FOLLICULAR T CELLS IN PERIPHERAL BLOOD: INCREASING COMPLEXITY AND KEY QUESTIONS

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Follicular helper (Tfh) and follicular regulatory (Tfr) T cells play critical roles in inducing and controlling B cell responses, including the generation of high-affinity humoral immunity, the antibody class-switching, and the prevention of autoreactivity. Successful Tfh responses are linked to robust vaccine-induced neutralizing antibody production and efficient clearance of various pathogens. Conversely, dysregulation of follicular T cells is often linked to autoimmune diseases and allergic reactions. Furthermore, these cells are implicated in the formation of ectopic lymphoid structures (ELS), contribute to certain vascular pathologies, and hold prognostic value in several cancers. Consequently, the analysis of follicular T cell subpopulations in human peripheral blood is increasingly utilized to investigate the mechanisms underlying various diseases. In this opinion article, the current understanding of follicular T cell subsets, their functions, and the evolving methods for analyzing their circulating counterparts in human blood are discussed. In the author's opinion, the central unresolved questions remaining in the field are the precise phenotypic definition of circulating Tfr cell subpopulations, the elucidation of their developmental trajectory from precursors cells to mature regulatory forms, and the identification of their anatomical differentiation niches. The collection and translation of these essential data into reliable cellular signatures for peripheral blood analysis are critical for advancing personalized patient prognosis and developing tailored therapies.

Keywords: follicular helper T cells (Tfh), follicular regulatory T cells (Tfr), circulating follicular T cells, infectious diseases, vaccines, allergies, autoimmune diseases, oncology

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ФОЛЛИКУЛЯРНЫЕ Т-КЛЕТКИ ПЕРИФЕРИЧЕСКОЙ КРОВИ: НАРАСТАЮЩАЯ СЛОЖНОСТЬ И ПЕРВООЧЕРЕДНЫЕ ВОПРОСЫ

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Фолликулярные Т-хелперы (Tfh) и фолликулярные регуляторные Т-клетки (Tfr) играют ключевую роль в индукции и контроле В-клеточных ответов, включая формирование высокоаффинного гуморального иммунитета, переключение классов антител и предотвращение аутореактивности. Успешный ответ Tfh ассоциирован с эффективной выработкой нейтрализующих антител после вакцинации и эффективной элиминацией различных патогенов. В то же время дизрегуляция фолликулярных Т-клеток часто связана с аутоиммунными заболеваниями и аллергическими реакциями. Нарушение их нормального функционирования связано также с формированием эктопических лимфоидных структур (ЭЛС), способствует развитию некоторых сосудистых патологий и имеет прогностическое значение при ряде онкологических заболеваний. В связи с этим анализ субпопуляций фолликулярных Т-клеток в периферической крови человека все чаще используют при изучении механизмов различных заболеваний. В данной статье обсуждаются современные данные о разных типах фолликулярных Т-клеток, их функциях и методах анализа субпопуляций фолликулярных Т-клеток, циркулирующих в крови человека. Согласно мнению автора, центральными нерешенными вопросами остаются точное фенотипическое определение циркулирующих субпопуляций Tfr-клеток, выяснение иерархии их развития от клеток-предшественников до зрелых регуляторных форм и установление их анатомических ниш дифференцировки. Накопление необходимых сведений и их преобразование в надежные сигнатуры для клеток периферической крови необходимы для развития подходов прогнозирования исхода заболевания у отдельных пациентов и разработки персонализированных терапевтических подходов.

Ключевые слова: фолликулярные хелперные Т-клетки, Tfh, фолликулярные регуляторные Т-клетки, Tfr, циркулирующие фолликулярные Т-клетки, инфекционные заболевания, вакцины, аллергия, аутоиммунные заболевания, онкологические заболевания

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Follicular T cells, which localize in B cell follicles and specifically assist in regulating B cell and antibody responses, were first reported in 1999 [1]. Since that time, they have attracted considerable scientific interest, gradually evolving into a field integrated with the understanding of various diseases and pathologies. Association analysis of the risk of development and progression of various diseases with impaired function and imbalance of follicular T cell subpopulations circulating in peripheral blood is increasingly becoming an important tool for prognosis and for establishing potential treatment targets for multiple diseases [2, 3].

