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# Definition of B cell helper T cells in rheumatoid arthritis and their behavior during treatment

Charlotte Lucas<sup>1,2,3</sup>, Aleth Perdriger<sup>2</sup>, Patricia Amé<sup>1,3</sup>

<sup>1</sup>INSERM U1236, Univ Rennes, Etablissement Français du Sang Bretagne, LabEx IGO, F-35000 Rennes, France; <sup>2</sup>CHU de Rennes, Service de rhumatologie, F-35000 Rennes, France; <sup>3</sup>CHU de Rennes, laboratoire SITI, F-35000 Rennes, France

## Correspondence:

Patricia Amé

INSERM U1236

Faculté de Médecine

2 Avenue du Pr Léon Bernard

F- 35043 Rennes

Phone number: +332.232.348.27

[patricia.ame@univ-rennes1.fr](mailto:patricia.ame@univ-rennes1.fr)

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## Abstract

**Background:** Immunopathogenesis of rheumatoid arthritis (RA) is not yet clearly defined. Besides known B-cell involvement, RA development and evolution involves CD4<sup>+</sup> T helper cell homeostasis dysregulation. Imbalance between Th17 cells and regulatory T cells have been reported and may contribute to sustained inflammation. Since the last decade, increasing reports focused on a newly described CD4<sup>+</sup> T helper cell subset, called T follicular helper (Tfh) cells, functionally distinct from other subsets due to their own property to provide help to B cells by enhancing their survival and differentiation into long-lasting antibody secreting cells. More recently, another B cell helper subset, named T peripheral helper (Tph) cells, has been specifically described in synovial tissues and blood of RA patients.

**Methods:** We conducted an exhaustive and careful literature search focusing our interest on T cells specialized on B cell help, Tfh cells and Tph cells, in RA pathogenesis and focused on their modulation during treatments.

**Results:** Tfh cells and in higher proportions Tph cells were described in synovial tissue and liquid of RA patients. In blood of RA patients, despite the use of diverse Tfh cell definitions, a vast majority of reports revealed an increase of circulating Tph and Tfh cell frequency, compared to healthy controls. However, discordant correlations between disease activity score and either effector or activated circulating Tfh cell subsets were highlighted, probably due to heterogeneity of cohort design in term of definition of disease activity, cohort size and use of different treatments. Indeed, frequencies of circulating Tfh and Tph cells are modulated depending on the nature and duration of RA treatment. Interestingly, it has been demonstrated that the proportion of ICOS-expressing blood Tfh cells before abatacept initiation was an independent and significant predictor of DAS28-ESR improvement at week 24. This report may encourage to conduct further investigations based on T-cell subsets as predictive biomarkers of response to biotherapies.

**Conclusion:** Mounting evidences hypothesize that circulating Tfh and Tph cells may be involved in RA pathogenesis and response to treatment.

## Keywords

Follicular helper T cells, Peripheral helper T cells, rheumatoid arthritis.

## Introduction

Immunopathogenesis of rheumatoid arthritis (RA) is not yet clearly defined. B cells play a crucial role by producing autoreactive antibodies such as anti-citrullinated peptide antibodies (ACPA), which are pathognomonic of RA. Rheumatoid factor (RF), another type of autoreactive antibody could also be detected and is associated to disease severity, like ACPA [1]. In mouse models of autoimmune arthritis, B cell deficiency result in arthritis development impairment after immunization with proteoglycan [2]. Another evidence of B cell involvement in RA pathogenesis is efficiency of B cell depletion therapy [3]. Besides known B-cell involvement, RA development and evolution involves CD4<sup>+</sup> helper T-cell homeostasis dysregulation. Infiltration of CD4<sup>+</sup> T helper cells at inflamed sites is a hallmark of autoimmune diseases and RA [4,5]. Proinflammatory Th1 cells secrete high amounts of interferon- $\gamma$  (IFN- $\gamma$ ) and tumor-necrosis factor- $\alpha$  (TNF- $\alpha$ ) allowing efficient activation of cytotoxic cells, whereas Th2 cells produce interleukine (IL)-4, IL-5, and IL-13. High frequencies of citrullinated specific Th1 cells have been reported in RA blood patients [6]. Moreover, an imbalance between Th17 / T regulatory (Treg) cell commitment is noted in autoimmune and inflammatory disorders [7]. Like Th1 cells, Th17 cells display pro-inflammatory functions by their IL-17 and IL-22 secretion. Several reports highlighted positive correlations between IL-17 or Th17 cell proportion and disease activity of RA patients [8,9]. Conversely, Tregs mediate immune response inhibition and immune tolerance by production of IL-10 and/or TGF- $\beta$  allowing to slow down excessive inflammatory states. Despite some contradictory data, most studies demonstrated decreased proportions of circulating Tregs [10,11]. Since the last decade, increasing reports focused on a newly described helper T cell subset, called T follicular helper (Tfh) cells, functionally distinct from other T helper cells due to their own property to provide a cognate help to B cells by enhancing their survival and differentiation into long-lasting antibody secreting cells [12].

