Immunology Letters 162 (2014) 39–47

Contents lists available at ScienceDirect

Immunology Letters

journal homepage: www.elsevier.com/locate/immlet

Review

Follicular helper T cell-mediated mucosal barrier maintenance

Colleen J. Winstead *

University of Alabama at Birmingham, Department of Pathology, Birmingham, AL, United States

ARTICLE INFO

Article history:
Available online 19 August 2014

Keywords:
Follicular helper T cells
Germinal center
Microbiota
IgA
Gut
Plasticity

ABSTRACT

The basic functions of the immune system are protection from pathogens and maintenance of tolerance to self. The maintenance of commensal microbiota at mucosal surfaces adds a layer of complexity to these basic functions. Recent reports suggest follicular helper T cells (Tfh), a CD4$^+$ T cell subset specialized to provide help to B cells undergoing isotype switching and affinity maturation in germinal centers (GC), interact with the microbiota and are essential to maintenance of mucosal barriers. Complicating the issue is ongoing controversy in the field regarding origin of the Tfh subset and its distinction from other effector CD4 T cell phenotypes (Th1/Th17/Treg). This review focuses on the differentiation, phenotypic plasticity, and function of CD4 T cells, with an emphasis on commensal-specific GC responses in the gut.

1. Introduction

There are one hundred trillion microorganisms housed in the human body and microbial genes in this microbiota outnumber ours 100 to 1 [1]. These microorganisms are maintained as commensals on the skin, in the airways, in the genital tract, and in the lumen of the gut by the immune system. The delicate balance with host immunity is sensitive to disruption both by competition between commensals and by environmental factors external to but which impact the microbiota, namely diet, antibiotic use, and pathogenic infection. Breach of the barrier constructed by mucosal immunity and exposure to the circulation can change commensals from mutualists to pathobionts and trigger an inflammatory, anti-microbial immune response [2].

Many types of adaptive immune cells contribute to biochemical and physical maintenance of mucosal barriers, however, as demonstrated by studies of mucosal barrier function in the context of immunodeficiency, CD4 T cells and B cells are crucial [3]. Microbial products play a positive role in differentiation and homeostasis of gut-resident CD4 T cells, notably, induction of the Th1, Th17, and regulatory T cell (Treg) phenotypes essential for control of inflammatory responses [4]. With the advent of studies on germinal centers (GCs) and importance of antibody to barrier maintenance, the importance of T cells that locate to GCs and facilitate this process is gaining appreciation. The follicular helper T cell (Tfh) field is relatively new and evolving. A controversial topic in the plasticity field, which is also debated extensively in a field devoted to pathology of infection and systemic immunity, is whether Tfh originate from naïve cells or differentiate functionally from other activated CD4 T cell phenotypes [5,6]. This is important in the context of mucosal vaccine development and the role of microbiota in complex disease aetiologies – both pathogen-driven and autoimmune. In this review, we will discuss recent developments in the study of mucosal adaptive immunity with a focus on the gut germinal center reaction, CD4 T cell phenotypic plasticity, and growing appreciation for the role of follicular helper T cells (Tfh) in barrier maintenance.

2. Establishing the mucosal barrier to commensal microbes

2.1. Composition of the gut microbiome

Through years of work characterizing and manipulating the mouse and human microbiomes, we have come to appreciate its importance in establishing and maintaining healthy mucosal immunity. The intestines house the greatest abundance and diversity of microbes, and the relationship of gut microbes with host immunity is truly mutualistic, in that microbes benefit from the nutrient-rich environment of the gut while simultaneously aiding the host with digestion and providing essential vitamins. The commensal microbiota also protects against mucosal pathogenic infection by virtue of competitive advantage – a concept termed 'colonization resistance' [7]. Taking up residence in the intestinal lumen, commensal microbes limit physical access of acquired pathogens to the gut epithelium. Microbial composition in the human gut is complex and individualized [8]. Heavily influenced by health and environment, increasing usage of antibiotics and global travel by humans has necessitated mechanistic studies

* Tel.: +12059346532.
E-mail addresses: winstecj@yahoo.com, winstecj@uab.edu

http://dx.doi.org/10.1016/j.imlet.2014.07.015
0165-2478/

modeling commensal dysbiosis and complex disease in mice [9]. The composition of the adult human gut microbiome known to modulate intestinal and systemic immunity is concentrated in two phyla: the Bacteroidetes and the Firmicutes. Three additional phyla found in many people are minor constituents and include the Actinobacteria, Proteobacteria, and Verrucomicrobia [8,10]. The genetic diversity of microbe species found within each phylum is so vast that researchers have found incorporation of functional diversity measures based on host age and disease state necessary for characterization and study of any given individual's microbiota [8]. In order to study autoimmune and/or pathogenic intestinal disease of which adaptive (i.e. antigen-specific) immunity to commensals is a major component, researchers have relied largely on monocolonization of mice with a limited number of microbes expressing known specific antigens for which a repertoire of B and T cells have been identified [11].

