Peripheral follicular helper T cells in acute viral diseases: a perspective on dengue

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Follicular helper T cells (T_{FH}) are a predominant subset of $CD4^+$ T cells specialized in providing help to B cells in germinal centers and necessary to generate T cell-dependent antibody responses. Peripheral T_{FH} (pT_{FH}) are the counterpart of T_{FH} found in the circulation, which resemble T_{FH} in many aspects of their phenotype and function. The $CD4^+$ pT_{FH} subset has received a lot of interest recently because they are easy to access and have the potential to serve as a biomarker for long-lasting humoral immunity. This review will discuss recent findings of pT_{FH} in human acute viral diseases with a focus on dengue infection.

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Four serotypes of dengue virus (DENV1–4) cause an estimated 100 million symptomatic infections each year resulting in a spectrum of disease, ranging from a nonspecific febrile syndrome to dengue fever to more severe illness, dengue hemorrhagic fever (DHF) and dengue shock syndrome [1]. There are many risk factors for developing severe disease, which include viral factors, host genetics, nutritional status and background immunity [2]. Infection with any serotype of DENV for the first time (primary infection) is believed to confer life-long homotypic immunity [3]. Epidemiologic evidence clearly indicates that sequential infection with a heterologous serotype of DENV is a major risk factor for developing DHF [4]. DHF can occur in primary dengue infection of infants born to dengue immune mothers [5]. Cross-reactive antibodies with low avidity and altered T cell immune responses to the current infecting serotype are hypothesized to contribute to severe dengue disease during a second infection with a heterologous serotype [6,7]. A goal of many researchers, therefore, is to develop effective strategies that can induce potent neutralizing antibody and cross-reactive T cell responses to all four DENV serotypes.

T helper subsets

$CD4^+$ T cells can contribute to protection against a number of pathogens. Naive $CD4^+$ T cells activated by dendritic cells (DCs) presenting pathogen-derived peptides can differentiate into memory and effector T cells through a complex process [8]. There are different subsets of antigen-primed $CD4^+$ T helper (T_H) cells classified as T_H1 , T_H2 , T_H9 , T_H17 , T_H22 , T follicular helper cells (T_{FH}) and T regulatory cells (Tregs) [8]. T_H subsets are defined based on the expression of surface molecules, production of cytokines and expression of transcription factors [8]. T_{FH} are a predominant subset of $CD4^+$ T cells specialized in providing help to B cells and necessary to generate T cell-dependent antibody responses in germinal centers (GCs) [9–11]. Peripheral T_{FH} (pT_{FH}) are the counterpart of T_{FH} found in circulation [12]. In this review, we will discuss recent findings of pT_{FH} following influenza and Ebola virus vaccination and natural DENV infection.

Features of T_{FH}

T_{FH} were identified for the first time in human tonsils and are characterized by high expression of the B cell zone-homing chemokine receptor CXCR5 that drives T_{FH} to B cell follicles, including GCs in response to chemokine ligand CXCL13 [9–11]. In addition to CXCR5 expression, T_{FH} express other surface markers such as PD-1, ICOS, OX40 and CD40L [9,10,13,14]. T_{FH} express low levels of the T cell zone-homing chemokine receptor CCR7 [9,10] and the IL-7R α [15]. DCs initiate the differentiation of T_{FH} in the T cell zone in secondary lymphoid organs.

After priming, T_H cells first interact with cognate B cells in the T-B cell border (pre- T_{FH}) and then T_{FH} position in the B cell follicles completing T_{FH} cell differentiation [16]. Furthermore, T_{FH} can form after prolonged antigen presentation by DCs in the absence of antigen presentation by B cells [17]. Already differentiated T_H1 , T_H2 or T_H17 cells can also reprogram to T_{FH} in GCs [14]. The master regulator of T_{FH} differentiation is the transcription factor BCL-6 [18]. Other transcription factors that regulate T_{FH} generation include IRF-4, BATE, c-MAF and the STAT3 [19–22]. BLIMP-1 can modulate T_{FH} generation by suppressing BCL-6 expression [23]. IL-21 is considered the hallmark cytokine secreted by T_{FH} . IL-21 helps B cells secrete antibodies and can also act in an autocrine manner to generate T_{FH} [24,25]. In addition to IL-21, T_{FH} can secrete IL-4, IL-10 and IFN- γ [26,27].