The central feature of follicular T cells is that they are driven by the master transcription factor Bcl6 and have elevated levels of the G-protein coupled receptor CXCR5 (Figure, Table). The CXCR5 receptor enables T cells to migrate into B cell follicles of secondary lymphoid organs (SLOs), where stromal

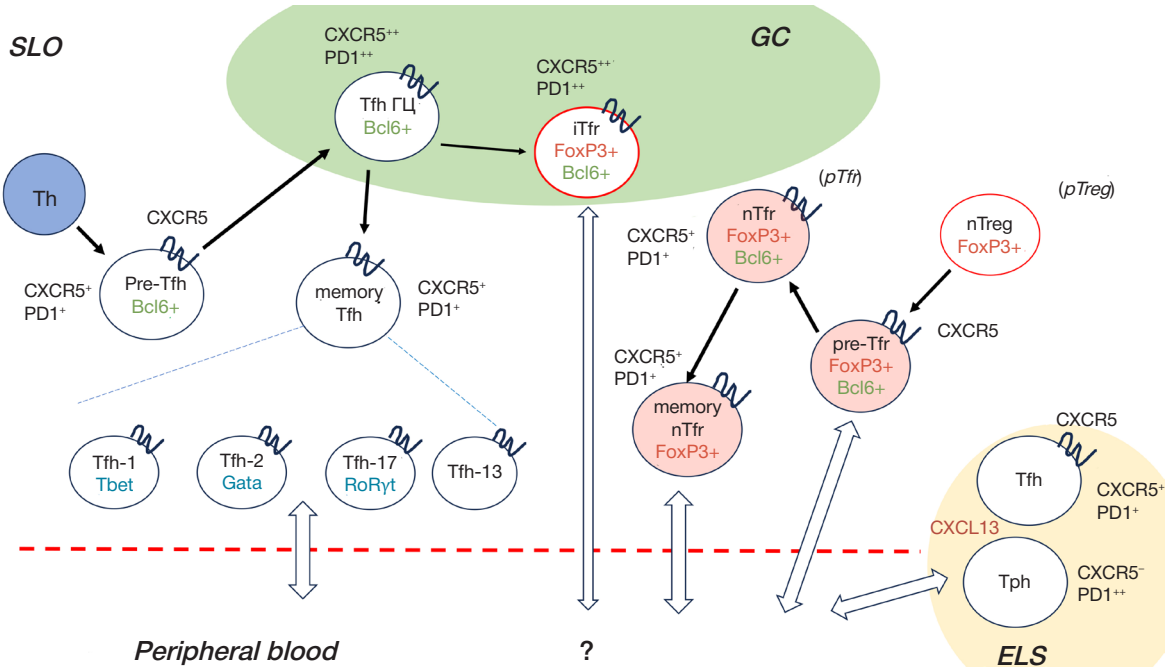

Fig. Major follicular and follicular-like T cells in humans. A schematic describing the generation in secondary lymphoid organs (SLOs) and possible recirculation into peripheral blood of the main follicular T cell subpopulations: helper (Tfh) and regulatory (Tfr). GC — germinal centers. ELS — ectopic lymphoid structures. Memory follicular T cells that acquire the ability to enter the circulation downregulate Bcl6 expression and surface levels of CXCR5 and PD1 compared to T cells in GCs. Peripheral helper T cells (Tph) and, to some extent, Tfh accumulate in ELS, where they produce the chemokine CXCL13, which can recruit B cells

cells express the CXCR5 chemokine ligand CXCL13. Two major types of follicular T cells that differentiate in response to antigenic challenge from CD4⁺ helper T cells (Th) and regulatory T cells (Tregs) are called follicular helper T cells (Tfh) and follicular regulatory T cells (Tfr) correspondingly.

Tfh cells are critical for supporting long-term, high-affinity B cell and antibody responses, as they are essential for germinal center (GC) reactions in SLOs. Tfh cells selectively help GC B cells that present the highest density of cognate peptide–MHCII complexes via T cell receptor recognition, delivering essential signals (CD40L, cytokines IL-21, and IL-4). These interactions ensure the survival and proliferation of high-affinity B cell clones and therefore drives GC B cells affinity maturation. Furthermore, Tfh support immunoglobulin class-switching, memory B-cell formation, and differentiation of B cells into plasma cells [4].

In contrast to Tfh, Tfr cells are involved in the tolerance control of B cell responses. In addition, Tfr cells suppress B cell isotype switching to IgA and IgE as well as differentiation into plasma cells. They also play an important role in fine-tuning B cell affinity maturation [5].

While the majority of Tfh cells are known to differentiate from foreign antigen-specific Th cells, the precursors of Tfr cells have long been suggested to be thymically-derived Tregs that express transcription factor FoxP3 and are specific to autoantigens or in some cases peripheral Tregs (pTregs, Th cells that upregulate FoxP3 in the periphery). However, more recent studies have also demonstrated the upregulation of FoxP3 and the formation of Tfr cells from Tfh cells in mice and human tonsils. These cells are now called induced Tfr (iTfr) [6, 7] (Figure, Table).

