Aus dem Institut für Immunologie am Biomedizinischen Centrum
Institut der Ludwig-Maximilians-Universität München
Leitung: Prof. Dr. rer. nat. Thomas Brocker

Human circulating T follicular helper cells during viral infection and autoimmunity

Dissertation

zum Erwerb des Doktorgrades der Naturwissenschaften
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität zu München

vorgelegt von

Johanna Huber

aus Prien am Chiemsee

2020

Mit Genehmigung der Medizinischen Fakultät
der Universität München

Mit Genehmigung der Medizinischen Fakultät der Universität München

Betreuer:	Prof. Dr. Dirk Baumjohann
Zweitgutachter(in):	PD Dr. rer. nat. Hanna-Mari Baldauf
Dekan:	Prof. Dr. med. dent. Reinhard Hickel

Tag der mündlichen Prüfung: 15.12.2020

Zwei Dinge sind zu unserer Arbeit nötig: Unermüdliche Ausdauer und die Bereitschaft, etwas, in das man viel Zeit und Arbeit gesteckt hat, wieder wegzuwerfen.

Albert Einstein

Eidesstattliche Versicherung

Huber, Johanna

Name, Vorname

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation mit dem Titel

Human circulating T follicular helper cells during viral infections and autoimmunity

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe. Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 07.01.2021 — Johanna Huber

Ort, Datum — Unterschrift Doktorandin

Summary

CD4$^+$ T helper cells orchestrate the adaptive immune response. The differentiation of naïve CD4$^+$ T cells into various functionally different subsets of T helper cells ensures the adaptation of the immune response to the invading pathogen. The T helper cell subset that is responsible for B cell help during affinity maturation in the germinal center (GC) reaction is called T follicular helper (Tfh) cells. Therefore, Tfh cells are crucial to develop long-lasting immunity by ensuring the generation of memory B cells and high-affinity antibody-producing plasma cells.

Blood-resident Tfh cells, so called circulating Tfh (cTfh) cells, can be used to investigate human Tfh cells instead of lymphoid tissue-resident Tfh cells, which are difficult to assess in humans. Lymphoid tissue-resident Tfh cells and cTfh cells both provide B cell help and share similarities in phenotype and gene expression. cTfh cells can express other CD4$^+$ T cell subset-defining chemokine receptors and can thereby be clustered into different subsets.

Although increased cTfh cell frequencies have been connected to better vaccination outcome and new insights into cTfh cell kinetics might improve the understanding of established vaccinations and impact future vaccine design, only few studies investigated cTfh kinetics after vaccination. Most conclusions were drawn from annual influenza vaccinations that allow for investigation of recall responses. Nevertheless, it is difficult to distinguish between the primary and secondary immune response as vaccinees have likely been in contact with influenza virus before vaccination and additionally influenza vaccination can have low efficacy. Therefore, I tracked and characterized cTfh and other blood-resident immune cells by flow cytometry after challenge with a live virus in the context of a vaccination against yellow fever.

Yellow fever virus (YFV) is endemic in tropical regions. Yellow fever vaccination elicits a strong, long-lasting immune response with neutralizing antibodies in almost all vaccinees. We were able to show that vaccination with the attenuated yellow fever virus elicited an increased frequency of activated cTfh cells from three days on after vaccination. The peak frequency of activated cTfh cells was detectable 14 days after vaccination. In addition, we observed a shift in the subset composition of cTfh cells during the immune response with cTfh1 cells as the most prevalent subpopulation. Those findings were confirmed by the detection of YFV-specific CD4$^+$ T cells in the blood with major histocompatibility complex (MHC) II tetramers for four known epitopes. Moreover, we found a correlation of frequencies of cTfh1 cells with the strength of the neutralizing antibody response, which might influence future vaccine design.

Tfh cells have also been implicated in the pathogenesis of several autoimmune diseases and are for example contained in ectopic lymphoid structures and implicated in the formation of autoantibodies. Multiple sclerosis (MS) often involves ectopic lymphoid structures and oligoclonal bands in the

cerebrospinal fluid and multiple studies point to a role of Tfh cells in multiple sclerosis. Yet, cTfh cells and the impact of immunomodulatory drugs are not well investigated in patients with MS. Therefore, I compared blood-resident T and B cell populations of patients with multiple sclerosis, that either received no treatment or different immunomodulatory drugs, with cells derived from healthy donors.

Although cTfh cells from MS patients were phenotypically not distinguishable from healthy donors, immunomodulatory treatment with the sphingosine-1-phosphate receptor (S1PR) 1 blocking drug fingolimod resulted in profoundly reduced frequencies of cTfh cells. Additionally, other T cells expressing the Tfh cell hallmark chemokine receptor CXCR5, such as T follicular regulatory cells and $CXCR5^{+}CD8^{+}$ T cells, were similarly affected. This provides insight into the migratory pattern of cTfh cells as well as a better understanding of the impact of fingolimod on blood-resident lymphocyte populations.

In summary, the findings I present in this thesis contribute to a better understanding of circulating Tfh cells after viral challenge and immunomodulation. This might have implications for vaccine design and for the treatment of autoimmune diseases.

Zusammenfassung

CD4$^+$ T-Helferzellen steuern die adaptive Immunantwort. Die Differenzierung von naiven CD4$^+$ T-Zellen in verschiedene T-Helferzell-Subtypen mit unterschiedlichen Funktionen sichert die spezifische Anpassung der Immunantwort auf das eindringende Pathogen. Follikuläre T-Helferzellen (Tfh) sind für die B-Zell-Hilfe während der Affinitätsreifung in der Keimzentrumsreaktion verantwortlich. Indem sie sicherstellen, dass B-Gedächtniszellen und hochaffine Antikörper-produzierende Plasmazellen gebildet werden, sind Tfh-Zellen essenziell für die Entwicklung langanhaltender Immunität.

Tfh-Zellen im Blut, so genannte zirkulierende („circulating“) Tfh-Zellen (cTfh), können dazu verwendet werden, humane Tfh-Zellen zu untersuchen, anstatt dafür Tfh-Zellen aus den Lymphknoten zu verwenden, deren Probennahme invasiv und daher schwierig ist. Tfh-Zellen in Lymphknoten und cTfh-Zellen leisten beide B-Zell-Hilfe und teilen phänotypische und genexpressionelle Gemeinsamkeiten. cTfh-Zellen können Chemokinrezeptoren anderer CD4$^+$ T-Zell-Subtypen exprimieren und dadurch ebenfalls in verschiedene Subtypen unterteilt werden.

Obwohl erhöhte cTfh-Zell-Frequenzen mit einem besseren Impfresultat in Verbindung gebracht wurden, neue Erkenntnisse über die Kinetik von cTfh-Zellen das Verständnis von etablierten Impfungen verbessern könnten und diese Erkenntnisse die Entwicklung neuer Impfstoffe beeinflussen könnten, haben sich bisher nur wenige Studien mit der cTfh Kinetik nach Impfungen beschäftigt. Die meisten Rückschlüsse wurden von jährlichen Grippeimpfungen gezogen, die es erlauben eine Gedächtnisantwort zu untersuchen. Allerdings ist es im Falle von Impfungen gegen Influenzaviren schwierig, zwischen einer primären und sekundären Immunantwort zu unterscheiden, da die geimpften Personen mit einer hohen Wahrscheinlichkeit schon zuvor mit Influenzaviren in Kontakt waren und Grippeimpfungen manchmal nicht vollen Impfschutz vermitteln. Daher habe ich cTfh-Zellen und andere Immunzellen im Blut nach einer Immunisierung mit einem lebenden Virus im Rahmen einer Gelbfieberimpfung mithilfe von Durchflusszytometrie untersucht.

Das Gelbfiebervirus ist endemisch in den Tropen. Die Impfung gegen Gelbfieber führt zu einer starken, langanhaltenden Immunantwort mit neutralisierenden Antikörpern bei beinahe allen geimpften Personen. Wir konnten zeigen, dass die Impfung mit dem attenuierten Gelbfiebervirus eine erhöhte Frequenz von aktivierten cTfh-Zellen ab Tag drei nach der Impfung zur Folge hatte. Die höchste Frequenz an aktivierten cTfh-Zellen wurde nach 14 Tagen erreicht. Zusätzlich konnten wir während der Immunantwort eine Veränderung der Zusammensetzung der cTfh-Zellen erkennen, bei der cTfh1-Zellen dominierten. Mithilfe von Haupthistokompatibilitätskomplex („major histocompatibility complex“, MHC) II Tetrameren, die mit vier bekannten Epitopen beladen waren, wurden diese Beobachtungen durch den Nachweis von Gelbfiebervirus-spezifischen CD4$^+$ T-Zellen im Blut bestätigt. Außerdem

fanden wir eine Korrelation zwischen der Frequenz von cTfh1-Zellen und der Stärke der neutralisierenden Antikörper Antwort, was die Entwicklung von Impfstoffen beeinflussen könnte.