2.2. Basic anatomy of the mucosal barrier

Intestinal homeostasis is maintained by a sophisticated anatomical and chemical barrier, co-evolved with microbes to which animals are constitutively exposed [12]. The first line of defense limiting direct contact of luminal microbial contents with the intestinal surface consists of glycoproteins forming a penetration-resistant mucus layer. The mucosal immune 'firewall' consists of an epithelial and intra-epithelial cell (IEC) layer, including mucus-secreting and anti-microbial protein-producing goblet and Paneth cells residing in the crypts, and through which commensal antigens and some whole microbes are sampled by underlying cells in the lamina propria (LP) [13]. Intestine draining mesenteric lymph nodes (MLN), Peyer's patches (PP), and isolated lymphoid follicles (ILF) are organized mucosa-associated lymphoid tissues (MALT) of the gut that constitute 'inductive' sites where T and B cells are activated by antigen transported from the LP to undergo differentiation and clonal expansion [14]. The LP serves as the mucosal 'effector' site, where cells are recruited from the inductive sites, and where the majority of affinity-matured, antibody-producing B cells reside. It is an area of diffuse connective tissue through which is transported mucus layer-bound anti-microbial peptides and commensal-specific secretory immunoglobulin (sIg), primarily IgA (discussed below).

2.3. B cell activation and the gut germinal center reaction

The Peyer's patches, major inductive sites of mucosal immunity in the gut, consist of multiple B cell follicles built on a network of specialized, follicle-restricted antigen-presenting cells (follicular dendritic cells; FDC). Inter-follicular regions or 'T cell zones' separating follicles contain T and dendritic cells tasked with constant sampling of antigens delivered through the overlaying epithelium. Components of the overlaying IEC, microfold cells (M cells) and goblet cells act as extensions of the follicle itself, mediating the transport of bacteria and luminal antigens to resident macrophages and FDC [13,15,16]. The germinal center (GC) reaction initiates as B cells are activated by antigen-loaded FDC and/or bacterial component interaction with pattern-recognition receptors (i.e. toll-like receptors; TLRs) in an area of the GC termed the 'light zone'. The activated B cells undergo rapid clonal expansion, forming a 'dark zone' area adjacent to the T cell zone, the border to which CD4 T cells activated in inter-follicular regions are recruited as helpers for proliferation and further B cell maturation [17]. T cell help-dependent extrafollicular plasmablasts are short-lived. Plasmablasts that develop in response to bacterial components in the absence of T cell help may live for months and, in part, sustain the GC response [18]. Full differentiation of selected GC B cells into long-lived immunoglobulin-secreting plasma cells requires expression of the enzyme activation-induced cytidine deaminase (AID), which catalyzes mutations in the immunoglobulin heavy chain gene essential for affinity maturation and isotype switching [19]. Some of the helped B cells are fated to fill the memory B cell niche, available to respond to recall antigen stimulation with rapid production of affinity-matured antibody.

