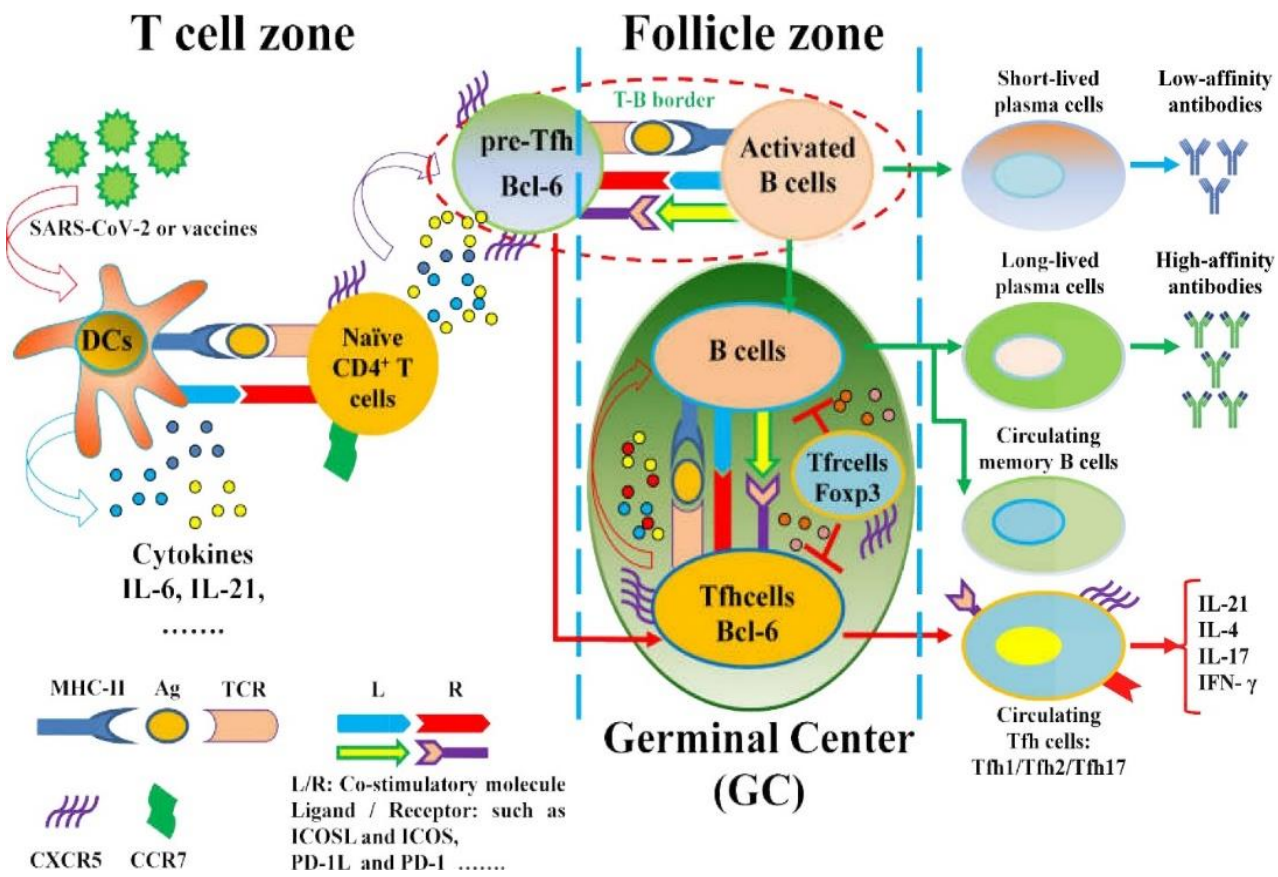

Fig. 2. The involvement of signaling pathways in controlling Tfh cell differentiation and function in SARS-CoV-2 infection with vaccines. When exposed to SARS-CoV-2 or viral antigens, naive CD4+ T cells were activated through APCs (DCs), which are triggered against antigen-specific Pre-Tfh cells by the association of MHC-II molecules between DCs with cognate TCR on CD4+ T cells, and the expression of costimulatory molecules with cytokine production, pre-Tfh cells associate with active B cells at the T-B boundary in the follicular zone, wherein they develop into diverse Tfh cell subtypes that move to the GC, wherein Tfh cells stimulate B cell differentiation and specific antibody production. Reproduced/adapted with permission [131]