Thus, both CD4<sup>+</sup> T helper cells and B cells seems to be required to RA development and progression. However, less is known about T and B cell crosstalk, and in consequence ectopic lymphoid structure formation and pathogenic antibody production. In this review, we focus our interest on Tfh cells and T

peripheral helper (Tph) cells in RA pathogenesis, compared to healthy controls and during RA treatment.

## Methods

We conducted a thorough literature search using PubMed database to identify English-written publications for studies published between 1990 to 2020. The selected keywords, such as rheumatoid arthritis, T helper cells, and T regulatory cells, were used, and we selected only studies in which the selected keywords were the major focus. References cited in this work are original articles, as well as review articles, published in peer-reviewed journals.

## Professional CD4<sup>+</sup> T cells in B cell help in secondary lymphoid organs: T follicular helper cells

T follicular helper (Tfh) cells were first proposed to be a proper T cell subset in 2000 [13]. They are now considered as a specific CD4<sup>+</sup> T helper cell subset, distinct from Th2, and characterized by their own transcription factor, B cell lymphoma 6 (Bcl-6) [14]. Tfh cell commitment is induced in secondary lymphoid organs (SLO) by an environment rich in IL-6 and IL-21 [15]. These Tfh cells have previously met antigenic peptides presented by dendritic cells, inducing their commitment in Tfh cell lineage before interacting with B cells. Tfh cells home to B cell follicles thanks to their expression of the CXCR5 chemokine receptor responding to CXCL13 gradients [13,16]. They also express high levels of programmed cell death protein 1 (PD-1), these two markers allowing their phenotypic segregation among CD4<sup>+</sup> T cells in SLO [17]. In addition, Tfh cells also express high levels of the co-stimulatory molecule inducible T cell co-stimulator (ICOS) leading motility and mature Tfh differentiation [17,18]. The vast majority of human Tfh cells secrete CXCL13 and IL-21, this latter is mandatory for B-cell differentiation [19]. Antigen-specific interactions of Tfh cells with B cells are essential for selection of high-affinity B cell clones in GC (*Figure 1*). Tfh cells provide B cell help by promoting their survival, class-switch recombination of immunoglobulin genes, and terminal differentiation leading to generation of memory B cells and long-lived plasma cells, and therefore

long-term antibody responses [20]. Of note, different subsets of Tfh cells has been described based on their cytokine secretion profile: IFN- $\gamma$  secretion for Tfh1, IL-4 for Tfh2 and IL-17 for Tfh17 cells [12].