Tfh-Zellen sind auch in der Pathogenese von Autoimmunkrankheiten beschrieben, kommen beispielsweise in ektopischen lymphoiden Strukturen vor und sind potenziell an der Bildung von Autoantikörpern beteiligt. Multiple Sklerose (MS) geht oft mit der Bildung ektopischer lymphoider Strukturen und oligoklonaler Banden im Liquor einher und mehrere Studien schreiben Tfh-Zellen eine Rolle in Multipler Sklerose zu. Allerdings sind cTfh-Zellen und der Einfluss immunmodulierender Medikamente bei MS Patienten nicht gut untersucht. Daher habe ich T-Zellen und B-Zellen im Blut von Patienten mit Multipler Sklerose, die entweder keine Behandlung oder verschiedene immunmodulatorische Medikamente erhielten, mit denen gesunder Spender verglichen.

Obwohl cTfh-Zellen von MS Patienten phänotypisch nicht von denen gesunder Spender zu unterscheiden waren, hatte eine Behandlung mit dem Sphingosine-1-Phosphat-Rezeptor (S1PR) 1-blockierenden Medikament Fingolimod zur Folge, dass die cTfh Frequenzen stark reduziert waren. Zusätzlich zeigte Fingolimod ähnliche Effekte auf andere T-Zellen, die ebenfalls den Tfh-Zell-definierenden Chemokinrezeptor CXCR5 exprimieren, wie etwa follikuläre regulatorische T-Zellen und $CXCR5^{+}CD8^{+}$ T-Zellen. Diese Beobachtungen lassen sowohl Rückschlüsse auf das Migrationsverhalten von cTfh-Zellen zu, als auch auf die Effekte von Fingolimod auf Lymphozyten im Blut.

Zusammenfassend tragen die Ergebnisse, die ich in dieser Arbeit präsentiere, zu einem besseren Verständnis von zirkulierenden Tfh-Zellen während einer antiviralen Immunantwort und während Immunmodulation bei. Dies könnte für die Entwicklung von Impfstoffen und für die Behandlung von Autoimmunerkrankungen von Bedeutung sein.

Table of Contents

Eidesstattliche Versicherung 4

Summary 6

Zusammenfassung 8

Abbreviations 12

1 Introduction 14

1.1 $CD4^+$ T helper cells 14

1.2 Tfh cells provide B cell help in the germinal center 14

1.3 Human Tfh cells in peripheral blood 16

1.4 The relationship of lymphoid tissue-resident and blood-resident Tfh cells 18

1.5 Human cTfh cells during viral challenge 19

1.6 Human cTfh cells in autoimmune diseases 20

2 Publication I 23

2.1 Contributions to publication I 23

3 Publication II 24

3.1 Contributions to publication II 24

4 References 25

5 Acknowledgements 30

6 List of publications 31

6.1 Earlier publications 31

6.2 Publications during the PhD project 31

Abbreviations

CNS	central nervous system
cTfh	circulating T follicular helper
cTfr	circulating T follicular regulatory
EAE	experimental autoimmune encephalomyelitis
ELS	ectopic lymphoid structures
GC	germinal center
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPV	human papilloma virus
IL	interleukin
LN	lymph node
MHC	major histocompatibility complex
MS	multiple sclerosis
RA	rheumatoid arthritis
S1PR	sphingosine-1-phosphate receptor
SLE	systemic lupus erythematosus
SS	Sjögren's syndrome
T-B	T cell – B cell
TCR	T cell receptor
Tfh	T follicular helper
Tfr	T follicular regulatory
Th	T helper
Treg	regulatory T
YFV	yellow fever virus

1 Introduction

1.1 CD4⁺ T helper cells

CD4⁺ T cells, so-called T helper (Th) cells, are a crucial component of the adaptive immune system. They shape the activity of other immune cells by releasing cytokines, promote or suppress immune responses, adapt the immune response to the invading pathogen, display cytotoxic effects either on their own or by supporting activation and proliferation of other cytotoxic cells, and help B cells during class switch and affinity maturation in the germinal center (GC) response.

Th cells can be grouped into different lineages depending on a combination of transcription factors, chemokine receptors, and cytokines expressed, which also determines their function during an immune response (Fig. 1). Th1, Th2, and Th17 cells are specialized in providing help during infection with certain classes of pathogens and one subset usually becomes predominant during an immune response. Th1 cells provide help during infections with pathogens that reside or replicate within macrophages like viruses or intracellular bacteria. Th2 cells help during infections with extracellular parasites. Th17 cells are the predominant Th cell subset during infections with extracellular bacteria and fungi. In contrast, regulatory T (Treg) cells suppress immune reactions and thereby prevent dysregulated immune responses and autoimmunity (Fig. 1).

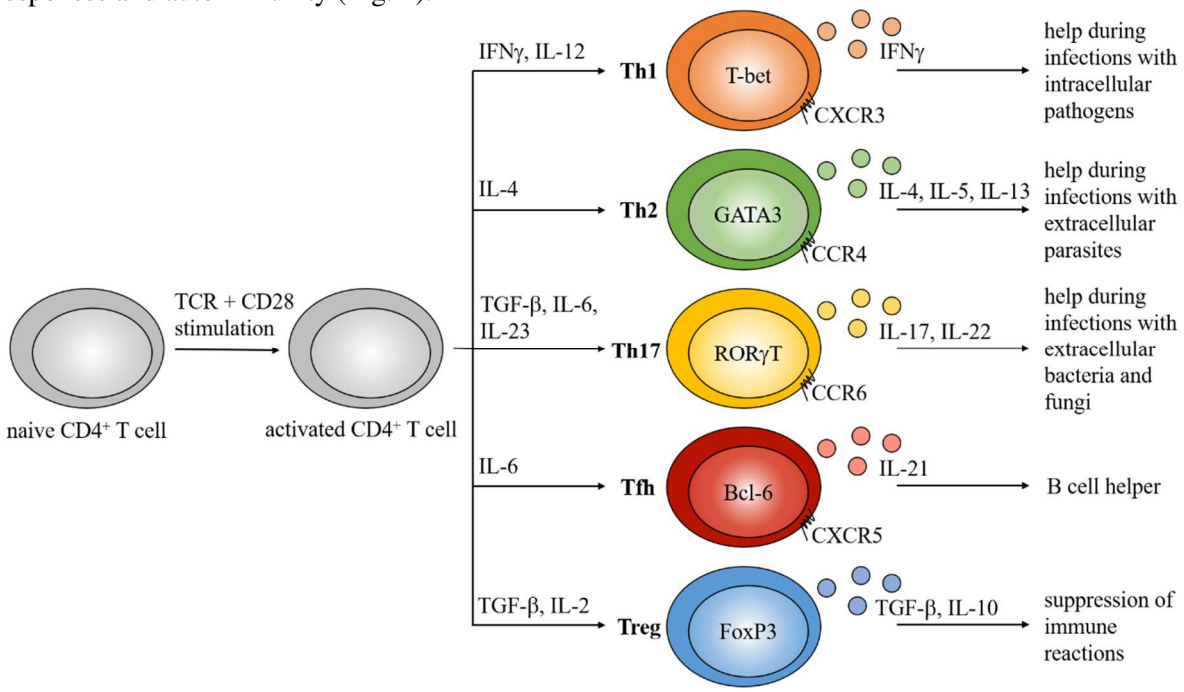

Figure 1. Overview over the major CD4⁺ T helper cell subsets, their cytokines needed for differentiation from naïve CD4⁺ T cells, their defining transcription factors, cytokines, and chemokines receptors, and their function.

1.2 Tfh cells provide B cell help in the germinal center

It was long believed that Th2 cells were the T helper cell subset responsible for providing help to B cells (1). In 1999 the first steps were made to challenge this view, when the expression of the chemokine

receptor CXCR5 on activated $CD4^+$ T cells was described to be responsible for the migration towards the chemokine CXCL13 in B cell follicles (2). Shortly after that, CXCR5-expressing $CD4^+$ T cells were described in B cell follicles of human tonsils, that promoted antibody-secretion and expressed costimulatory molecules like CD40L and ICOS (3-5). It wasn't until 2009 that T follicular helper (Tfh) cells were fully accepted as a separate T helper cell lineage when three groups simultaneously described the Tfh hallmark transcription factor Bcl-6 (6-8).