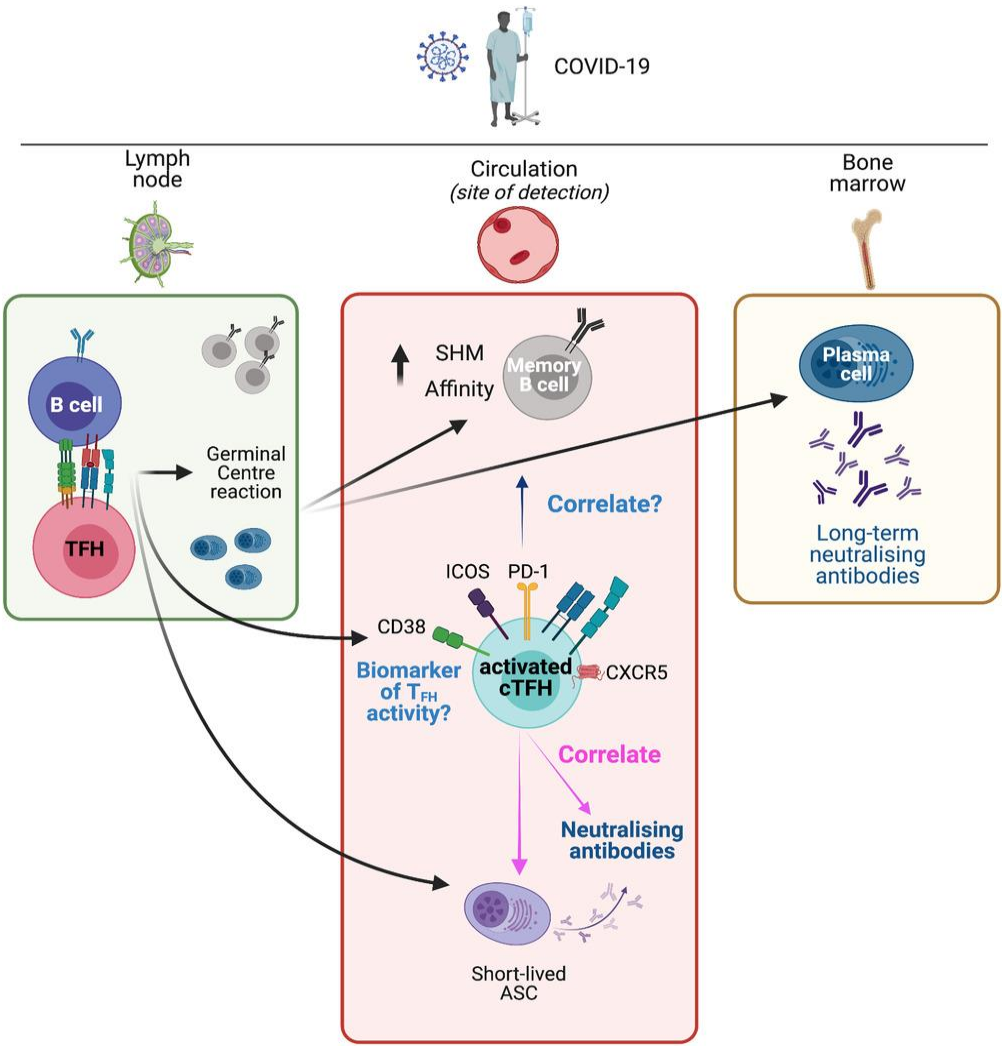

Fig. 3. Lymphoid and circulating TFH responses in COVID-19. SARS-CoV-2 antigen in the lymph nodes activates antigen-specific B cells and TFH cells. Their interaction leads to the initiation of the germinal centre reaction. This results in the development of memory B cells with increased somatic hypermutation (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 rare source of neutralizing antibodies. Concurrently, a population of activated (CD38+, PD-1+, ICOS+) cTfh cells appears in the circulation. This population contains antigen-specific cTfh cells (not depicted). Although memory B cells and ASCs are primarily located in lymphoid tissues, they are typically measured in blood samples, which correlate with activated cTfh cells. Activated cTfh cells also correlate with the development of neutralizing antibodies. These cTfh cells are a potential biomarker of TFH activity in lymphoid tissues. However, it remains to be determined if this population of cTfh cells are predictive of long-term neutralizing antibodies or the development of long-lived plasma cells and the prolonged evolution of the MBC pool. Reproduced/adapted with permission [135].

Rapid advances have been made in designing and developing SARS-CoV-2 vaccines, such as inactivated DNA, mRNA, and specific SARS-CoV-2 proteins [123]. The mRNA-1273 vaccine can effectively activate Th1 and interleukin-21 expressing CXCR5+PD1+ICOS+Tfh cellular responses as well as trigger powerful SARS-CoV-2 neutralizing potential, providing quick protection against SARS-CoV-2 infection in the vertical and horizontal airways of Rhesus Macaques [4]. In contrast to the SARS-CoV-2 recombinant SARS-CoV-2 receptor-binding domain (rRBD) developed for the AddaVax (rRBD-AddaVax) protein vaccine gene, the