### **Peripheral blood counterpart of secondary lymphoid organ Tfh cells: circulating Tfh cells**

A subset of CXCR5<sup>+</sup> CD4<sup>+</sup> T cells sharing functional properties with SLO Tfh cells have been recently described in blood [21]. They are considered as blood circulating Tfh (cTfh) cells, even if they do not express detectable levels of the Tfh lineage master regulator Bcl-6 [21]. Compared to GC Tfh cells, only a fraction of these cTfh cells express PD-1 or ICOS, depending on their activation status [22]. It has been demonstrated that ICOS and PD-1 transient expression on cTfh cells is related to recently activated cells, as elegantly demonstrated after influenza vaccination [23]. Like their OLS counterpart, cTfh cells could secrete IL-21. For *Morita et al*, differential expression of the chemokine receptors CXCR3 and CCR6 defines three major unique subsets of cTfh cells: CXCR3<sup>+</sup>CCR6<sup>-</sup>, CXCR3<sup>-</sup>CCR6<sup>-</sup> and CXCR3<sup>-</sup>CCR6<sup>+</sup> cTfh cells called cTfh1, cTfh2 and cTfh17, respectively [21]. Like Th1 cells, cTfh1 cells have been described as IFN- $\gamma$  secreting cells. Several reports show that cTfh1 cells are important for the initiation of high-affinity antibody responses against influenza after vaccination (reviewed in [24]). *In vitro*, only activated cTfh1 cells have the capacity to promote Ig production from memory B cells, and not from naïve B cells [25]. Thus, cTfh1 have limited capacity to B-cell help in term of antibody production, compared to the other cTfh subsets. Indeed, cTfh2 and cTfh17 cells have been described as efficient helpers of B cell maturation into antibody-secreting cells for both naïve and memory B cells, regardless of their activation status [25]. In addition, cTfh2 and cTfh17 resemble Th2 and Th17 cells based on their shared cytokine secretion profile: IL-4, IL-15, IL-13 for Th2 and cTfh2, and IL-17 and IL-22 for Th17 and cTfh17 [26].

### **B cell-helper T cells in rheumatoid arthritis**

CD4<sup>+</sup> T cell differentiation implicate a cognate interaction with antigenic peptide-presenting cells, costimulatory molecule cooperation and cytokine infusion. All these essential signals are abundant in rheumatoid joints, allowing development of local sites of CD4<sup>+</sup> T cell differentiation (Figure 2). Due to their physiological capacity to induce high-affinity humoral responses by allowing long-lived plasma cells and memory B cells differentiation, Tfh cells have been hypothesized to be involved in autoantibody production.

In RA, synovial tissue could be considered as a tertiary lymphoid structure (TLS) or an ectopic lymphoid organ as it can display well-organized lymphoid organ-like structures with T and B-cell differentiation sites. This synovial lymphoid neogenesis and maintenance is a reversible consequence of chronic inflammation found in RA, like in other inflamed sites of several auto-immune diseases. Nevertheless, TLS does not seem to be a major determinant of rheumatoid arthritis autoantibody responses as no association between lymphoid neogenesis and local production of ACPA or RF has been highlighted in rheumatoid joints [27]. In addition, patients presenting fully-organized structures with specialized stromal cells called follicular dendritic cells are rather rare with a prevalence of 6 to 25%, depending on the studied cohorts and the treatment regimens [5,27-30]. Of note, patients with these fully-organized structures display similar clinical and biological parameters [30].

Accordingly, CD4<sup>+</sup> T cells sharing all phenotypic and functional properties of SLO Tfh cells have been described in synovial tissues of RA patients. Recent SF investigations based on small cohorts revealed that total Tfh, Tfh2 and Tfh17 cell frequencies were decreased in SF compared to peripheral blood [31] and that no activated PD-1<sup>hi</sup> Tfh cells were detectable [32], whereas active sites of B-cell differentiation may occur. Nevertheless, CXCR5-expressing cells represent a weak proportion of whole CD4<sup>+</sup> T cell compartment, despite frequent B and plasma cells and a CXCL13-rich environment in synovial fluid and tissue of RA patients [33,34]. In 2008, a CXCR5<sup>-</sup> BCL-6<sup>-</sup> Tfh-like cell subset secreting CXCL-13 was identified closed to B cells in RA synovial tissues both in lymphocyte aggregates and diffuse synovitis [35]. More recently, a pathologically expansion of CXCR5<sup>-</sup> CD4<sup>+</sup> helper T cell subset driving B cell differentiation into antibody secreting cells has been highlighted [36]. These cells, called peripheral helper T (Tph) cells, express inflammatory chemokine receptors such as CCR2, CX3CR1 and CCR5, that drive their migration to inflamed sites. Tph cells also display