Tfh cells are now defined by the expression of CXCR5, which recruits them to the CXCL13 (ligand of CXCR5) rich follicles where they interact with B cells. Additionally, Tfh cells are characterized by the expression of their master transcription factor Bcl-6, which acts as a transcriptional repressor, as well as by the expression of high levels of PD-1 and ICOS, and by the secretion of interleukin (IL)-21 (9).

Interestingly, Tfh cells also display a certain degree of plasticity and diversity as they express cytokines and transcription factors typical of other Th cell subsets. This influences the class switch towards certain isotypes in naïve B cells. Additionally, it has been shown that Th2 (10) and Treg cells (11) can contribute to the Tfh cell pool. Yet, the plasticity of Tfh cells remains elusive.

Tfh cell differentiation requires multiple steps. First, a naïve T helper cell is primed towards the Tfh cell fate by antigen-presenting cells (mostly dendritic cells) in the T cell zone. These pre-Tfh cells upregulate Bcl-6 expression, and consequently CXCR5 expression, while downregulating CCR7 expression, and migrate to the T cell – B cell (T-B) border. Upon antigen recognition, naïve B cells increase the expression of major histocompatibility complex (MHC) II and also migrate to the T-B border to interact with pre-Tfh cells (9).

B cells that have a high affinity to an antigen and therefore express more MHCII on their surface, are more likely to get T cell help (12-14). B cells and Tfh cells then form a GC where B cells cycle between a light zone, with now fully differentiated GC Tfh cells and follicular dendritic cells, and a dark zone where they undergo cell division and somatic hypermutation. GC Tfh cells and GC B cells are co-dependent, where the GC Tfh cell provides proliferation and differentiation signals to the GC B cells via CD40L and cytokines such as IL-21, and at a later GC stage also IL-4 (6, 9). GC Tfh cells are indispensable for the GC reaction (8). Without Tfh cells, GCs neither form nor persist. GC B cells then differentiate into either memory B cells or antibody-producing plasma cells, which seems to depend on low/intermediate versus high antigen-affinity of the GC B cell, and consequently the resulting strength of GC Tfh cell signals (9) (Fig. 2).

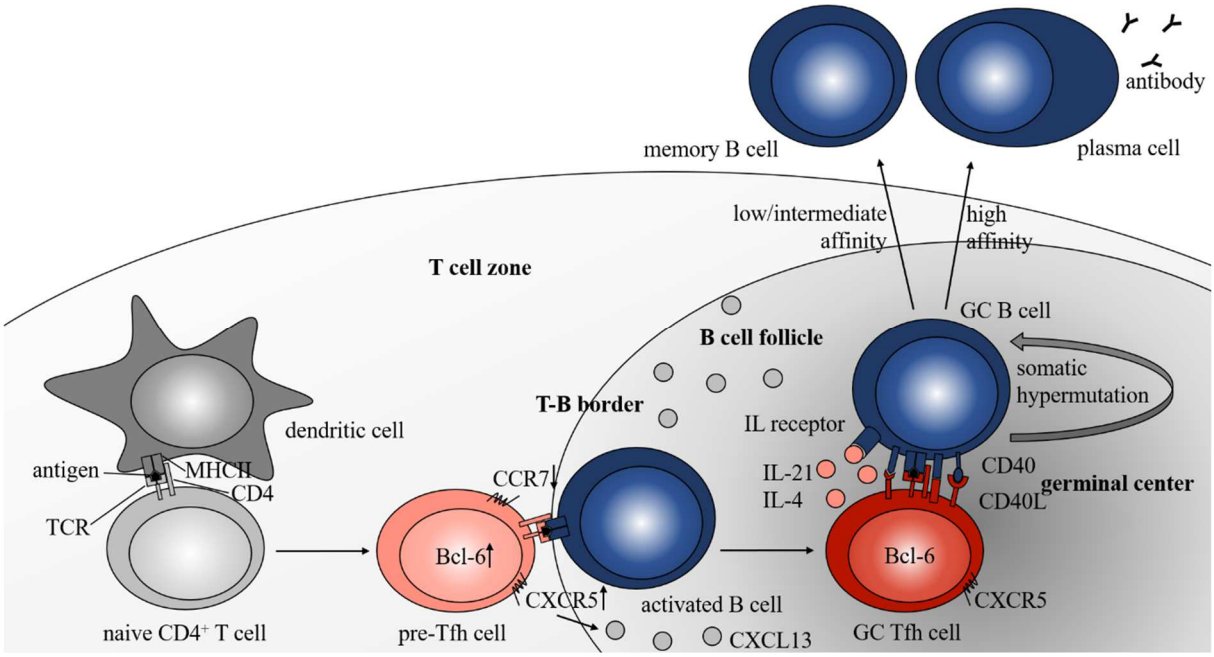

Figure 2. Schematic overview of the GC reaction. First, a naïve CD4⁺ T cell is primed by a dendritic cell to upregulate Bcl-6 and CXCR5 and downregulate CCR7. Consequently, this pre-Tfh cell migrates due to its CXCR5 expression to the CXCL13-rich T-B border and interacts with activated B cells. Tfh cells and B cells then form a GC, where the B cell undergoes affinity maturation and becomes either a memory B cell or a plasma cell.

Dysregulated germinal center reactions with excessive proliferation of GC Tfh and GC B cells can lead to several pathological conditions and therefore need to be controlled by regulatory T cells. The so-called T follicular regulatory (Tfr) cells share features of Treg and Tfh cells and express regulatory markers such as FoxP3 and IL-10 as well as follicular markers like CXCR5, ICOS, PD-1, Bcl-6 (15-18). They originate from thymic-derived FoxP3⁺ precursor cells and enter the GC in a similar way as Tfh cells by upregulating CXCR5 and downregulating CCR7 in the T cell zone upon activation (15). Tfr cells were shown to control the germinal center reaction by directly suppressing GC Tfh and B cells, limiting GC T and B cell numbers, and altering the antibody output (15-17, 19).

1.3 Human Tfh cells in peripheral blood

While it is easy to investigate follicular cells in mice, as lymphoid tissue is readily available, human lymphoid tissue is harder to obtain routinely, especially if multiple time points need to be compared.

Blood-resident CXCR5-expressing cells were first reported in 1994 (20) and then also mentioned in the 2000/2001 papers that first described CXCR5-expressing cells in human tonsils (3-5). In 2011, two papers compared this CXCR5⁺ T helper cell subset to blood Th cells that did not express CXCR5 (21, 22). The so-called circulating Tfh (cTfh) cells showed classical Tfh cell abilities as they were superior to their CXCR5⁻ counterparts in providing help to B cells as well as secreting IL-21 and IL-10 *in vitro* (21, 22). In contrast to GC Tfh cells, cTfh cells did not express Bcl-6 and only few cTfh cells expressed GC Tfh activation markers such as ICOS, which indicates that they are in a resting memory state (21-

26). But when co-cultured with naïve B cells, cTfh cells induced class-switch and immunoglobulin secretion which was dependent on cognate interaction of cTfh and B cells (21, 22).

Moreover, Morita et al. defined different subsets of cTfh cells according to their expression of the chemokine receptors CXCR3, which is typical for Th1 cells (27-29), and CCR6, which is mostly expressed on Th17 cells (30-32). $CXCR3^{+}CCR6^{-}$ cTfh cells, called cTfh1 cells, expressed the classical Th1-defining markers T-bet and IFNγ, while $CXCR3^{-}CCR6^{-}$ cTfh cells, called cTfh2 cells, expressed the Th2-defining markers GATA3, IL-4, IL-5, and IL-13, and $CXCR3^{-}CCR6^{+}$ cTfh17 cells expressed the classical Th17 markers Rorγt and IL-17 (21). Furthermore, the subsets differed in their IL-21 secretion as well as their ability to induce class-switch and immunoglobulin secretion of naïve B cells *in vitro*, with cTfh1 cells expressing low amounts of IL-21 and displaying insufficient B helper cell abilities, in contrast to cTfh2 and cTfh17 cells (21, 24).

Besides Tfh cells, other follicular cells can be found in the blood as well. Circulating Tfr (cTfr) cells can be identified according to their expression of regulatory markers as well as by CXCR5. They also suppress B and Tfh cells *in vitro*, although at a lower capacity than their lymphoid-resident counterparts (33). Even though the exact relationship between lymphoid tissue-resident Tfr cells and blood cTfr cells is still unclear, altered ratios of cTfh to cTfr cells have been reported for multiple pathological conditions (34-36).