SARS-CoV-2 mRNA vaccine gene encodes RBD as well as full-length spike protein efficiently to prompt SARS-CoV-2-specific GC B and Tfh cellular responses, which also enhance specific neutralizing antibody responses in inoculated mice. Surprisingly, the rRBD-AddaVax Vaccination produced a significant proportion of IL-4$^+$ Tfh cells [124]. The BNT162b2 mRNA vaccine against SARS-CoV-2 produced significant AIM$^+$CXCR5$^+$CD45RA-CD3$^+$cTfh cell responses in humans. AIM (activation-induced marker) cells are including CD69$^+$OX40$^+$ or CD69$^+$CD40L$^+$ or CD69$^+$4-1BB$^+$ or OX40$^+$4-1BB$^+$ or CD40L$^+$4-1BB$^+$ or CD40L$^+$4-1BB$^+$ or CD40L [4]. The above results show that SARS-CoV-2 mRNA vaccines can help enhance antigen-specific Tfh cell differentiation and B cell responses, as well as the production of protective immune responses by producing antibodies, making them especially potential for eliciting high-quality adaptive immune responses to regulate as well as eradicate SARS-CoV-2 infection.

Moreover, “target protein vaccines such as the SARS-CoV-2 subunit vaccine (NVX-CoV2373) with both the full-length Spike (S)-protein, StriFK-FH002C, as well as the Spike (S)/receptor binding domain (RBD) protein subunit vaccine remarkably stimulate specific cTfh cell but also GC B cell responses, leading to high SARS-CoV-2” [125-127] neutralizing levels of antibodies (Table 2). Several human clinical studies show that immobilized SARS-CoV-2 vaccinations can produce sufficient high neutralizing levels of antibodies to decrease the rate of patients developing severe COVID-19 [127-129]. Such findings imply that SARS-CoV-2 vaccinations can boost host immune response, increase neutralizing antibody levels, and reduce the death rate of critically ill patients.

Meanwhile, in serious COVID-19 patients, the absence of GC structures lowers Bcl-6+ Tfh cells. Interestingly, SARS-CoV-2-specific Tfh cells significantly increased in moderate and asymptomatic COVID-19 patients. Vaccines also can stimulate Tfh cell differentiation and GC production, including defensive antibody responses.