T_{FH} function

The principal function of T_{FH} is to provide help to B cells in a cell-to-cell contact manner through ligands/receptors such as CD40L and ICOS and cytokines such as IL-4 and IL-21 in secondary lymphoid organs [13,24,26,28,29]. T_{FH} are critical for GC formation and maintenance of GC responses [23,30–32]. T_{FH} provides signals for B cell survival and proliferation [33,34], support memory B cell differentiation [35], Ig isotype class switching [36] and plasma cell differentiation [24]. T_{FH} can kill B cells that fail to present cognate antigen in the absence of prosurvival signals via Fas–FasL interactions [14].

T_{FH} in the circulation

Germinal center T_{FH} (GC T_{FH}) share many phenotypic and functional properties with $CD4^+ CXCR5^+$ T cells in the circulation (p T_{FH}) but there is considerable debate about cell surface markers that clearly define p T_{FH} . Given the ease of access to p T_{FH} and their potential to serve as a biomarker for monitoring antibody responses to vaccination or infection, several studies in humans have evaluated p T_{FH} in the last decade. The majority of p T_{FH} express CXCR5 and are in a quiescent state [37] with minimal to no expression of BCL-6 [12,14,27,38]. Memory GC T_{FH} can exit the GC, express markers similar to central memory T_H ($CCR7^+ CD62L^+$) and circulate in different organs including the spleen, lymphoid nodes, bone marrow and blood [27,39]. Based on the expression of CCR7, it is possible that p T_{FH} are memory GC T_{FH} found in the circulation [9,10]. Some studies suggest that p T_{FH} may represent bona fide GC T_{FH} in the circulation while others suggest they are a distinct T cell subset [40,41]. Circulating p T_{FH} are further classified into three main subpopulations based on the expression of the chemokine receptors CXCR3 and CCR6, as p $T_{FH}1$ ($CXCR3^+ CCR6^-$), p $T_{FH}2$ ($CXCR3^- CCR6^-$) and p $T_{FH}17$ ($CXCR3^- CCR6^+$) [12,37]. Expression profiling of transcription factors and cytokine production demonstrated that p $T_{FH}1$ express T-bet and produce IFN- γ ; p $T_{FH}2$ express GATA3 and produce IL-4, IL-5, and IL-13 and p $T_{FH}17$ express ROR γ T and produce IL-17 and IL-22. The p $T_{FH}17$ subset is the main producer of IL-21 with lower production by other subsets [12]. This classification is not intended for GC T_{FH} since very few GC T_{FH} produce cytokines other than IL-21 and IL-4 [19,42]. *In vitro* studies have found varying capacity of p T_{FH} subsets to help naive B cells – p $T_{FH}2$ and p $T_{FH}17$ subsets efficiently help B cells while the p $T_{FH}1$ subset is less efficient at inducing antibody secretion from B cells [12,43,44]. Furthermore, p $T_{FH}2$ induce naive B cells to produce IgG and IgE isotype antibodies while the p $T_{FH}17$ subset induces IgG and IgA isotype secretion from naive B cells [12].

Other follicular T cells

The heterogeneity of T_{FH} has been evaluated in the last 5 years and new subsets have been identified. T follicular regulatory cells (T_{FR}) express some Treg-related molecules including CD25, CTLA-4, IL-10, TGF- β and the transcription factor FOXP3 in addition to T_{FH} -related molecules CXCR5, PD-1, ICOS and BCL-6 allowing the cells to migrate to the GCs [45]. T_{FR} however lack expression of CD40L, IL-4 and IL-21, do not provide help to B cells and restrain humoral immune responses [46–48]. T_{FR} form after infection or immunization and can be derived from thymus-derived (natural) FOXP3 $^+$ Tregs [46–48] and from naive T cells [49]. The ratio between T_{FH} and T_{FR} is important in the GC reaction [50,51]. A potential role proposed for T_{FR} is to control the magnitude of the GC reaction [46–48]. T_{FR} can be detected in blood (p T_{FR}) but these cells are not fully competent [52,53]. Other human follicular T cell populations that participate in the regulation of antibody responses include follicular CD8 T cells [54], follicular helper natural killer T (NKT $_{FH}$) cells [55,56], and follicular gamma delta ($\gamma\delta$) T cells [57]. Follicular CD8 T cells, follicular helper natural killer T and follicular $\gamma\delta$ T cells have not been studied during human acute viral infections and are therefore not covered in this review.