2.4. Commensal-specific IgA

Unlike under conditions of systemic pathogenic infection, GCs in the PPs and MLNs are constantly maintained by a variety of antigens from the gut. A major mechanism of gut barrier maintenance is secretion of commensal-specific IgA by B cells [20,21]. By far the most abundant antibody isotype produced in the body, secretory IgA (sIgA) plays a crucial role in 'immune exclusion' of the intestinal microbiota [22]. Secreted as microbe-coating dimers by plasma cells in the lamina propria, they target the intestinal lumen. Upon binding to the polymeric immunoglobulin receptor at the basolateral side of IECs, the complexes are actively transcytosed to the apical membrane, where the receptor is cleaved. The sIgA released constitutes a hybrid of the polymeric IgA and the secretory component of the receptor [23]. Among their many activities, sIgA complexes entrap antigens – from food, environmental toxins, pathogens, and commensals – in the mucus layer, preventing exposure to epithelial cell surface receptors [24]. They also inhibit bacterial motility and down-regulate flagellar gene expression [25]. This is essential to maintenance of the intestinal barrier, as mutualism of phyla in the gut lumen – Bacteriodetes, Firmicutes, and Proteobacteria – is maintained through suppression of their flagellar motility. The vaginal microbiome, passed to an otherwise germ-free neonate at birth, is important for the health of both mother and offspring. IgA initially delivered to neonates through their mother's milk prior to full development of their own immunity helps to establish a healthy immune barrier to and regulate establishment of the gut microbiome [26]. Full maturation of B cells into high-affinity IgA-secreting plasma cells in the PPs helps to maintain the immune barrier through weaning and into adulthood. This process is dependent on CD4 T cell help in the form of cell-to-cell interactions and provision of cytokine. The expression of AID and induction of affinity maturation by germinal center B cells is critical to the regulation of intestinal microflora [19]. AID expression is dependent on signaling through the TNF superfamily co-stimulatory molecule CD40, the ligand for which is expressed on activated T cells – particularly CD4 helper T cells bearing a follicular phenotype [27,28]. It is unknown whether TLR-stimulated B cells that appear activated without engagement of BCR survive to seed the memory B cell pool, or whether these cells are terminally differentiated as plasma cells secreting Ig, particularly IgA [29,30]. Some isotype-switched but low affinity Ig, including IgA, is known to be T-independent and may arise from GC-independent memory B cells in the ILF and LP induced to express AID by toll-like receptor ligation and/or inflammatory cytokine signaling [22,31,32].

2.5. Activation and B cell helper function of commensal-specific CD4+ T cells

An individual's CD4 T cell repertoire is established as a barrier to infection. CD4 T cell selection occurs in the thymus and is dependent on T cell antigen receptor (TCR) recognition of self-antigens presented by specialized antigen-presenting cells (APCs) in the thymic medulla [33]. Cells that recognize self-antigen too strongly are deleted. Those that survive selection, including cells that have low affinity for self and/or self-like commensal or food antigens, migrate to the periphery as a naïve population with highly diverse antigen specificity [34,35]. Naïve CD4 T cells are activated and phenotypic differentiation is initiated upon contact with MHCII

presented by activated, antigen-bearing dendritic cells in the secondary lymphoid tissues. Commensal-specific naïve CD4 T cells are activated and begin to differentiate in the MLNs upon interaction with commensal antigen-bearing migratory DCs transported to these inductive sites from the PP and ILF via the afferent lymphatics. These migratory DCs also upregulate gut-homing molecules on the activated T cells, which then exit via efferent lymphatics into the circulation and home to the gut, where they complete polarization to effector phenotypes. In the gut, activated CD4 T cells encounter mucosal dendritic cells and resident macrophages that have sampled commensal antigens. They, and other innate cells essential to this process, produce a host of factors that influence T cell differentiation, including stimulatory factors IL-6 and IL-23, and regulatory factors TGF-β, retinoic acid (RA), and IL-10 [36]. Under homeostatic/non-infectious conditions, it is believed these recently activated, commensal-specific CD4 T cells overwhelmingly adopt one of three effector phenotypes – Th1/Th17/Foxp3+ regulatory CD4 T cell (Treg) – each characterized by expression of a lineage-defining transcription factor and production of a particular anti-microbial cytokine. Distinct from these three phenotypes are the follicular helper T cells (Tfh). Specifically suited for interaction with B cells, Tfh cells express unique surface proteins conferring the ability to migrate into B cell areas of the MALT. The acquisition of a follicular phenotype in CD4 T cells happens in parallel with development of the germinal center B cell response. In a mutual exchange, Tfh cells reinforce AID expression in GC B cells (discussed above) while at the same time GC B cells provide re-encounter with antigen and co-stimulation that reinforces the molecular program of Tfh cells initiated upon encounter of commensal antigen-bearing DCs.

3. Follicular helper T cells in the germinal center: what we know of their phenotype and function

The Tfh was first described more than 13 years ago in human tonsil, a GC-rich MALT draining the nasopharynx and upper respiratory tract. Found in close proximity to CD40-expressing B cells undergoing affinity maturation in the GC, these antigen-experienced CD4 T cells expressed high levels of CD40L and the chemokine receptor CXCR5 [37–39]. The expression of CD40L on memory T cells was known to be essential to GC B cell survival [40]. However, prior to this discovery CXCR5 was only known as essential for B cell responsiveness to FDC secreting the chemokine CXCL13 and the formation of organized follicles. Our current understanding of the follicular helper T cell (Tfh) phenotype is largely based on anatomical location and the functional consequences of cell-to-cell interactions (T to B) within lymphoid follicles [41,42].