5. HUMAN CIRCULATING T FOLLICULAR HELPER CELLS (cTfh) RESPONSE AGAINST COVID-19

Activated cTfh (PD-1$^+$ICOS$^+$) [41,43,132-134] with enhanced expression of CD38 [135] but also decreased expression of CCR7 [43] is seen in the blood during acute infection. Such activated PD-1$^+$ICOS$^+$ cTfh cells appear spontaneously throughout the infection but typically decrease 14 days after symptom onset. As a result, antigen-specific T cell tests (activation-induced marker (AIM) and intracellular cytokine staining) were crucial to evaluating SARS-CoV-2-specific cTfh responses throughout recovery. These studies have also shown that S-specific cTfh cells that arise following acute infection [136] remain in convalescent patients for at least 6 months [32], with a half-life of approximately 129 days [50].

The link between cTfh frequencies, morphological and functional polarization, and SARS-CoV-2 serologic responses have been investigated. Most of the PD1$^+$ICOS$^+$ cTfh populations formed during the acute stage of COVID-19 are CXCR3$^+$cTfh1 cells [132,134], similar to influenza infection [137]. Moreover, the investigation of S-specific cTfh showed a completely dominant population of CXCR3$^-$ CCR6$^+$ cells [46,49,136]. Remarkably, the fraction of CXCR3$^-$CCR6$^+$ S-specific cTfh cells during late convalescence (6 months) is more significant than that in initial convalescence (1–2 months) or even during the acute stage [49,136]. Despite the presence of CXCR3CCR6$^+$ cTfh17 cells, antigen-specific cTfh cells from COVID-19 patients routinely release IFN and IL-21, even when IL-17 is not present, according to multiple independent studies [46,48-50,133,136,137]. The development of effective neutralizing antibody responses has been associated with a phenotypic polarization of cTfh. Furthermore, high levels of serum spike binding and neutralizing antibodies were strongly associated with CXCR3$^+$ cTfh1 cells. This was true not only for the overall ICOS$^+$PD-1$^+$ cTfh1 populations (which corresponds to ASC responses and plasma CXCL13) [43,132-134], but also for S-specific cTfh1 [46]. The activation of cTfh1 cells in acute COVID-19 significantly corresponds with both the confirmatory testing and antibody production of RBD-specific IgM antibodies [46]. “The association between cTfh2, cTfh17, and antibody response, on the other hand, varies among cohorts and tests. The discovery of S-specific cTfh led to the conclusion that the occurrence of cTfh2 responses was positively correlated with increasing neutralizing levels of antibodies, whereas S-specific cTfh17 showed a significant negative correlation with neutralizing activity” [46]. Overall, ICOS$^+$PD1$^+$ cTfh2/17 cells were significantly negatively correlated with antibody response in different populations [43,46,133,134]. Therefore, the present

findings show that CXCR3$^+$ cTfh1 cells are a significant correlate of neutralizing as well as overall antibodies towards SARS-CoV-2, but the roles of CXCR3$^-$cTfh2 and cTfh17 cells, as well as the differences between total ICOS$^+$PD-1$^+$ and AIM$^+$ cTfh cells, deserve additional exploration.

Whereas most research has concentrated on S-specific cTfh cells, N and M-specific cTfh cells were also investigated [53,136,138]. The proportion of cTfh cells specific for S, N, and M was shown to be favourably linked to increased plasma neutralization activity with N-specific IgG antibodies [53]. Remarkably, for cTfh cells specific towards various SARS-CoV-2 antigens, polarisation throughout cTfh1, cTfh2, and cTfh17 subgroups has been shown to change [138], but the relevance of this result is still unknown. A possible decrease of TFH cells has also been identified in some patients with acute COVID-19. In particular, GC-B cells and TFH cells were shown to be reduced in lymphoid tissues of a subgroup of deceased COVID-19 patients [45,53]. “Furthermore, a population with cTfh cells expressing cytotoxicity-associated genes, including PRF1 and GZMB (encoding perforin and granzyme B), was enhanced in hospitalized vs. nonhospitalized patients. It was also linked with decreased antibody levels to S” [139]. Such findings contradict the more significant antibodies found in acute COVID-19 [132] because decreased TFH function would be predicted to decrease antibody levels. Such findings highlight the need for more research into the nature and function of TFH cells in acute COVID-19 and whether they correspond to a specific population of patients.