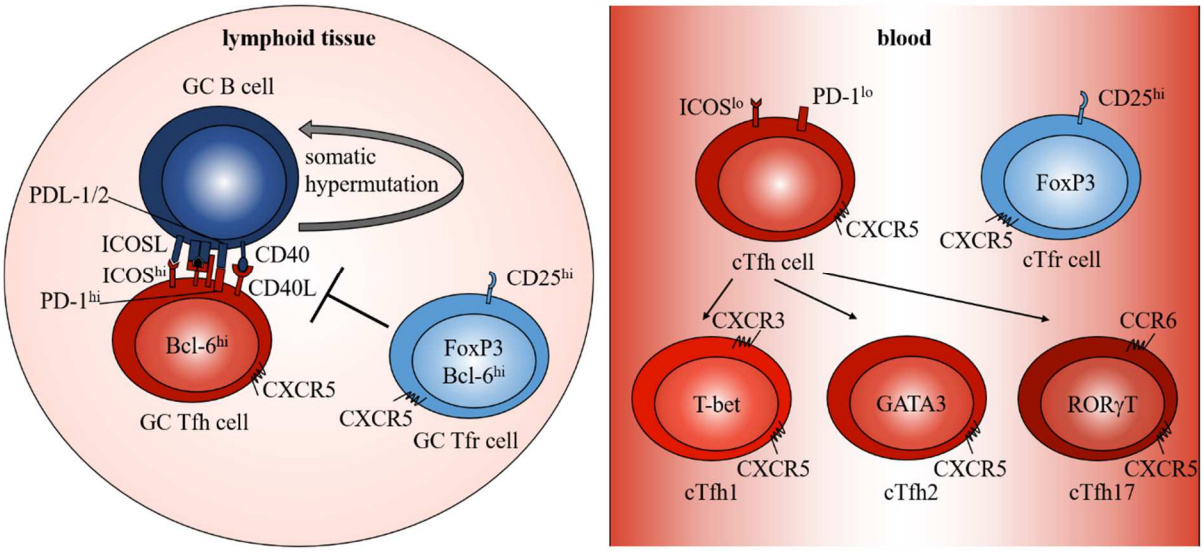

Figure 3. Comparison of lymphoid tissue- and blood-resident Tfh and Tfr cells.

There are several indications that support the potential role of cTfh cells as the circulating memory compartment of Tfh cells: cTfh cells contribute to the pool of antigen-specific memory cells, which are detectable even more than a decade after vaccination; certain subsets of Tfh cells expand upon antigen stimulation; and cTfh cells are able to recall their Tfh-specific effector functions after secondary antigen exposure (21-26, 37, 38). Additionally, it was shown that annual influenza vaccinations generated a

recurrent oligoclonal repertoire of vaccine-responsive, activated cTfh cells (37). This repertoire persisted in the non-activated cTfh compartment in the subsequent year (37).

1.4 The relationship of lymphoid tissue-resident and blood-resident Tfh cells

Although functional similarities are evident, the exact relationship between cTfh cells and GC Tfh cells is still unclear. From mouse studies it is known that GC Tfh cells can exit the GC (39, 40) and contribute to the pool of circulating $CXCR5^+$ T helper cells (23, 41, 42). Efforts have been made recently to investigate the origin, trafficking, and function of human cTfh cells as well. Patients with CD40L or ICOS deficiencies that lead to impaired GC generation, displayed decreased numbers of cTfh cells in their blood (43), which was a first indication that cTfh cells depend on GCs. Furthermore, activated cTfh cells correlated with the magnitude of newly generated Tfh cells in lymphoid tissue (26).

Several other papers provided evidence that activated cTfh cells are related to GC Tfh cells (25, 44-46). Comparisons of tonsillar GC Tfh cells and $PD\text{-}1^+$ cTfh cells not only showed that the two Tfh cell populations were similar in phenotype, function, and gene expression (21, 25, 26), but that they also were clonally related as they shared a common T cell receptor (TCR) repertoire, while non-Tfh cells were clearly clonally distinguishable (44, 45). Further evidence about the origin of cTfh cells was provided by a study that compared Tfh cells from lymph nodes (LN), blood, and the thoracic duct, the largest lymphatic vessel in the human body, which transports lymphocytes from multiple lymph nodes and releases them directly into the bloodstream (46). Tfh cells from the thoracic duct showed an intermediate phenotype between LN and blood Tfh cells with higher expression of migration-associated molecules like CXCR3 and CD69 and retention of GC marker expression. Furthermore, they showed that while Tfh cells from the thoracic duct and LN were transcriptionally and epigenetically similar, only the fraction of activated $ICOS^+CD38^+$ cTfh cells showed a similar transcriptional profile, whereas total blood Tfh cells differed from that signature. Additionally, Tfh cells from the thoracic duct and the blood shared common TCR sequences.

These findings were able to establish a direct connection between activated cTfh cells and GC Tfh cells with cTfh cells as the circulating memory compartment of lymphoid Tfh cells (23). This correlation between GC Tfh cells and circulating Tfh cells enabled further studies based on the investigation of cTfh cells during, for example, vaccinations, chronic infections, and autoimmune diseases. Nevertheless, not all cTfh cells might directly be derived from the GC reaction and are rather in a quiescent, recirculating cell state. Whether those quiescent cTfh cells are derived from extrafollicular germinal centers, obtain a quiescent phenotype after circulating in peripheral blood for a long time, are derived from $CXCR5^-$ memory T helper cells, or represent a separate lineage still remains an active field of investigation (26, 47).

1.5 Human cTfh cells during viral challenge

Due to their involvement in antibody generation, Tfh cells are critical players in the immune response and promising targets for therapeutic approaches and vaccine design (48). To gain insight into Tfh cell kinetics during primary and recall immune responses, vaccinations are an ideal model. Knowledge about cTfh cells during immune responses is mostly derived from influenza vaccinations that have been investigated for the contribution of Tfh cells to the immune response and memory formation (48, 49). Interestingly, cTfh1-polarized cells play a central role in the immune response after annual influenza vaccination. Activated cTfh1 cells transiently increased one week after vaccination, were highly enriched in influenza-specific cells, and correlated with antibody titers one week after vaccination (24, 26, 37, 50, 51). Impaired cTfh1 activation in elderly or human immunodeficiency virus (HIV)-infected people, however, lead to suboptimal antibody generation (52, 53). Similar observations were made after vaccination against human papilloma virus (HPV) where an increase in activated cTfh1 cells was observed one week after vaccination and this correlated with memory B cell frequencies one month later (54).

In the paper titled "Dynamic changes in circulating T follicular helper cell composition predict neutralizing antibody responses after yellow fever vaccination" we made use of the immune response to the yellow fever vaccine to investigate a primary viral immune response. As one of the most efficient vaccinations with 94% of vaccinees developing neutralizing antibodies it creates an ideal model to investigate an efficient immune response to a live virus in humans (55). Furthermore, vaccinees normally have not been in contact with the virus prior to immunization as the yellow fever virus (YFV) is endemic to tropical regions of South America and Africa. Therefore, in Germany, the vaccination usually induces a primary immune response. This is an advantage compared to studies with influenza vaccination as immune responses to influenza strains are usually mixed recall and primary immune responses.

Yellow fever still poses a threat today, as shown by the strong outbreak in Brazil in August 2018, which was one of the largest in decades (56). Infection with YFV causes flu-like symptoms, which can progress to hemorrhagic fever and organ failure. Vaccination with the attenuated YFV strain 17D has been used since the 1930s and can efficiently prevent infection (57).

A single vaccine shot provides life-long immunity and protective antibody titers can still be detected decades after immunization (58). Production of long-lasting titers of highly specific antibodies suggests the involvement of the GC. For that reason, I set out to analyze the cTfh cell response at various time points after vaccination by flow cytometry and correlated the results with the development of neutralizing antibodies. As for influenza vaccinations, the cTfh1 cell subset as well as activated cTfh1 cells expanded in frequency, although, this occurred two weeks after vaccination compared to one week after influenza vaccinations. YFV-specific CD4-positive T cells, as tested by tetramer staining, were detectable at the peak of the immune response in the blood and were predominantly Tfh1-polarized as

well. Furthermore, we observed an increase in the frequency of antibody-producing cells, which also peaked at day 14 post vaccination. Interestingly, the frequency of cTfh1 cells and activated cTfh1 cells correlated positively with the amount of neutralizing antibody on day 28 post vaccination while the frequency of cTfh17 and activated cTfh17 cells correlated negatively. This study provides valuable insight into the role of cTfh cells after yellow fever vaccination and sheds a new light on the role of cTfh1 cells, which were mostly described as cTfh cells with insufficient B helper cell qualities. This might contribute to the development of improved vaccination strategies.