T_{FH} have been implicated in different immune system disorders [58]. An increase in the frequency of T_{FH} is associated with autoimmunity and T cell lymphoma while a decrease is associated with immunodeficiency [58].

In human chronic viral diseases, pT_{FH} have been evaluated following Hepatitis B [59], Hepatitis C [60] and HIV infections [44,61]. Circulating T_{FH} have recently been studied after influenza and Ebola vaccination and natural DENV infection.

Peripheral follicular helper T cell responses following vaccination

Influenza vaccination

Protection against influenza viral disease can be mediated by neutralizing antibodies specific to the hemagglutinin (HA) protein induced by natural infection or vaccination [62] although cellular immunity may also contribute to protection. To prevent seasonal influenza, an annual vaccination is recommended for all persons older than 6 months of age [63]. However, there are still some gaps in eliciting vaccine efficacy in pregnant women, children between 6 months to 5 years and older adults [64]. Many studies have reported increased pT_{FH} [65–68] and pT_{FR} (FOXP3⁺ and CD25⁺) frequencies postvaccination [69,70]. After vaccination with the standard dose of influenza vaccine, reduced frequencies of pT_{FH} were found in the peripheral blood of elderly individuals [71,72], with a decreased ability to provide B cell help [71]. However, when a high dose influenza vaccine was administered in elderly individuals, an increased frequency and activation of pT_{FH} was observed. The frequency of pT_{FH} correlated with the frequency of plasmablasts in this study [73]. In the elderly, therefore, modulation of the dose of influenza vaccine could improve the induction and activation of pT_{FH} responses. Some studies suggest that CD4⁺ T cells or pT_{FH} do not express BCL-6 [65,74] while others suggest pT_{FH} (ICOS⁺CD38⁺) express mRNA and/or protein for BCL-6 [69,71]. Administration of an inactivated influenza vaccine (TIV) increased frequencies of the pT_{FH}1 subset [65,74] with no increase in the frequencies of pT_{FH}2 and pT_{FH}17 T cells [65]. Although pT_{FH} have reactivity to multiple influenza antigens, the majority of pT_{FH} are specific to HA antigens [66,69,75]. The frequency of pT_{FH} specific for influenza antigens was associated with circulating plasmablast frequencies, the frequency of CD21^{lo}CD27⁺ and CD21^{hi}CD27⁺ memory B cells and protective antibody responses [66,67,69]. The frequency of pT_{FH} (ICOS⁺PD-1⁺CXCR3⁺) contributed to the generation of high-avidity antibodies after the administration of inactivated influenza vaccine [66]. However, pT_{FH} frequencies correlated with pre-existing antibody titers and not with the induction of primary antibody responses. In this study, purified CD4⁺ICOS⁺CXCR5⁺CXCR3⁺ T cells were most efficient at inducing memory B cells to differentiate into plasma cells to produce influenza-specific antibodies [65].

Ebola vaccination

Preclinical studies in nonhuman primates and mouse models have suggested that antibodies and CD4⁺ T cells mediated by a vaccine candidate based on recombinant vesicular stomatitis virus (rVSV) expressing the Zaire Ebola virus (ZEBOV) glycoprotein are involved in protection against Zaire Ebolavirus [76,77]. A single dose of the recombinant vesicular stomatitis virus-ZEBOV vaccine candidate induced ZEBOV-GP-specific pT_{FH} at day 28 and pT_{FH} frequencies were dependent on the vaccine dose and maintained at day 56. Interestingly, in this study, the pT_{FH}17 subset was the predominant subset induced followed by pT_{FH}2 and pT_{FH}1 cell subsets. The frequency of pT_{FH}17 but not pT_{FH}2 or pT_{FH}1 subsets correlated with antibody titers at day 28 postvaccination [78].

Peripheral follicular helper T cell responses following DENV infection

Fewer published studies have evaluated pT_{FH} during DENV infection. Rivino *et al.* identified a small population of DENV-specific CD4⁺CD45RA⁺CXCR5⁺ memory T cells that secreted hallmark T_{FH} cytokine IL-21 by stimulating convalescent peripheral blood mononuclear cells (PBMC) with dengue peptides. IL-21 was only produced by CXCR5⁺CD4⁺ T cells while both CXCR5⁺ and CXCR5⁺CD4⁺ T cells produced IFN- γ . The ability of DENV-specific pT_{FH} to provide B cell help was not assessed in this study; however, the results support other *in vitro* studies, which clearly demonstrate that CD4⁺CXCR5⁺CD45RA⁺ T cells in the circulation can help B cells to produce antibody [79]. This study differs from recently published work which suggest that CXCR5⁺PD1^{hi}CD4⁺ T cells express factors including IL-21, CXCL13, ICOS and MAF and can provide help to B cells in patients with rheumatoid arthritis [80].