Finally, total stimulation of cTfh cells and associated phenotypic polarisation throughout COVID-19 are indicators of B-cell reaction neutralization (Fig. 3). Furthermore, the phenotypic traits of the different cTfh subgroups, including their link with GC Tfh activities, must be clarified. It is crucial to investigate the potential for cTfh cells as indicators of the formation and recall of humoral immunity to SARS-CoV-2, particularly in developing variants of concern (VOCs) with a higher potential for evasion of humoral immunity. It is essential to investigate the potential for cTfh cells as indicators of the formation and recall of humoral immunity to SARS-CoV-2, particularly in developing variants of concern (VOCs) with a higher potential for evasion of humoral immunity.

6. HUMAN CIRCULATING T-FOLLICULAR HELPER CELLS (cTfh) RESPONSES AFTER SARS-CoV-2 VACCINATION

Vaccination with certified COVID-19 vaccines produces antibody responses linked to infection prevention. Following mRNA vaccination, examination of axillary draining lymph nodes showed substantial GC responses that were maintained for a minimum of 12 weeks following booster vaccination [140]. S-specific Tfh cells were significantly produced at those sites because they were associated with S-specific GC B cells [124,141]. According to an evaluation of associated lymph nodes and blood samples, S-specific cTfh cells show an activated phenotype (CD38+HLA-DR+ICOS+) increase during the first month before reverting to a resting phenotype and then decreasing in frequency. Within a minimum of 60 days, the frequency of S-specific Tfh cells within lymph nodes is majorly consistent [141]. Despite being confined to a limited number of donors, our results indicate that mRNA vaccines trigger significant GC responses, which may highlight the vaccine's outstanding immunological characteristics. Vaccination of naive (previously uninfected) humans produces S-specific cTfh cells [142-148] with such a CXCR3+ phenotype [142] as well as the potential to produce IFN, although without IL-17A [4]. At a minimum, S-specific cTFH cell frequency increases approximately one month after immunization and subsequently decreases, compared with S-specific TH1 cell frequency, which remains constant for a minimum of 6 months [144]. At two weeks following Vaccination, the number of S-specific cTfh cells and S-specific conventional CD4+ TH1 cells corresponds to neutralizing antibodies against Spike and VOCs and S with RBD-specific MBC responses [86]. This suggests that cTfh cells serve as indicators again for the production of neutralizing antibodies with MBCs and subsequent spike vaccination. Vaccinating people who have recovered from COVID-19 usually results in higher S-specific cTfh responses versus naïve people [4]. Significantly, the number of S-specific cTfh cells among recovering patients before Vaccination increases significantly post-vaccination, neutralizing antibody levels observed in both the ancestor's viruses and VOC [4,143]. Therefore, it indicates that cTfh cells play a significant function as biomarkers of innate immunity after SARS-CoV-2 vaccination. This will be critical to better classifying and correctly understanding lymphoid and circulating Tfh cell responses after