membrane expression of activation markers like CD69, and shared PD-1 and ICOS expression with Tfh cells. Like Tfh cells, their B-cell helper capacity results in part from CXCL-13 and IL-21 secretion. However, they display a low BCL6/BLIMP1 protein expression ratio, allowing to definitively distinguish them from Tfh cells. Interestingly, high PD-1 expression was detected on SF Tph cells of seropositive RA patients, unlike SF Tfh cells. This suggest that SF Tph cells, more than SF Tfh cells, could be the most important B-cell helpers specifically in RA synovial tissues.

Considering circulating compartments, several studies investigated cTfh cell frequency in blood of RA patients, but no consensual phenotypic definition was used impairing strict comparison and conclusive statements. Indeed, some authors defined cTfh cells only by the expression of the chemokine receptor CXCR5, whereas others only considered a small fraction of CD45RA<sup>-</sup> cTfh expressing ICOS and/or high levels of PD-1 (Table 1). Overall, a higher proportion of cTfh cells was reported in patients with active RA compared to healthy controls, unless cTfh phenotypic definition (Table 1). Considering cTfh cell subsets, a specific higher proportion of effector memory cTfh, cTfh2, and IL-21-secreting cTfh cells were pointed [31,37,38]. Moreover, higher ICOS<sup>hi</sup> and PD-1<sup>+</sup>, or ICOS and PD-1 co-expressing cTfh were reported [39-41]. Taking into account CD4<sup>+</sup> blood concentration variations, Liu *et al* also described an increase of CXCR5<sup>+</sup> and CXCR5<sup>+</sup> PD-1<sup>hi</sup> cTfh cell absolute numbers, compared to healthy controls [42]. Finally, contradictory results were obtained considering IL-21 plasma concentrations [39-41,43-46], cytokine reflecting activation of not only Tfh cells, but also Th17 cells. Several reports observed a positive correlation between proportion of CD4<sup>+</sup> CXCR5<sup>+</sup> or ICOS<sup>+</sup> or PD-1<sup>+</sup> cTfh cells and ACPA titer [39,41,45,47]. Lui *et al* first described in 2012 a positive correlation between CXCR5<sup>+</sup> PD-1<sup>+</sup> cTfh cell frequencies and disease activity calculated with DAS28 score [46]. Thereafter, other reports highlighted discordant positive correlations between disease activity score and either effector, ICOS- and/or PD-1-expressing cTfh cell subsets [31,37,40,45]. These discrepancies may be related to cohort design in term of definition of disease activity, cohort size and use of different treatment regimens for patients. Nevertheless, these findings hypothesize Tfh cell involvement in RA pathogenesis. In addition, Deng *et al.* found that proportion of pSTAT3 in Tfh cells correlated positively with disease activity and highlighted an IL-6 – pSTAT3 – Tfh cell axis in regulating immune responses in RA patients [43].



Beside these classically explored cTfh cells, memory PD1<sup>hi</sup> CRXR5<sup>-</sup> CD4<sup>+</sup> T cells, circulating counterpart of synovial Tph cells, have been recently evidenced in blood of RA patients, albeit at much lower frequencies [36]. Like PD1<sup>hi</sup> cTfh cells, these cells show high ICOS expression and secrete high amounts of IL-21 [36]. cTph cells proportions were increased in blood of seropositive early RA patients, compared to controls [32,36]. Finally, they exhibit comparable B-cell helper function than activated PD1<sup>hi</sup> cTfh cells, in term of *in vitro* plasma cell differentiation and IgG secretion induction [36].

These studies highlight the importance of B-T cell crosstalk in RA inflamed tissues. However, there is no evidence of the precise role of Tph cells in TLS organization and maintenance. On the one hand, after their recruitment within inflamed sites, Tph cells could be considered as initiators of TLS organization by their CXCL-13 secretion allowing B and Tfh cell attraction, and their B-cell differentiation capacity. On the other hand, Tph cells could be considered as activated CD4<sup>+</sup> T cells recruited in inflammatory TLS gathering B and Tfh cells, committed in a specific lineage using similar molecules than Tfh cells for B-cell help, but displaying a different gene expression program.