Another distinguishing Tfh feature held in common with GC B cells is expression of the master regulating transcription factor Bcl-6 [43]. Expression of Bcl-6 by both cells is essential, in that the GC reaction is completely abolished in its absence [44,45]. Closely correlated with expression of CXCR5, intrinsic Bcl-6 expression by recently activated CD4 T cells reinforces the Tfh differentiation program through repression of miRNAs targeting expression of Tfh phenotypic markers (e.g. inducible co-stimulator (ICOS), programmed death-1 (PD-1), and CXCR5) and by repression of its antagonist, the non-follicular effector T cell-associated transcription factor Blimp-1 [43,46,47].

Cell surface expression of inhibitory PD-1 by Tfh cells, commonly used to identify the GC-specific follicular phenotype, has cell-intrinsic functional as well as phenotypic consequences [48]. Closely correlated with expression of Bcl-6, PD-1 is essential for the generation of gut commensal-specific, affinity-matured IgA via its role in differentiation, regulation of proliferation, and cytokine production by Tfh cells [49–51]. It is necessary for somatic hyper-mutation (SHM) and affinity maturation of B cells in PP GCs draining the gut. PD-1 signaling restrains Tfh differentiation, effectively regulating the helper capacity of these cells to GC B cells [52]. Deficiency in PD-1 results in breach of the commensal barrier and a systemic inflammatory response, implying that regulated Tfh cells expressing high levels of PD-1 are essential for mucosal barrier maintenance.

In order to enter the follicle, CD4 T cells adopting a follicular phenotype down-regulate expression of the T cell zone chemokine receptor CCR7 [53]. Accumulating evidence suggests that Tfh also down-regulate expression of P-selectin glycoprotein ligand 1 (PSGL-1) as part of their differentiation program. As PSGL-1 can bind CCR7 ligands CCL19 and CCL21, down-regulation prevents migration away from GCs to T cell zones [54–57]. Physical interaction with GC B cells is facilitated by Tfh expression of co-stimulators CD40L and ICOS as well as by the expression of the myeloid lineage regulatory Ig protein CD200 and the signaling adapter protein SAP [58,59]. Co-expression of CXCR4 in some Tfh is correlated with high expression of CD40L and IL-21, and may serve as an additional cell surface marker of Tfh function [60].

IL-21, a member of the common γ family of cytokines of which IL-2 is also a member, is the signature cytokine produced by Tfh cells. In much the same way that provision of IL-2 can serve as a rheostat for survival and proliferation versus activated-induced cell death of responders, secretion of IL-21 by Tfh cells has pleiotropic effects on responding cells. Production of IL-21 is regulated, in part, by PD-1 expression on Tfh. PD-1 deficiency results in expansion of Tfh that do not produce IL-21, impairing B cell maturation and immunoglobulin production [61,62]. Direct IL-21 signaling in B cells is essential for isotype switching to IgA, in that it is essential for development of extrafollicular plasmablasts (the majority of which in the gut produce IgA). It also enhances B cell expression of Bcl-6, which in turn enhances their survival and memory potential [61,63–66]. IL-21 is also necessary for maintenance of the Th17 phenotype, and Th17 cells resident to the small intestine lamina propria, themselves able to produce some IL-21, are reportedly crucial for production of high-affinity T cell-dependent IgA and assist Tfh in maintenance of the gut immune barrier (discussed below) [67].

Similar in function to PD-1, expression of the inhibitory receptor B- and T-lymphocyte attenuator (BTLA) on Tfh cells may regulate IL-21 expression. BTLA expression is not required for the Tfh phenotype, although absence leads to hyperglobulinemia and autoimmunity. This suggests it could be an important mediator for commensal-specific reactions, where Tfhs are constantly being stimulated, but where a memory/inflammatory reaction to them (generating IgG) would be detrimental [68,69].