As a shared first author of the paper "Dynamic changes in circulating T follicular helper cell composition predict neutralizing antibody responses after yellow fever vaccination" I designed, performed and analyzed all flow cytometry experiments while my co-first author conducted the study. I additionally wrote most of the initial manuscript draft. The exact contributions to the paper are stated under 2.1.

Further reports about cTfh cells in patients chronically infected with HIV or hepatitis C virus (HCV) suggest that targeting cTfh cells might be a promising therapeutical approach. In HIV^+ patients, it was shown that PD1-expressing $CXCR3^-$ cTfh cell frequencies correlated with the development of broadly neutralizing antibodies (25). In contrast, in chronically HCV-infected patients it was shown that $CXCR3^+$ cTfh cells correlated with the magnitude of the neutralizing antibody response (59). Taken together, those results indicate that the polarization of cTfh cells has a great impact on the development of a long-lasting and highly specific antibody response and that the ideal polarization may be dependent on the pathogen.

1.6 Human cTfh cells in autoimmune diseases

Increased frequencies of cTfh cells or an altered polarization have been described in various disease settings (60, 61). An imbalance of cTfh cell subsets (increased frequencies of cTfh2 and/or cTfh17 cells) observed in juvenile dermatomyositis, adult systemic lupus erythematosus (SLE), and Sjögren's syndrome (SS) patients was associated with disease activity, auto-antibody serum levels, and the frequency of pathogenic B cells (21, 62, 63).

Furthermore, the involvement of auto-antibodies and ectopic lymphoid structures (ELS) in the pathogenesis of multiple autoimmune diseases suggests a role of the GC and Tfh cells. SLE, SS, and rheumatoid arthritis (RA) are examples of autoimmune diseases in which ELS are formed in affected tissue (64-66) and the abundance of auto-antibodies is connected to disease activity (67-69). Additionally, disease activity or severity in SLE, SS, and RA were linked to increased numbers of one or both PD-1- and ICOS-expressing cTfh cells (70-73). Increased cTfh frequencies and/or their correlation with higher disease activity have also been described in patients with autoimmune thyroid disease (74), myasthenia gravis (75), systemic sclerosis (76), and Graves' disease (77), amongst other autoimmune diseases (61). Even though the precise contribution of Tfh cells to the pathogenesis of most

autoimmune diseases is still an active field of research, the generation of auto-antibody-secreting or pro-inflammatory, pathogenic B cells during an aberrant GC reaction implies defective Tfh cell help. It was shown that autoreactive B cells are derived from a GC reaction in SLE (78), that activated cTfh cell frequencies correlate with pathogenic B cell frequencies (79), and that auto-antibodies display a high degree of affinity and are class-switched (61).

Multiple sclerosis (MS) is an autoimmune disease characterized by damage to the central nervous system (CNS) caused by both cellular (T cell-mediated) and humoral (antibody-mediated) immune responses against various components of the CNS (80). This results in neurological damage and disability affecting 2.5 million people worldwide (81). The main autoreactive T helper cell subset involved in the pathogenesis of MS and its animal model experimental autoimmune encephalomyelitis (EAE) is Th17 cells. CCR6-expressing Th17 cells enter the CNS via a CCL20 (ligand of CCR6) gradient in the choroid plexus (82). Furthermore, Th17 cells express an array of proinflammatory cytokines such as IL-17, IL-23, GM-CSF, and IFNγ, and thereby act as encephalitogenic cells that disrupt the blood brain barrier or instruct phagocytes to damage CNS tissue (83, 84).

Although MS is generally not considered an autoantibody-mediated autoimmune disease, the presence of oligoclonal bands in the cerebrospinal fluid and of ELS in the meninges of MS patients, which correlate with disease progression in secondary progressive MS patients (85), suggest an involvement of Tfh cells.

Moreover, B cells are believed to contribute to MS pathology and an overrepresentation of proinflammatory B cell subsets in the peripheral blood of MS patients, an infiltration of proinflammatory cytokine-secreting B cells into the CNS, and the success of B cell depleting MS therapies by anti-CD20 administration are strong indications for the involvement of B cells in MS (86-90).

In the paper titled “Fingolimod profoundly reduces frequencies and alters subset composition of circulating T follicular helper cells in multiple sclerosis patients” we showed that the frequencies of cTfh and other CXCR5-expressing T cells in the blood of MS patients treated with the sphingosine-1-phosphate receptor (S1PR) 1 modulator fingolimod, were preferentially reduced compared to other T cell subsets. This effect was not observed with other immunomodulatory drugs that targeted lymphocyte migration via integrin α4 or depleted CD20-expressing B cells. Fingolimod treatment further changed the composition of cTfh cells and lead to increased frequencies of activated and cTfh1-polarized cells. Furthermore, B cell subsets considered as pathogenic, were reduced in frequency in the blood after fingolimod treatment, whereas regulatory B cells were increased in frequency. We were not able to confirm differences in circulating Tfh cells of MS patients and healthy controls as suggested by another study (85). Nevertheless, with our study we were able to provide insights into the mechanism of an established immunomodulatory drug. Additionally, the study provides insights into the migration behavior of cTfh cells and other $CD4^+$ T cell subsets residing in peripheral blood.

I designed, performed and analyzed all experiments shown in the paper “Fingolimod profoundly reduces frequencies and alters subset composition of circulating T follicular helper cells in multiple sclerosis patients” and wrote the initial manuscript draft. The exact contributions are stated under 3.1.

In general, with the results shown in this thesis, I contributed to a better understanding of cTfh cells in response to viral challenge and immunomodulation, the potential role of their polarization for the humoral immune response, and their migration potential to secondary lymphoid tissue. This will hopefully add to new therapeutic approaches for vaccine design and treatment of autoimmune diseases.

2 Publication I

Dynamic changes in circulating T follicular helper cell composition predict neutralising antibody responses after yellow fever vaccination

Johanna E. Huber, Julia Ahlfeld, Magdalena K. Scheck, Magdalena Zaucha, Klaus Witter, Lisa Lehmann, Hadi Karimzadeh, Michael Pritsch, Michael Hoelscher, Frank von Sonnenburg, Andrea Dick, Giovanna Barba-Spaeth, Anne B. Krug, Simon Rothenfußer, Dirk Baumjohann

Clinical & Translational Immunology; 2020; 9: e1129

DOI: https://doi.org/10.1002/cti2.1129

2.1 Contributions to publication I

As a shared co-first author, I performed and designed all flow cytometry experiments and analyzed the data derived from these experiments, while my co-first author Julia Ahlfeld coordinated the previously established yellow fever vaccine study cohort. Simon Rothenfußer and Anne Krug initiated and established the yellow fever vaccine study cohort. Dirk Baumjohann formulated the research question on studying Tfh cells in the context of the yellow fever vaccine, guided the study design, provided funding, and initiated contact with the collaborators in the clinical department. Dirk Baumjohann and I contributed to the interpretation of data and conception of the study with contributions from Julia Ahlfeld and Simon Rothenfußer. Dirk Baumjohann and I had the idea to use tetramers for the detection of yellow fever-specific $CD4^+$ T cells and through literature research and investigation of the prevalent HLA types of previous study participants, I found suitable tetramers and established the staining with them. Furthermore, I wrote most of the initial manuscript draft with contributions to the introduction and methods section by Julia Ahlfeld. I also created all figures used in the paper. The draft was adapted, corrected, and finalized together with Dirk Baumjohann, Julia Ahlfeld, and Simon Rothenfußer with minor contributions from Anne Krug. Magdalena Scheck performed preliminary measurements of the antibody titers and contributed to the methods section of the manuscript. The measurement of neutralizing antibodies was performed by Magdalena Zaucha and Lisa Lehmann. Giovanna Barba-Spaeth established the method for the measurement of the neutralizing antibodies. Klaus Witter and Andrea Dick performed the HLA sequencing. Vaccinations and collection of the blood samples were performed by Michael Pritsch under supervision of Michael Hoelscher and Frank von Sonnenburg. Hadi Karimzadeh helped with the interpretation of the antibody data.