### **Modulation of B cell-helper T cells in rheumatoid arthritis during treatment**

One weak point for the comparison of results obtained for the different studies depicted above is the heterogeneity of cohorts in term of RA treatment during T cell and cytokine assessment; the most homogeneous cohorts gathering only non-treated patients, whereas others gathered patients with several different therapies, disease-modify anti-rheumatic drugs (DMARDs) and/or biologics. To date, only few reports studied B-cell helper T cell modulations as a consequence of RA treatment. *Zang et al* [44] followed 9 RA patients treated with disease-modify anti-rheumatic drug (DMARD) therapy, essentially methotrexate monotherapy. These patients presented high disease activity before treatment, and a drastic reduction after 24 weeks of methotrexate therapy. At the same time, a significant reduction of serum IL-21 concentration was observed, whereas no change in cTfh cell frequency was found. In addition, *Wang et al* [48] followed 9 RA patients treated with DMARDs and *T. Wilfordii*. Unlike non-responding patients, PD-1<sup>+</sup> cTfh cell frequencies and serum IL-21 concentrations were significantly reduced in responding patients after 1 month of treatment, compared with baseline

values. However, there was no correlation between ICOS<sup>+</sup> or PD-1<sup>+</sup> cTfh cell frequencies and ACPA concentrations or DAS28 activity score after 1 month of treatment.

*Nakayamada et al* [47] followed a big cohort of 108 bio-naïve RA patients non-responsive to conventional DMARDs, before initiation and after 24 weeks of various biotherapies: anti-TNF (n=46), abatacept (n=40) or tocilizumab (n=22). Anti-TNF induced a significant increase of ICOS<sup>+</sup> cTfh cell frequency whereas abatacept resulted in a decrease of ICOS<sup>+</sup> cTfh cell frequency after 24 weeks of biotherapy. *Takeshita et al.* analyzed 49 naïve patients treated with methotrexate alone, 46 patients with a combination of methotrexate and infliximab, and 28 patients with methotrexate and tocilizumab [31]. They observed an increase of Tfh2 and Tfh17 during a 12-month follow-up after methotrexate treatment initiation. Combination of methotrexate with tocilizumab or infliximab induced a Tfh2 or a Tfh17 increase, respectively. Data of these two latter studies demonstrate that the nature of biotherapy could have opposite consequences on cTfh cell compartments.

*Singh et al* first hypothesized that cTfh cell frequency may have a predictive value of disease progression during treatment [49]. They followed 16 patients with active RA treated with DMARDs before and after more than 6 months of anti-TNF therapy. Despite decreased activity score, enrolled patients presented similar cTfh cell frequencies after 5-month of anti-TNF. Focusing on cTfh17 cells, which correlate with baseline disease activity in active RA before treatment, the authors did not demonstrate a predictive value for therapy response. Nevertheless, a positive correlation between baseline cTfh17 and swollen joints at 5 months post anti-TNF was highlighted, even though absolute numbers of swollen joints decreased for most subjects. In addition, subjects without any swollen joints after 1-year of anti-TNF had lower proportions of cTfh17 cells before treatment. More interestingly, *Nakayamada et al* showed that the proportion of ICOS<sup>+</sup> cTfh cells before abatacept initiation was an independent and significant predictor of DAS28-ESR improvement at week 24 [47]. These two studies featured new insights of B-cell helpers and may promote new investigations based on T-cell subsets as predictor of treatment response.

Finally, considering cTph cells, only few reports explored this new-described subset. *Rao et al.* [36] found no differences in term of cTph cell frequencies depending on methotrexate, anti-TNF or biologics treatment used for a cohort of 38 RA patients. *Fortea-Gordo et al.* [32] showed in a smaller

cohort of 9 early RA patients a significant decrease of cTph and ICOS<sup>+</sup> cTph cell proportions associated to a decrease of RF titers and DAS28 score in response to a 12 month-methotrexate treatment. Conversely, no differences were highlighted for cTfh cells.