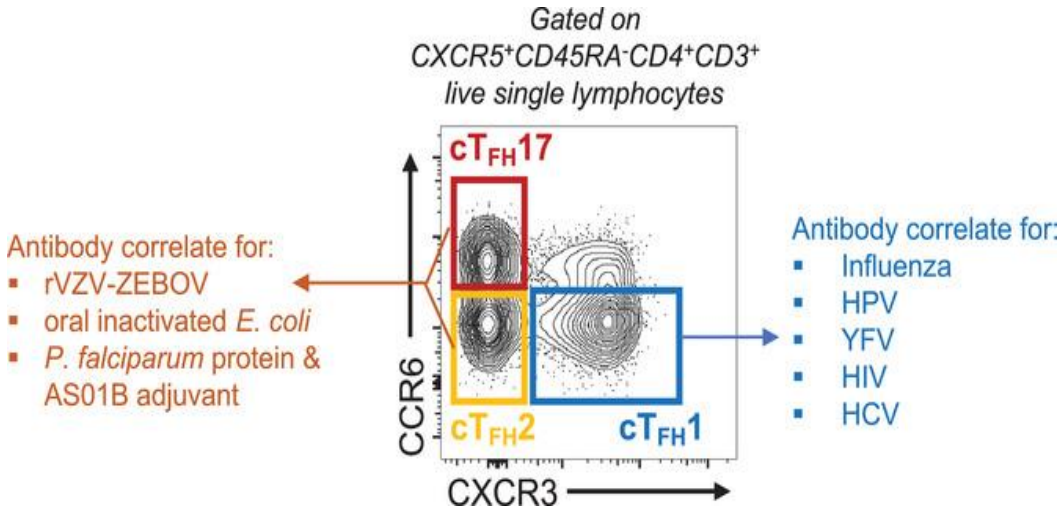

Fig. 4. Circulating TFH subsets as correlates of antibody responses. Flow cytometry visualization of cTfh subgroups depending on CXCR3 as well as CCR6 expression and its relationship with antibody responses in different configurations. Reproduced/adapted with permission [135].

administration of various newly invented vaccine systems, as well as whether/how they differently activate such responses, particularly in the context of heterologous prime-boost Vaccination.

7. FUTURE PROSPECTIVE OF cTfh-MEDIATED WITH OTHER THERAPIES FOR SARS-CoV-2 INFECTION

The future prospects of cTfh-mediated therapies in the context of SARS-CoV-2 infection are promising, particularly in light of the remarkable adaptability of these cells to emerging variants. As our understanding of cTfh cell dynamics evolves, it becomes increasingly clear that their ability to modulate immune responses could be harnessed in conjunction with other therapeutic approaches. Integrating cTfh-mediated strategies with innovative hormonal therapies may enhance vaccine efficacy and promote robust long-term immunity. By targeting the plasticity of cTfh cells, future interventions could be designed to optimize immune responses to current variants and potential future mutations of SARS-CoV-2, thereby improving clinical outcomes and overall public health strategies.

8. CONCLUSION

There are strong indications that the cTfh study can provide valuable information on the quantitative and qualitative characteristics of neutralizing antibodies against SARS-CoV-2 infection during Vaccination (Fig. 4). This evidence suggests that cTfh cells, specifically the cTfh1 subgroup, were valuable indicators for producing neutralizing antibodies and MBCs targeting both wild-type Spike and VOCs. However, critical issues exist regarding the involvement of CXCR3/CCR6 cTfh subgroups in generating neutralizing antibodies or maintaining spike-specific cTfh memory. To obtain specific results on Tfh's ability to produce significant neutralizing antibody responses via Vaccination, it will be crucial to understand why only specific cTfh subgroups correlate positively to antibody levels and how Tfh quality may be modified using innovative vaccine platforms. Furthermore, whereas the cTfh initiation, as well as the frequency of antigen-specific cTfh cells, are biomarkers of neutralizing antibodies in the acute as well as early phases of COVID-19 infection but also Vaccination, it is unknown whether cT cells indicate the development of long-lived plasma cells or long-term neutralizing antibodies. Researchers should start understanding more about the duration of GC TFH responses and their relationship to cTFH frequencies and phenotype as research aims to comprehend the immunologic processes underpinning the persistent development of the MBC pool [145-147]. Resolving these concerns is critical to realizing their promise of developing efficient vaccination approaches.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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