3 Publication II

Fingolimod Profoundly Reduces Frequencies and Alters Subset Composition of Circulating T Follicular Helper Cells in Multiple Sclerosis Patients

Johanna E. Huber, Yinshui Chang, Ingrid Meinl, Tania Kümpfel, Edgar Meinl and Dirk Baumjohann

The Journal of Immunology; March 1, 2020; 204 (5) 1101-1110

DOI: https://doi.org/10.4049/jimmunol.1900955

3.1 Contributions to publication II

For this study, I performed all experiments and data analysis of those experiments. I also designed the flow cytometry panel used in this study. Additionally, I found the connection between fingolimod treatment and loss of CCR7-expressing cells through literature research and had the idea to analyze different $CD4^+$ T cell subsets from an already published RNA-sequencing dataset according to their expression of various migration markers. This dataset was then re-analyzed by Yinshui Chang. Furthermore, I wrote most of the initial manuscript draft with contributions to the introduction by Dirk Baumjohann. I also created all figures used in the paper. The manuscript draft was adapted, corrected, and finalized together with Dirk Baumjohann and Edgar Meinl. Ingrid Meinl and Tania Kümpfel provided the clinical samples. Dirk Baumjohann and I contributed to the interpretation of data and conception of the study with contributions from Tania Kümpfel and Edgar Meinl. Dirk Baumjohann formulated the research question, guided the study design, provided funding, and initiated contact with the collaborators in the clinical department.

4 References

1. Mosmann TR, Coffman RL. TH1 and TH2 Cells: Different Patterns of Lymphokine Secretion Lead to Different Functional Properties. Annual Review of Immunology. 1989;7(1):145-73.
2. Ansel KM, McHeyzer-Williams LJ, Ngo VN, McHeyzer-Williams MG, Cyster JG. In Vivo–Activated Cd4 T Cells Upregulate Cxc Chemokine Receptor 5 and Reprogram Their Response to Lymphoid Chemokines. Journal of Experimental Medicine. 1999;190(8):1123-34.
3. Breitfeld D, Ohl L, Kremmer E, Ellwart J, Sallusto F, Lipp M, et al. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. J Exp Med. 2000;192(11):1545-52.
4. Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of CXCR5+ T cells: B helper activity is focused in a germinal center-localized subset of CXCR5+ T cells. J Exp Med. 2001;193(12):1373-81.
5. Schaerli P, Willimann K, Lang AB, Lipp M, Loetscher P, Moser B. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. J Exp Med. 2000;192(11):1553-62.
6. Johnston RJ, Poholek AC, DiToro D, Yusuf I, Eto D, Barnett B, et al. Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. Science. 2009;325(5943):1006-10.
7. Nurieva RI, Chung Y, Martinez GJ, Yang XO, Tanaka S, Matskevitch TD, et al. Bcl6 mediates the development of T follicular helper cells. Science. 2009;325(5943):1001-5.
8. Yu D, Rao S, Tsai LM, Lee SK, He Y, Sutcliffe EL, et al. The transcriptional repressor Bcl-6 directs T follicular helper cell lineage commitment. Immunity. 2009;31(3):457-68.
9. Crotty S. T Follicular Helper Cell Biology: A Decade of Discovery and Diseases. Immunity. 2019;50(5):1132-48.
10. Glatman Zaretsky A, Taylor JJ, King IL, Marshall FA, Mohrs M, Pearce EJ. T follicular helper cells differentiate from Th2 cells in response to helminth antigens. J Exp Med. 2009;206(5):991-9.
11. Tsuji M, Komatsu N, Kawamoto S, Suzuki K, Kanagawa O, Honjo T, et al. Preferential generation of follicular B helper T cells from Foxp3+ T cells in gut Peyer's patches. Science. 2009;323(5920):1488-92.
12. Yeh C-H, Nojima T, Kuraoka M, Kelsoe G. Germinal center entry not selection of B cells is controlled by peptide-MHCII complex density. Nature Communications. 2018;9(1).
13. Schwickert TA, Victora GD, Fooksman DR, Kamphorst AO, Mugnier MR, Gitlin AD, et al. A dynamic T cell–limited checkpoint regulates affinity-dependent B cell entry into the germinal center. 2011;208(6):1243-52.
14. Abbott RK, Lee JH, Menis S, Skog P, Rossi M, Ota T, et al. Precursor Frequency and Affinity Determine B Cell Competitive Fitness in Germinal Centers, Tested with Germline-Targeting HIV Vaccine Immunogens. Immunity. 2018;48(1):133-46.e6.
15. Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3+ follicular regulatory T cells control the germinal center response. Nature medicine. 2011;17(8):975-82.
16. Chung Y, Tanaka S, Chu F, Nurieva RI, Martinez GJ, Rawal S, et al. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. Nature medicine. 2011;17(8):983-8.
17. Wollenberg I, Agua-Doce A, Hernandez A, Almeida C, Oliveira VG, Faro J, et al. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. J Immunol. 2011;187(9):4553-60.
18. Sage PT, Francisco LM, Carman CV, Sharpe AH. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. Nat Immunol. 2013;14(2):152-61.
19. Lim HW, Hillsamer P, Banham AH, Kim CH. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. J Immunol. 2005;175(7):4180-3.
20. Forster R, Emrich T, Kremmer E, Lipp M. Expression of the G-protein--coupled receptor BLR1 defines mature, recirculating B cells and a subset of T-helper memory cells. Blood. 1994;84(3):830-40.

21. Morita R, Schmitt N, Bentebibel S-E, Ranganathan R, Bourdery L, Zurawski G, et al. Human Blood CXCR5+CD4+ T Cells Are Counterparts of T Follicular Cells and Contain Specific Subsets that Differentially Support Antibody Secretion. Immunity. 2011;34(1):108-21.
22. Chevalier N, Jarrossay D, Ho E, Avery DT, Ma CS, Yu D, et al. CXCR5 Expressing Human Central Memory CD4 T Cells and Their Relevance for Humoral Immune Responses. The Journal of Immunology. 2011;186(10):5556.
23. Hale JS, Ahmed R. Memory T follicular helper CD4 T cells. Front Immunol. 2015;6:16.
24. Bentebibel SE, Lopez S, Obermoser G, Schmitt N, Mueller C, Harrod C, et al. Induction of ICOS+CXCR3+CXCR5+ TH cells correlates with antibody responses to influenza vaccination. Sci Transl Med. 2013;5(176):176ra32.
25. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, et al. Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. Immunity. 2013;39(4):758-69.
26. He J, Louis, Yew, Hu X, Cindy, Chevalier N, et al. Circulating Precursor CCR7loPD-1hi CXCR5+ CD4+ T Cells Indicate Tfh Cell Activity and Promote Antibody Responses upon Antigen Reexposure. Immunity. 2013;39(4):770-81.
27. Sallusto F, Lanzavecchia A, Mackay CR. Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. Immunol Today. 1998;19(12):568-74.
28. Bonecchi R, Bianchi G, Bordignon PP, D'Ambrosio D, Lang R, Borsatti A, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. J Exp Med. 1998;187(1):129-34.
29. Rabin RL, Alston MA, Sircus JC, Knollmann-Ritschel B, Moratz C, Ngo D, et al. CXCR3 is induced early on the pathway of CD4+ T cell differentiation and bridges central and peripheral functions. J Immunol. 2003;171(6):2812-24.
30. Acosta-Rodriguez EV, Rivino L, Geginat J, Jarrossay D, Gattorno M, Lanzavecchia A, et al. Surface phenotype and antigenic specificity of human interleukin 17-producing T helper memory cells. Nat Immunol. 2007;8(6):639-46.
31. Annunziato F, Cosmi L, Santarlasci V, Maggi L, Liotta F, Mazzinghi B, et al. Phenotypic and functional features of human Th17 cells. J Exp Med. 2007;204(8):1849-61.
32. Singh SP, Zhang HH, Foley JF, Hedrick MN, Farber JM. Human T cells that are able to produce IL-17 express the chemokine receptor CCR6. J Immunol. 2008;180(1):214-21.
33. Sage PT, Alvarez D, Godec J, von Andrian UH, Sharpe AH. Circulating T follicular regulatory and helper cells have memory-like properties. The Journal of Clinical Investigation. 2014;124(12):5191-204.
34. Xu B, Wang S, Zhou M, Huang Y, Fu R, Guo C, et al. The ratio of circulating follicular T helper cell to follicular T regulatory cell is correlated with disease activity in systemic lupus erythematosus. Clinical immunology (Orlando, Fla). 2017;183:46-53.
35. Yan L, Li Y, Li Y, Wu X, Wang X, Wang L, et al. Increased circulating Tfh to Tfr ratio in chronic renal allograft dysfunction: a pilot study. BMC Immunol. 2019;20(1):26-.
36. Wang X, Yang C, Xu F, Qi L, Wang J, Yang P. Imbalance of circulating Tfr/Tfh ratio in patients with rheumatoid arthritis. Clin Exp Med. 2019;19(1):55-64.
37. Herati RS, Muselman A, Vella L, Bengsch B, Parkhouse K, Del Alcazar D, et al. Successive annual influenza vaccination induces a recurrent oligoclonotypic memory response in circulating T follicular helper cells. Sci Immunol. 2017;2(8).
38. Rivino L, Messi M, Jarrossay D, Lanzavecchia A, Sallusto F, Geginat J. Chemokine receptor expression identifies pre–T helper (Th) 1, pre–Th2, and nonpolarized cells among human CD4+ central memory T cells. The Journal of experimental medicine. 2004;200(6):725-35.
39. Shulman Z, Gitlin AD, Targ S, Jankovic M, Pasqual G, Nussenzweig MC, et al. T follicular helper cell dynamics in germinal centers. Science. 2013;341(6146):673-7.
40. Suan D, Nguyen A, Moran I, Bourne K, Hermes JR, Arshi M, et al. T follicular helper cells have distinct modes of migration and molecular signatures in naive and memory immune responses. Immunity. 2015;42(4):704-18.
41. Marshall HD, Chandele A, Jung YW, Meng H, Poholek AC, Parish IA, et al. Differential expression of Ly6C and T-bet distinguish effector and memory Th1 CD4(+) cell properties during viral infection. Immunity. 2011;35(4):633-46.