### **Conclusion and perspectives**

Despite conflicting results due to non-homogeneous phenotypic definition of Tfh and Tph cells, these two B cell-helpers T cell subsets are now assumed to be involved in RA pathogenesis and disease evolution. Nevertheless, increasing recent studies focused on circulating T follicular regulatory (cTfr) cells, the blood counterpart of SLO Tfr cells, specialized in GC reaction regulation. A modulation of cTfh/cTfr ratio has been reported, with a decrease of cTfr cells in balance of increased frequencies of cTfh cells in peripheral blood of RA patients [37,48,50]. Nevertheless, to date, no studies take into account cTfh, cTfr but also cTph cells in peripheral blood of individual patients to really appreciate the different actors of B-cell help.

## LIST OF ABBREVIATIONS

ACPA: anti-citrullinated peptide antibodies

Bcl-6: B cell lymphoma 6

CD: cluster of differentiation

cTfh cells: circulating T follicular helper cells

cTfr cells: circulating T follicular regulatory cells

DMARDs: disease-modify anti-rheumatic drugs

GC: germinal center

ICOS: inducible T cell co-stimulator

IFN- $\gamma$ : interferon- $\gamma$

IL: interleukin

PD-1: programmed cell death protein 1

RA: rheumatoid arthritis

RF: rheumatoid factor

SLO: secondary lymphoid structure

Tfh cells: T follicular helper cells

TGF- $\beta$ : transforming growth factor- $\beta$

Th: T helper cells

TLS: tertiary lymphoid structure

TNF- $\alpha$ : tumor-necrosis factor- $\alpha$

Tph cells: T peripheral helper cells

Treg cells: T regulatory cells

## FIGURE LEGENDS

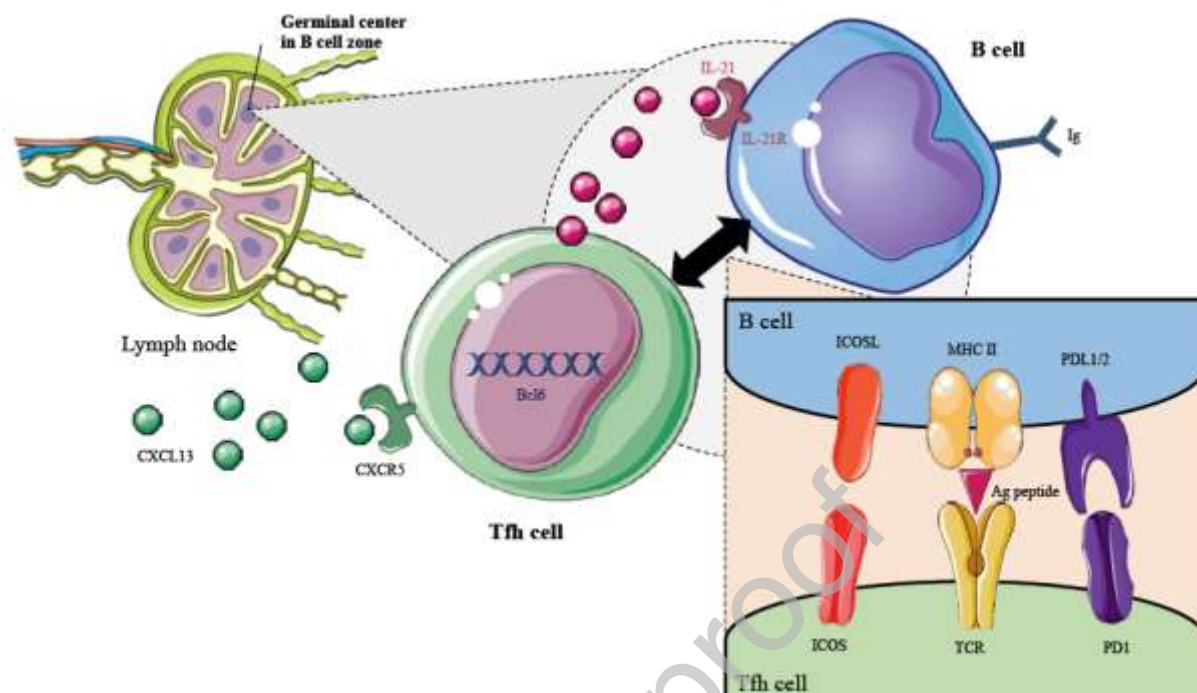


Figure1: B-Tfh cell crosstalk in germinal centers. Tfh cells and B cells interact in germinal centers localized in B-cell zone of lymph nodes. Herein, Tfh cells are characterized by the expression of CXCR5 responding to CXCL13 gradients, and their high secretion levels of IL-21 mandatory for B-cell differentiation. In addition, Tfh cells and B cells interact by their respective expression of ICOS, TCR, PD-1 and ICOSL, MHC-II, PDL-1/2. MHC: major histocompatibility complex; ICOSL: ICOS ligand, TCR: T-cell receptor, Ig: immunoglobulin; PDL-1/2: programmed death-ligand1/2.

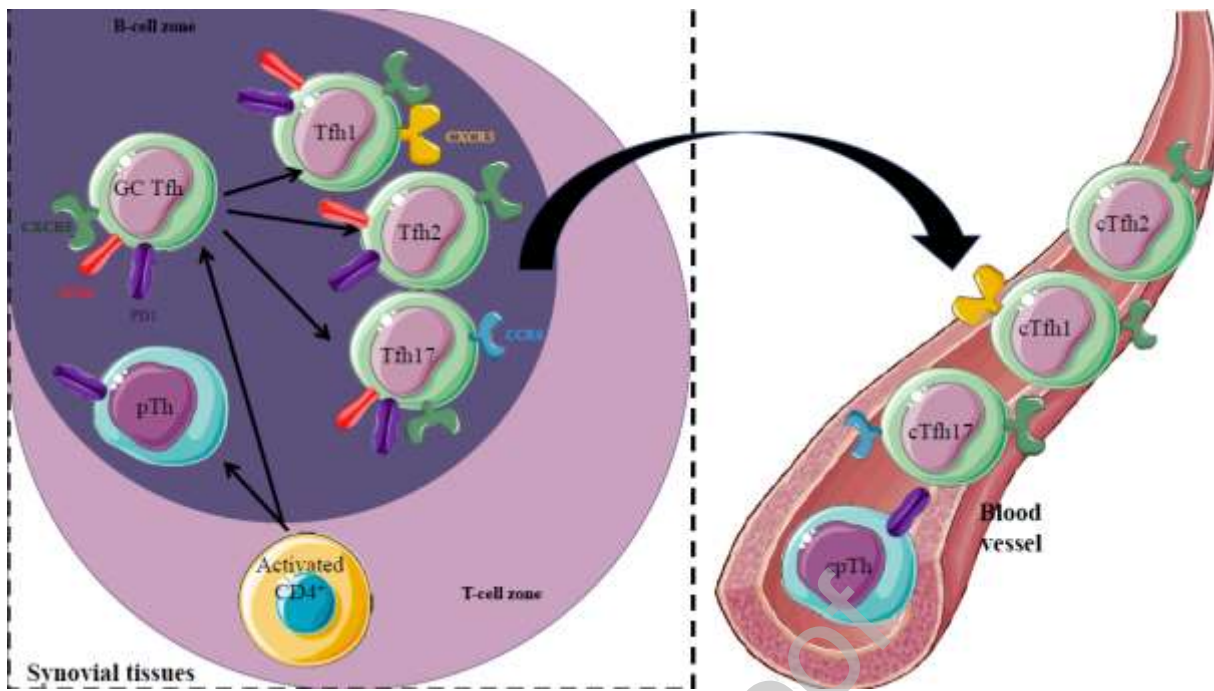


Figure 2: B-cell helper T cells in RA. Differential characterization of pTh, Tfh1, Tfh2 and Tfh17 cells localized in B-cell zones of synovial tissues, and definition of their blood counterparts (cpTh, cTfh1, cTfh2 and cTfh17).