Given the critical role of Tfh and Tfr cells in regulating B cell responses, forming memory B cells, and generating high-affinity humoral immunity, their contribution is extensively studied in both animal models and in human diseases. The functions of Tfh cells have been studied in detail using animal models of various chronic and acute infections [2, 3]. In parallel, human studies have examined their roles in infections including Streptococcus pyogenes, malaria, HIV, flaviviruses, COVID-19, and other infections [2, 3, 8]. Vaccination studies in humans, particularly following seasonal influenza and SARS-CoV-2 mRNA immunization, have further assessed Tfh cell responses in relation to neutralizing antibody outcomes [2, 3, 9]. Dysregulation of follicular T cells is also a major focus of investigation in autoimmune diseases, spanning both autoantibody-mediated and T cell-dependent pathologies, as well as in allergies. Furthermore, Tfh cells have been linked to the development of a number of cardiovascular pathologies, as well as to a positive prognosis in certain cancers when present within the tumor [2, 3, 9, 10].

The analysis of follicular T cell dynamics in humans during the normal or pathological immune responses has been carried out predominantly on the subpopulations that circulate in peripheral blood. Since GC Tfh cells are confined to SLOs, the majority of blood-circulating CXCR5⁺ T cells represent Bcl6- memory Tfh cells that have partially downregulated expression of CXCR5 (Figure, Table). These circulating Tfh (cTfh) can be further classified into functional subsets Th1-, Th2-, and Th17-associated, based on chemokine receptor expression (CCR6, CXCR3) and their underlying transcription factor profiles (Table) [11]. Additionally, cytokine profiling has identified IL-13⁺ cTfh cells, a subset associated with IgE with high-affinity to allergens [12].

Notably, human cTfh cells themselves express high levels of CXCL13. This has led to the hypothesis that cTfh cells that access peripheral tissues may help initiate ectopic lymphoid structures (ELS) by creating a chemokine gradient, which attracts B cells and promotes further lymphoid organization. However, recent studies have also revealed enrichment of a distinct CXCL13⁺ T-cell subpopulation with a CXCR5⁻ PD1^{high} IL-21⁺ phenotype in ELS. These cells, named peripheral helper T cells (Tph), are phenotypically similar to Tfh and can also support B-cell responses despite lacking the CXCR5 receptor. Furthermore, a strong association has been established between the circulating Tph and diseases such as rheumatoid arthritis and systemic lupus erythematosus. Current evidence does not clarify the cellular origin of Tph [2, 3]. Nevertheless, Tph have now become a standard component in the analysis

Table 1. Major types of follicular and follicular-like T cells that regulate B cell responses.

Cell type	Precursor cells	antigen	Major TF	Cytokines	Surface markers	Localization	Peripheral Blood
Pre-Tfh	Th	foreign antigen	Bcl6+		CXCR5+ PD1+ ICOS+	SLO	None
Tfh GC	Pre-Tfh	foreign antigen	Bcl6+	IL21 IL4 CXCL13	CXCR5++ PD1++ ICOS++	GC in SLO	None
Tfh memory cells	Tfh GC	foreign antigen	Bcl6-	CXCL13	CXCR5+ PD1+ ICOS+	follicles in SLO/ELS	cTfh
Tfh-1			Tbet+	IFNγ	CXCR3+ CCR6-	SLO	cTfh-1
Tfh-2			Gata+	IL4, IL5	CXCR3- CCR6-		cTfh-2
Tfh-17			RoRγt+	IL17, IL22	CXCR3- CCR6+		cTfh-17
Tfh-13		allergen		IL4, IL13			cTfh-13
Tph			Blimp1+ MAF	IL21 CXCL13	CXCR5- PD1++ CR2	ELS/inflamed tissues	cTph
Pre-Tfr	nTreg	auto-antigen	Bcl6+ FoxP3+		CXCR5+ PD1+ ICOS+ CD45RA+	SLO	None
Pre-Tfr memory cells	Pre-Tfr?		Bcl6- FoxP3+		CXCR5+ PD1+ ICOS+ CD45RA+	SLO	cPre-Tfr
nTfr	Pre-Tfr	auto-antigen	Bcl6+ FoxP3+ Blimp1+	IL10	CXCR5+ PD1+ ICOS+ CD45RA- CD25high	follicles in SLO, around GC	None
nTfr memory cells	nTfr	auto-antigen	Bcl6- FoxP3+		CXCR5+ PD1+ ICOS+ CD45RA- CD25high	SLO	cTfr?
iTfr	Tfh GC	auto-antigen	Bcl6+ FoxP3+		CXCR5++ PD1++ CD38+	GC	?