42. Pepper M, Pagan AJ, Igyarto BZ, Taylor JJ, Jenkins MK. Opposing signals from the Bcl6 transcription factor and the interleukin-2 receptor generate T helper 1 central and effector memory cells. Immunity. 2011;35(4):583-95.
43. Bossaller L, Burger J, Draeger R, Grimbacher B, Knoth R, Plebani A, et al. ICOS deficiency is associated with a severe reduction of CXCR5+CD4 germinal center Th cells. J Immunol. 2006;177(7):4927-32.
44. Brenna E, Davydov AN, Ladell K, McLaren JE, Bonaiuti P, Metsger M, et al. CD4+ T Follicular Helper Cells in Human Tonsils and Blood Are Clonally Convergent but Divergent from Non-Tfh CD4+ Cells. Cell Reports. 2020;30(1):137-52.e5.
45. Heit A, Schmitz F, Gerdts S, Flach B, Moore MS, Perkins JA, et al. Vaccination establishes clonal relatives of germinal center T cells in the blood of humans. The Journal of Experimental Medicine. 2017;214(7):2139-52.
46. Vella LA, Buggert M, Manne S, Herati RS, Sayin I, Kuri-Cervantes L, et al. T follicular helper cells in human efferent lymph retain lymphoid characteristics. Journal of Clinical Investigation. 2019.
47. Boswell KL, Paris R, Boritz E, Ambrozak D, Yamamoto T, Darko S, et al. Loss of circulating CD4 T cells with B cell helper function during chronic HIV infection. PLoS Pathog. 2014;10(1):e1003853.
48. Ueno H. Tfh cell response in influenza vaccines in humans: what is visible and what is invisible. Curr Opin Immunol. 2019;59:9-14.
49. Aljurayyan A, Puksuriwong S, Ahmed M, Sharma R, Krishnan M, Sood S, et al. Activation and Induction of Antigen-Specific T Follicular Helper Cells Play a Critical Role in Live-Attenuated Influenza Vaccine-Induced Human Mucosal Anti-influenza Antibody Response. J Virol. 2018;92(11).
50. Bentebibel SE, Khurana S, Schmitt N, Kurup P, Mueller C, Obermoser G, et al. ICOS(+)PD-1(+)CXCR3(+) T follicular helper cells contribute to the generation of high-avidity antibodies following influenza vaccination. Sci Rep. 2016;6:26494.
51. Koutsakos M, Wheatley AK, Loh L, Clemens EB, Sant S, Nussing S, et al. Circulating TFH cells, serological memory, and tissue compartmentalization shape human influenza-specific B cell immunity. Sci Transl Med. 2018;10(428).
52. Herati RS, Reuter MA, Dolfi DV, Mansfield KD, Aung H, Badwan OZ, et al. Circulating CXCR5+PD-1+ response predicts influenza vaccine antibody responses in young adults but not elderly adults. J Immunol. 2014;193(7):3528-37.
53. George VK, Pallikkuth S, Parmigiani A, Alcaide M, Fischl M, Arheart KL, et al. HIV infection Worsens Age-Associated Defects in Antibody Responses to Influenza Vaccine. J Infect Dis. 2015;211(12):1959-68.
54. Matsui K, Adelsberger JW, Kemp TJ, Baseler MW, Ledgerwood JE, Pinto LA. Circulating CXCR5(+)CD4(+) T Follicular-Like Helper Cell and Memory B Cell Responses to Human Papillomavirus Vaccines. PloS one. 2015;10(9):e0137195.
55. Niedrig M, Lademann M, Emmerich P, Lafrenz M. Assessment of IgG antibodies against yellow fever virus after vaccination with 17D by different assays: neutralization test, haemagglutination inhibition test, immunofluorescence assay and ELISA. Trop Med Int Health. 1999;4(12):867-71.
56. Faria NR, Kraemer MUG, Hill SC, Goes de Jesus J, Aguiar RS, Iani FCM, et al. Genomic and epidemiological monitoring of yellow fever virus transmission potential. Science. 2018;361(6405):894-9.
57. Theiler M, Smith HH. THE USE OF YELLOW FEVER VIRUS MODIFIED BY IN VITRO CULTIVATION FOR HUMAN IMMUNIZATION. J Exp Med. 1937;65(6):787-800.
58. Poland JD, Calisher CH, Monath TP, Downs WG, Murphy K. Persistence of neutralizing antibody 30-35 years after immunization with 17D yellow fever vaccine. Bull World Health Organ. 1981;59(6):895-900.
59. Zhang J, Liu W, Wen B, Xie T, Tang P, Hu Y, et al. Circulating CXCR3(+) Tfh cells positively correlate with neutralizing antibody responses in HCV-infected patients. Scientific reports. 2019;9(1):10090-.
60. Ueno H, Banchereau J, Vinuesa CG. Pathophysiology of T follicular helper cells in humans and mice. Nature Immunology. 2015;16(2):142-52.
61. Gensous N, Charrier M, Duluc D, Contin-Bordes C, Truchetet ME, Lazaro E, et al. T Follicular Helper Cells in Autoimmune Disorders. Front Immunol. 2018;9:1637.