## DECLARATIONS

- Competing interests

The authors declare that they have no competing interests

- Authors' contributions

CL, AP and PA: literature search and reading, study design, data interpretation, and manuscript writing.

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Table 1: List of different studies related to cTfh in RA

First author	Year	Ref N°	RA cohort	Controls	Tfh phenotypic definition	Tfh cell frequency	Serum IL-21 concentration	Positive correlation DAS28 /%Tfh
Takeshita M	2019	[1]	53 untreated RA *	30 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup>	↗ Tfh, Tfh2, EM Tfh	-	Yes for EM Tfh
Fortea-Gordo P	2019	[2]	56 early and untreated RA	47 HC	CD4 <sup>+</sup> CD45RA <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>hi</sup>	↗ Tfh	-	No
Cao G	2019	[3]	30 early and untreated RA	30 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup>	↗ Tfh and ICOS <sup>+</sup> Tfh	↗	-
Zhou H	2019	[4]	44 established and untreated RA	37 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RA <sup>+</sup> CXCR5 <sup>+</sup>	↗ PD1 <sup>+</sup> ICOS <sup>+</sup> Tfh	No differences	Yes for PD1 <sup>+</sup> ICOS <sup>+</sup> Tfh
Sun WK	2019	[5]	45 RA (24 high activity, 21 low activity) *, **	20 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup>	↗ Tfh2 and Tfh17	-	-
Wang X	2019	[6]	24 established and treated (DMARDs +/- CTC) and untreated RA	20 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>hi</sup>	↗	-	Yes
Niu Q	2018	[7]	44 established RA (20 active, 24 inactive) **	20 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup> FOPX3 <sup>+</sup>	↗ in active RA	-	Yes for PD1 <sup>+</sup> Tfh
Liu C	2018	[8]	78 established (39 active, 39 remission), untreated and treated (DMARDs) RA	33 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup>	↗ Tfh and PD1 <sup>hi</sup> Tfh in active RA	-	-
Deng J	2018	[9]	31 early and established, treated and untreated (4 untreated, 27 DMARDs +/- CTC) RA	30 HC	CD4 <sup>+</sup> CD25 <sup>+</sup> CD45RA <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup>	↗	No differences	Yes
Nakayamada S	2018	[10]	108 established and treated (DMARDs + biotherapies +/- CTC) RA	33 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup> ICOS <sup>+</sup>	↗	-	No
Singh D	2016	[11]	44 established and treated (DMARDs +/- biotherapies +/- CTC) RA	10 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup> CD45RO <sup>+</sup>	No differences	-	No
Zhang Y	2015	[12]	54 untreated RA *	16 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup> ICOS <sup>hi</sup>	↗ in RA patients with high and moderate DAS28	↗	Yes

Arroyo-Villa I	2014	[13]	34 established and treated (DMARDs) RA (17 active and 17 in remission)	34 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup> ICOS <sup>+</sup>	↗ cTfh ↗(Tfh2+Tfh17)/Tfh1 ratio in active RA	-	No
Wang J	2013	[14]	25 early and untreated RA	15 HC	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR5 <sup>+</sup>	↗	↗	Yes for ICOS <sup>+</sup> Tfh
Liu R	2012	[15]	120 established and treated (DMARDs +/- CTC +/- others) RA	80 HC	CD4 <sup>+</sup> CXCR5 <sup>+</sup> PD-1 <sup>+</sup>	↗	↗	Yes
Ma J	2012	[16]	53 early and untreated RA	31 HC	CXCR5 <sup>+</sup>	↗ Tfh and ICOS <sup>hi</sup> Tfh	↗	-

Ref N°: reference number; PB: peripheral blood; RA: rheumatoid arthritis; HC: healthy controls; cDMARDs: conventional disease-modifying anti-rheumatic drugs; EM: effector memory cells; CTC: corticotherapy.

\* disease duration not mentioned.

\*\* treatment not mentioned

Early: <6 months

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