Note: GC — germinal centers, TF — transcription factors, SLO — secondary lymphoid organs, ELS — ectopic lymphoid structures.

of peripheral blood follicular T lymphocytes in various conditions [11].

The somewhat delayed discovery of FoxP3$^+$ Tfr cells necessitated reassessment of the earlier analysis of circulating follicular T cells, in which all CXCR5$^+$ T cells were presumed to be of Tfh origin. CD4$^+$ CXCR5$^+$ FoxP3$^+$ circulating Tfr cells were first identified in murine blood shortly after immunization [13]. Similarly, human studies have demonstrated a significant increase in circulating Tfr frequency following vaccination [9]. These cells are now understood to represent a circulating memory Tfr population (cTfr), capable of long-term persistence *in vivo* and potential recruitment into SLO upon rechallenge. The important role of Tfr in immune response regulation warrants significant attention to their abundance in peripheral blood. A key predictive parameter is believed to be the cTfr/cTfh ratio, which has been shown in some studies to be more predictive of dysregulated antibody responses than either subset alone [14].

More in-depth analysis of circulating Tfr has now revealed that a significant fraction of CD4$^+$CXCR5$^+$FoxP3$^+$ cells in the blood are CD45RA$^+$ cells that formed in SLOs in response to immunological challenge but represent an intermediate, not fully differentiated form of Tfr. Referred to as "pre-Tfr," these long-lived, recirculating cells can, upon stimulation, mature into potent regulators of B cell responses and also acquire some capacity for wound repair [9]. In addition to these pre-Tfr, more mature "effector-like" CD45RA$^-$CD4$^+$CXCR5$^+$FoxP3$^+$ cTfr cells are found in peripheral blood (Figure, Table).

One of the recent studies has separately assessed these Tfr subpopulations in the peripheral blood of COVID-19 patients. This was achieved using high-dimensional mass cytometry (CyTOF), which enabled the simultaneous analysis of multiple immune cell types in patient samples. The approach revealed extensive correlative networks between various cellular parameters, including the negative associations between cTfr levels and populations such as plasma cells or Tph cells [10]. Collectively, accumulating evidence supports the establishment of multiparametric signatures describing the diversity of follicular T cells in the blood, which have prognostic value and are indicative of specific dysfunctions in B cell and antibody responses. Initially, generating such signatures will require data from single-cell transcriptomics/proteomics, potentially combined with CyTOF. This could later be adapted into a reduced panel for flow cytometry analysis.

While increasing resolution of analysis of follicular T cells in peripheral blood deepens our understanding of ongoing disease processes, the portrait of circulating Tfr cells remains

incomplete. The recent identification of iTfr cells, which arise in SLOs from Tfh cells and help contract GC responses, raises important questions. Does this foreign antigen-specific Tfr subset enter the circulation? If this is the case, how can we differentiate between iTfr, which suppress foreign antigen/pathogen-driven B-cell responses, and nTfr (of nTreg origin), which are biased toward autoantigen recognition and control of autoreactive B-cell responses? We propose that future studies should explicitly investigate the presence of iTfr in human blood and define a molecular signature to distinguish circulating iTfr from their nTfr counterparts. Furthermore, research is needed to determine the stability of the Treg phenotype in iTfr cells.

Another key question is whether circulating Tfr cells retain a molecular imprint of their tissue of origin or a homing bias toward specific organs. Tissue-resident Tregs have been shown to acquire specialized receptor profiles that guide their localization [15]. Profiling the chemokine receptor and adhesion molecule signatures imprinted during Tfr differentiation in SLO could reveal their site of formation and predict their preferred tissue destinations. Such insight would further clarify Tfr specialization and function across different physiological and pathological contexts.

CONCLUSION

To summarize, within the past decade, significant progress has been made in dissecting various types of follicular T cells, their development, function, and association with humoral responses to pathogens, allergies, and various autoimmune diseases. However, despite accumulated knowledge, it is evident that intensive research is required to develop a phenotypic and functional profile of human peripheral blood follicular T cells and their interactions with other immune cell types. In particular, two key questions remain unresolved: defining the molecular signatures of various Tfr cell subpopulations in blood that originate from different precursor cells, and elucidating their tissue of origin. It is anticipated that the development of such cellular biomarker signatures could improve the prediction of immune status and enable personalized therapy selection for each patient.

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