4. Origin of the follicular helper CD4 T cell phenotype at mucosal barrier sites

Whether the follicular CD4 T cell phenotype is completely distinct from or reflects plasticity with other effector CD4 T cell phenotypes is a controversial topic in the field of T cell biology [70]. Difficulty in identifying endogenous naïve, commensal-specific CD4 T cells has made studies of their differentiation to the Tfh phenotype for promotion of GC responses a challenge and has necessitated studies employing adoptive transfer of cells specific for viral and pathogenic bacterial antigens. On a very basic level, the ability of T cells to interconvert between phenotypes is an important compensatory mechanism to maintain mucosal barriers to the constantly fluctuating commensal composition [71]. In an era focused on design of targeted therapy to regulate/generate/alter phenotypic immune responses, studies of CD4 T cell phenotypic plasticity under a variety of homeostatic and inflammatory

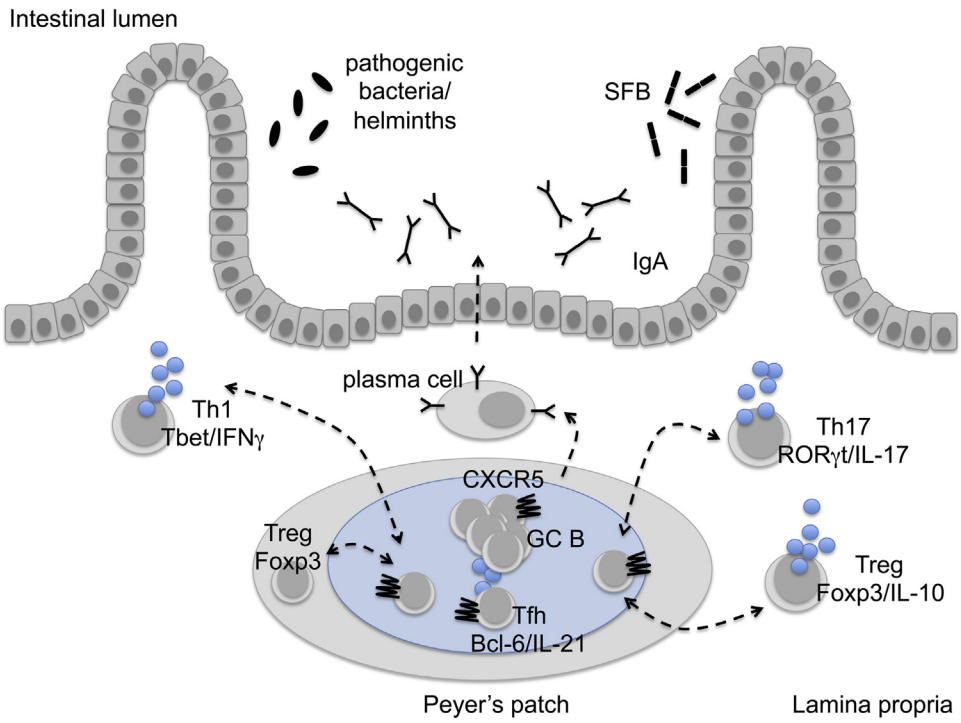

Fig. 1. Germinal center-dependent plasticity of follicular phenotype cells with other helper CD4+ T cell phenotypes in the intestinal mucosae. Naïve CD4+ T cells specific for pathogenic or commensal bacteria (e.g. SFB) are activated upon antigen encounter in gut-draining lymphoid tissue. These activated cells migrate to the lamina propria where they complete their differentiation to Th1, Th17, and Foxp3+ regulatory phenotypes, expressing anti-bacterial cytokines in an effort to maintain the barrier and reduce inflammation-associated damage. Demonstrated influence of each phenotype on plasma cell production of IgA suggests an as yet unknown degree of plasticity with the germinal center-localizing follicular helper phenotype.

contexts is warranted, but difficult to resolve. Complicating the matter is the fact that many characteristics of the follicular phenotype overlap with non-Tfh effector cells, including Bcl-6 expression. Though its antagonist Blimp-1 is expressed highly in all non-Tfh effector cells, serving as a distinguishing feature from the follicular phenotype, it is clear from sampling of human blood that Tfh-like cells may be maintained in the absence of Bcl-6 expression [72]. It is also clear that an anti-microbial cytokine attributed to one particular helper T cell phenotype may be expressed to some degree by cells of another phenotype [73]. In the case of the follicular helper phenotype – distinguished from other effector phenotypes primarily by location – plasticity based on cytokine expression is germinal center dependent and has been reported for Th1, Th17, and activated Tregs in multiple in vivo infectious and non-infectious contexts (Fig. 1).