62. Le Coz C, Joublin A, Pasquali JL, Korganow AS, Dumortier H, Monneaux F. Circulating TFH subset distribution is strongly affected in lupus patients with an active disease. PloS one. 2013;8(9):e75319.
63. Li XY, Wu ZB, Ding J, Zheng ZH, Li XY, Chen LN, et al. Role of the frequency of blood CD4(+) CXCR5(+) CCR6(+) T cells in autoimmunity in patients with Sjogren's syndrome. Biochem Biophys Res Commun. 2012;422(2):238-44.
64. Chang A, Henderson SG, Brandt D, Liu N, Guttikonda R, Hsieh C, et al. In situ B cell-mediated immune responses and tubulointerstitial inflammation in human lupus nephritis. J Immunol. 2011;186(3):1849-60.
65. Corsiero E, Nerviani A, Bombardieri M, Pitzalis C. Ectopic Lymphoid Structures: Powerhouse of Autoimmunity. Frontiers in immunology. 2016;7:430-.
66. Nocturne G, Mariette X. B cells in the pathogenesis of primary Sjogren syndrome. Nat Rev Rheumatol. 2018;14(3):133-45.
67. Xing R, Sun L, Wu D, Jin Y, Li C, Liu X, et al. Autoantibodies against interleukin-21 correlate with disease activity in patients with rheumatoid arthritis. Clin Rheumatol. 2018;37(1):75-80.
68. Elsenosy FM, Mousa SG, Mohamed NA, Mohamed RH. Study of Antichromatin Antibodies As A Marker of Lupus Activity and Lupus Nephritis. Egypt J Immunol. 2018;25(1):113-24.
69. Nigolian H, Ribi C, Courvoisier DS, Pagano S, Alvarez M, Trendelenburg M, et al. Anti-apolipoprotein A-1 autoantibodies correlate with disease activity in systemic lupus erythematosus. Rheumatology (Oxford). 2019.
70. Simpson N, Gatenby PA, Wilson A, Malik S, Fulcher DA, Tangye SG, et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum. 2010;62(1):234-44.
71. Wang J, Shan Y, Jiang Z, Feng J, Li C, Ma L, et al. High frequencies of activated B cells and T follicular helper cells are correlated with disease activity in patients with new-onset rheumatoid arthritis. Clin Exp Immunol. 2013;174(2):212-20.
72. Szabo K, Papp G, Barath S, Gyimesi E, Szanto A, Zeher M. Follicular helper T cells may play an important role in the severity of primary Sjogren's syndrome. Clin Immunol. 2013;147(2):95-104.
73. Brokstad KA, Fredriksen M, Zhou F, Bergum B, Brun JG, Cox RJ, et al. T follicular-like helper cells in the peripheral blood of patients with primary Sjogren's syndrome. Scand J Immunol. 2018:e12679.
74. Zhu C, Ma J, Liu Y, Tong J, Tian J, Chen J, et al. Increased frequency of follicular helper T cells in patients with autoimmune thyroid disease. J Clin Endocrinol Metab. 2012;97(3):943-50.
75. Luo C, Li Y, Liu W, Feng H, Wang H, Huang X, et al. Expansion of circulating counterparts of follicular helper T cells in patients with myasthenia gravis. J Neuroimmunol. 2013;256(1-2):55-61.
76. Ricard L, Jachiet V, Malard F, Ye Y, Stocker N, Riviere S, et al. Circulating follicular helper T cells are increased in systemic sclerosis and promote plasmablast differentiation through the IL-21 pathway which can be inhibited by ruxolitinib. Ann Rheum Dis. 2019;78(4):539-50.
77. Liu Y, Yuan X, Li X, Cui D, Xie J. Constitutive Changes in Circulating Follicular Helper T Cells and Their Subsets in Patients with Graves' Disease. J Immunol Res. 2018;2018:8972572.
78. Hostmann A, Jacobi AM, Mei H, Hiepe F, Dorner T. Peripheral B cell abnormalities and disease activity in systemic lupus erythematosus. Lupus. 2008;17(12):1064-9.
79. Choi JY, Ho JH, Pasoto SG, Bunin V, Kim ST, Carrasco S, et al. Circulating follicular helper-like T cells in systemic lupus erythematosus: association with disease activity. Arthritis Rheumatol. 2015;67(4):988-99.
80. Hemmer B, Kerschensteiner M, Korn T. Role of the innate and adaptive immune responses in the course of multiple sclerosis. Lancet Neurol. 2015;14(4):406-19.
81. Frohman EM, Racke MK, Raine CS. Multiple sclerosis--the plaque and its pathogenesis. The New England journal of medicine. 2006;354(9):942-55.
82. Reboldi A, Coisne C, Baumjohann D, Benvenuto F, Bottinelli D, Lira S, et al. C-C chemokine receptor 6-regulated entry of TH-17 cells into the CNS through the choroid plexus is required for the initiation of EAE. Nat Immunol. 2009;10(5):514-23.
83. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, Bernard M, et al. Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nature medicine. 2007;13(10):1173-5.

84. Croxford AL, Spath S, Becher B. GM-CSF in Neuroinflammation: Licensing Myeloid Cells for Tissue Damage. Trends in Immunology. 2015;36(10):651-62.
85. Romme Christensen J, Bornsen L, Ratzer R, Piehl F, Khademi M, Olsson T, et al. Systemic inflammation in progressive multiple sclerosis involves follicular T-helper, Th17- and activated B-cells and correlates with progression. PloS one. 2013;8(3):e57820.
86. Montalban X, Hauser SL, Kappos L, Arnold DL, Bar-Or A, Comi G, et al. Ocrelizumab versus Placebo in Primary Progressive Multiple Sclerosis. The New England journal of medicine. 2017;376(3):209-20.
87. Hauser SL, Bar-Or A, Comi G, Giovannoni G, Hartung HP, Hemmer B, et al. Ocrelizumab versus Interferon Beta-1a in Relapsing Multiple Sclerosis. The New England journal of medicine. 2017;376(3):221-34.
88. Hauser SL, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. The New England journal of medicine. 2008;358(7):676-88.
89. Brändle SM, Obermeier B, Senel M, Bruder J, Mentele R, Khademi M, et al. Distinct oligoclonal band antibodies in multiple sclerosis recognize ubiquitous self-proteins. Proceedings of the National Academy of Sciences of the United States of America. 2016;113(28):7864-9.
90. Quinn JL, Axtell RC. Emerging Role of Follicular T Helper Cells in Multiple Sclerosis and Experimental Autoimmune Encephalomyelitis. International journal of molecular sciences. 2018;19(10).

5 Acknowledgements

I would like to take the opportunity to thank all people that supported me during my PhD project.

First, I would like to thank my supervisor Prof. Dr. Dirk Baumjohann for the guidance throughout the project, unlimited support, valuable advice on the projects, always acknowledging and respecting my input, the freedom to develop own ideas, and the creation of an amazing working atmosphere. I wish you all the best for Bonn.

Next, I would like to thank my co-author Dr. Julia Ahlfeld for organising the yellow fever study, always accommodating my requests, support for the publication, and a very enjoyable collaboration. Furthermore, I would like to thank Prof. Dr. Simon Rothenfußer for letting us participate in the study and for the input. I would also like to thank Dr. Magdalena Zaucha for measuring the neutralizing antibody titers, which were essential for the paper and all the other co-authors of the paper for providing samples and input.

I would also like to thank the people involved in the multiple sclerosis project. First and foremost, Prof. Dr. Edgar Meinl, who provided valuable input on the B cell part and to the writing of the paper. And I would like to thank Dr. Ingrid Meinl, Prof. Dr. Tania Kümpfl, and clinic staff for providing me with the samples and the nice collaboration.

Furthermore, I would like to thank my thesis advisory committee Prof. Dr. Anne Krug, Prof. Dr. Thomas Brocker, and Prof. Dr. Veit Hornung for advice and guidance during my project.

I would also like to thank everyone at the Institute for Immunology for a very cooperative and friendly working environment and for always helping when needed.

Next, I would of course like to thank my group who were the most amazing co-workers I could have wished for. First, my rotational student Eva Maria Eckl who was of great help to me during my first projects, which didn't make it in my thesis though. Then, I would like to thank Dr. Angelika Schmidt for very helpful discussions and input for my papers. Yinshui Chang for the contribution to our paper and for always instantly jumping to help when needed. And especially Frank Dahlström, Dominik Alterauge, and Julia Zeiträg who I spent a great time with starting the group and setting up the lab. I am very grateful for the lab management, input, and teaching you provided. Thank you all for always supporting me and making work fun. The working atmosphere you created was incredible and carried me through bad days and I will miss working with you all dearly.

Last, but not least, I would like to thank my family and friends, especially Regina and Hans Huber. I am extremely grateful for your constant and enormous support, encouragement, and motivation in hard times as well as the joy you shared with me about my achievements. Without the balance and stability you gave me I would have never been able to finish my PhD.

6 List of publications

6.1 Earlier publications

- **Tunneling nanotubes enable intercellular transfer of MHC class I molecules.**

  Schiller C, Huber JE, Diakopoulos KN, Weiss EH.
  Hum Immunol. 2013 Apr; 74(4): 412-6.
  doi: 10.1016/j.humimm.2012.11.026. [Epub 2012 Dec 7]

6.2 Publications during the PhD project

- **Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition)**

  Cossarizza A, Chang HD, Radbruch A, …, Baumjohann D, …, Huber JE, …, Zielinski C, Zimmermann J, Zychlinsky A.
  Eur J Immunol. 2019 Oct; 49(10):1457-1973.
  doi: 10.1002/eji.201970107.

- **Complex human adenoid tissue-based ex vivo culture systems reveal anti-inflammatory drug effects on germinal center T and B cells**

  Schmidt A, Huber JE, Sercan Alp Ö, Gürkov R, Reichel CA, Herrmann M, Keppler OT, Leeuw T, Baumjohann D.
  EBioMedicine. 2020 Feb 27; 53:102684.
  doi: 10.1016/j.ebiom.2020.102684. [Epub ahead of print]

- **Fingolimod Profoundly Reduces Frequencies and Alters Subset Composition of Circulating T Follicular Helper Cells in Multiple Sclerosis Patients**

  Huber JE, Chang Y, Meinl I, Kümpfel T, Meinl E, Baumjohann D.
  J Immunol. 2020 Mar 1; 204(5): 1101-1110.
  doi: 10.4049/jimmunol.1900955

- **Dynamic changes in circulating T follicular helper cell composition predict neutralising antibody responses after yellow fever vaccination**

  Huber JE, Ahlfeld J, Scheck MK, Zaucha M, Witter K, Lehmann L, Karimzadeh H, Pritsch M, Hoelscher M, von Sonnenburg F, Dick A, Barba-Spaeth G, Krug AB, Rothenfußer S, Baumjohann D.
  Clin Transl Immunol. 2020 May 13; 9: e1129.
  doi:10.1002/cti2.1129