Vivanco-Cid *et al.* measured IL-21 levels in the sera of patients undergoing acute DENV infections (1–7 days after disease onset) and in early convalescence (8–10 days after disease onset) and found elevated levels in acute sera compared with healthy controls. Interestingly, they found the highest levels of IL-21 in sera of patients obtained during the early convalescent phase of primary DENV infection. A positive correlation between IL-21 levels and production of DENV-specific IgM and IgG antibodies was reported. In addition, significant differences in IL-21

Figure 1. Differentiation of follicular helper T cells during dengue virus infection. Infection of dermal DCs by DENV leads to the activation of RIG-1 and MDA-5 with type 1 IFN (IFN- α/β) and IL-27 production. DCs that present dengue peptides on MHC class II complexes prime naïve DENV-specific CD4 T cells to proliferate and differentiate into T_{FH} which express CXCR5, PD-1, BCL-6 and IL-21. Cross-talk between T_{FH} and cognate B cells requires IL-21 and helps to stimulate IgM and IgG antibody secretion. DC: Dendritic cell; DENV: Dengue virus; ICOS: Inducible T cell co-stimulator; ICOSL: ICOS ligand; TCR: T cell receptor.

levels were detected based on the clinical phase and the severity of DENV infection. Their findings suggest that IL-21 may have a protective role supporting B cell antibody production since T_{FH} are the main producers of IL-21 [81].

Our group recently evaluated frequencies of pT_{FH} in PBMC from Thai children. PBMC were collected during and after acute DENV infection at febrile (fever days -5 to -1), critical (fever days 0 to +1), early convalescence (fever days +3 to +8), and healthy (6-months to 2-years postenrollment) time points. We detected elevated frequencies of activated pT_{FH} (PD-1^{hi} pT_{FH} and PD-1⁺CD38⁺ pT_{FH}) in PBMC obtained during acute DENV infection with the highest frequencies of pT_{FH} activation detected during the critical phase of illness. The numbers of activated pT_{FH} were higher in PBMC obtained from patients with secondary compared with primary infections and in PBMC from patients with more severe disease [82]. We further phenotyped pT_{FH} in a subset of PBMC based on their expression of CXCR3 and CCR6 and the majority of pT_{FH} expressed CXCR3 but not CCR6. To determine whether there was a correlation between pT_{FH} frequencies and B-cell activation *in vivo*, we measured the frequency of plasmablasts at acute time points and found a positive correlation between the frequency of pT_{FH} activation and the frequency of plasmablasts. The clinical studies using samples from dengue patients are encouraging and warrant larger studies to evaluate the function of pT_{FH} in more detail.

DENV induces the generation of T_{FH}

A recent *in vitro* study found that DENV could drive the formation of pT_{FH} and subsequent antibody production [83]. DENV replication in human monocyte-derived DCs (mdDCs) and dermal DCs activated RIG-I and MDA-5 cytoplasmic RNA sensors leading to IFN- α/β transcription and IFN- α/β R activation. DENV infection specifically phosphorylated STAT1 to modulate IFN- α/β R signaling and drive IL-27 production. Co-culture of DENV-infected mdDCs with naïve CD4⁺ T cells lead to increased expression of CXCR5, PD-1 and BCL-6, and the formation of IL-21 secreting T_{FH} (Figure 1). T_{FH} formation was abrogated by neutralizing antibodies against IL-27, indicating that T_{FH} polarization was dependent on IL-27. Co-culture of the differentiated T cells with CD19⁺ B cells induced the secretion of IgM and IgG antibodies [83]. This study concluded that the infection of DCs with DENV drives T_{FH} polarization, and subsequently supports antibody production.

Conclusion & future perspective

The data to date suggest that pT_{FH} subsets and the key hallmark cytokine IL-21 are detected in the circulation during acute natural DENV infections (Figure 2). Our data found the majority of pT_{FH} detected in the peripheral blood during the critical phase of acute dengue infection express markers associated with the inefficient helper

Figure 2. Peripheral follicular helper T cells subsets during dengue virus infection. The expression of chemokine receptors CXCR3 and CCR6 defines three subsets of pT_{FH}: pT_{FH}1 (CXCR3⁺CCR6⁻), pT_{FH}2 (CXCR3⁻CCR6⁻) and pT_{FH}17 (CXCR3⁻CCR6⁺). The main subset produced during the critical phase of DENV infection is pT_{FH}1, which has been associated with inducing nonspecific B-cell activation. Frequencies of pT_{FH} are higher in PBMC obtained from patients with secondary (2°) compared with primary (1°) dengue infection as well as in patients with DHF compared with DF during the critical phase of infection. IL-21 is elevated during clinical course of DENV infections. DF: Dengue fever; DHF: Dengue hemorrhagic fever; PBMC: Peripheral blood mononuclear cell.

pT_{FH}1 subset while other pT_{FH} subsets (pT_{FH}2 and pT_{FH}17) more efficient at inducing naive B cells to secrete Ig and isotype switch were less dominant [12,37]. These results are similar to findings in patients with acute malaria [84]. It is tempting to suggest that vaccination strategies should focus on generating more efficient helper T cells, namely pT_{FH}2 or pT_{FH}17 subsets. Next generation adjuvants that enhance antibody responses are associated with increased frequencies of T_{FH} in animal models [85–88]. A number of questions, however, still need to be first answered as T_{FH} have also been implicated as key drivers in many autoimmune diseases [89]. Specifically, in response to DENV infection or vaccination we need to know:

- Does the increased frequency of pT_{FH}1 subsets detected during acute dengue impact the quality of DENV-specific neutralizing antibodies generated during primary as well as secondary DENV infections?
- Do pT_{FH} frequencies correlate with the ability to generate DENV-specific memory B-cells?
- What about the specificity of the T_{FH}? Is recognition limited to specific nonstructural proteins? Are pT_{FH} capable of producing IL-21 during natural DENV infection?
- Which immunoglobulin isotypes are induced by DENV-specific pT_{FH} subsets?
- What is the role of T_{FR} in DENV infection?
- Are pT_{FH} and pT_{FR} detected following vaccination with live attenuated, protein-based, inactivated vaccine candidates? Do pT_{FH} frequencies correlate with vaccine-induced antibody responses? Which pT_{FH} subsets are induced after DENV vaccination?

Considerable progress has been made with phenotyping and characterizing pT_{FH} during acute viral infections. The number of studies on dengue and pT_{FH} are limited, but the initial findings are encouraging and highlight the potential for pT_{FH} to serve as a biomarker for eliciting strong antibody responses in response to natural dengue infection. Given the number of ongoing Phase II and III dengue vaccine trials, using pT_{FH} subsets as a biomarker of vaccine efficacy is an attractive possibility.

Executive summary

- Follicular helper T cells (T_{FH}) are a distinct subset of effector T cells characterized by the expression of chemokine receptor CXCR5, the transcription factor BCL-6 and secretion of the cytokine IL-21 that are predominantly located in germinal centers in secondary lymphoid organs.
- Peripheral T_{FH} (pT_{FH}) characterized by CXCR5 and PD-1 expression are the counterpart of T_{FH} found in circulation.
- T_{FH} and pT_{FH} provide help to B cells to generate T cell-dependent antibody responses.
- Three pT_{FH} subsets can be distinguished based on the expression of chemokine receptors CXCR3 and CCR6 as pT_{FH}1 (CXCR3⁺CCR6⁻), pT_{FH}2 (CXCR3⁻CCR6⁻), and pT_{FH}17 (CXCR3⁻CCR6⁺). pT_{FH}2 and pT_{FH}17 subsets efficiently help B cells.
- T_{FR} are a recently described cell population that can regulate the antibody production. Influenza vaccines induce pT_{FR} and pT_{FH}. Influenza hemagglutinin-specific pT_{FH} produce IL-21 and express markers associated with the pT_{FH}1 subset.
- Ebola vaccine induces glycoprotein-specific pT_{FH}, and expresses markers associated with pT_{FH}17 which correlate with antibody responses.
- DENV induces the differentiation of naive CD4 T cells into T_{FH} and stimulates antibody production from naive B cells.
- High frequencies of pT_{FH} detected during the critical phase of dengue contribute to disease evolution.
- The majority of pT_{FH} found during the critical phase of dengue illness express markers associated with the pT_{FH}1 subset.
- IL-21 serum levels are elevated during the early convalescent phase of primary DENV infections and correlate with the production of DENV-specific IgM and IgG antibodies.

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