4.1. Th1 and Tfh: distinct phenotypes?

Th1 cells, important for cytotoxic immunity to viruses and intracellular pathogens, are identified by expression of the transcription factor Tbet and their signature cytokine IFNγ. Found in abundance in the gut, Th1 cells have demonstrated phenotypic plasticity with and, under inflammatory conditions associated with pathogenicity inducing expression of IL-12, Tbet, and Runx transcription factors, may arise from Th17 cells [74,75]. To this end, Th1 cells expressing IL-21 in response to abundant inflammation-driven IL-12 signaling have been noted in the intestines of both mice and humans with inflammatory bowel disease [76]. Despite the fact that they were defined and characterized decades ago, we are just beginning to understand the relationship of Th1s to the relatively newly described Tfh phenotype.

Initial characterization of plasticity between the Th1 and Tfh phenotypes focused on the molecular mechanisms of fate determination and suggested a complete bifurcation of phenotypes immediately upon naïve CD4 T cell activation by DCs. In a series of studies published in Immunity in 2011, evidence presented for early bifurcation of phenotypes emphasized the role cell-to-cell interaction with B cells in the GC plays in maintaining bifurcation through reinforcement of the Tfh phenotype and balance of transcription factors Bcl-6 and Blimp-1 in CD4 T cells responding to acute viral infection or protein immunization [77–79]. In a study published in 2013 by Choi et al. [80] using the same acute viral infection system, this early commitment to a Tfh fate was confirmed stable and maintained into memory after pathogen clearance. Another study published in 2011 by Nakayamada et al. [81] emphasized the similarity in transcriptional profiles between Th1 and Tfh cells and suggested cells fated for the Th1 differentiation pathway acquired a profile in common with Tfh, but suppressed Tfh functions via Tbet in mice infected systemically with the obligate intracellular parasite *Toxoplasma gondii*. The contributions of acute cytokine signaling to the molecular program and fate determination of CD4 T cells followed in published studies a year later. A careful, mechanistic study conducted by Johnston et al. [82] on the role of STAT5 signaling in cells responding to viral infection suggested IL-2 signaling in the presence of Blimp-1 expression blocked Bcl-6 and differentiation of the Tfh phenotype. In a complementary study, Oestreich et al. [83] suggested IL-2 signaling in CD4 T cells polarized in vitro to a Th1 phenotype inhibited Bcl-6 expression and enforced a Tbet and Blimp-1 expression profile resulting in suppression of CXCR5 and the Tfh phenotype. These findings were confirmed shortly after in a clinically relevant in vivo setting in which mice infected with influenza developed defective lung MALT GC B cell responses when treated systemically with IL-2 [84]. The results of a study published by Tubo et al. [85] regarding the importance of TCR signaling to Th1/Tfh fate determinations suggest dwell time of TCR with p-MHCII presented by APCs dictates CD4 T differentiation fate, in that shorter dwell times result in Th1 differentiation whereas prolonged interaction between TCR and APC

program a Tfh fate. This is consistent with a 2011 study published by Fahey et al. [86] suggesting the persistence of viral antigen can redirect Th1 cells to a Tfh phenotype. This divergence of fate based on kinetics of TCR signaling makes logical sense if one considers the dichotomy of a pathogenic infection requiring sensitive and rapid activation of anti-microbicidal Th1 function, culled after pathogen clearance, versus chronic antigen exposure provided by commensal bacteria. In support of this rationale, a recently published study using repeated exposure of mice to antigen from the intestinal pathogen Salmonella typhi in the form of a vaccine elicited IFNγ-producing follicular helper T cell responses that promoted B cell memory responses and the generation of protective antibody [87].

4.2. Plasticity of Th17 cells with Th1/Tfh/Treg phenotypes

Appreciation for the importance of Th17 cells to host defense and their association with the pathogenesis of autoimmunity has gained momentum in recent years as our understanding of the form and function of the commensal microbiome has increased [88]. Th17 cells home to the LP where they reinforce the barrier through expression of the transcription factor RORγt and production of signature cytokines IL-22 and IL-17 [89]. Under steady-state conditions, Th17 cells are non-pathogenic and demonstrate phenotypic plasticity with Tregs. This plasticity is maintained in the small intestine LP by continuously sampled commensal antigen and TGFβ, a required cytokine for induction of both phenotypes [90,91]. Under conditions where regulation is impaired (e.g. inflammatory bowel disease or IBD) these cells may become pathogenic. Pathogenic Th17 cells, especially those resident to the gut, demonstrate phenotypic and functional plasticity with Th1 cells [74]. Co-expression of the transcription factors RORγt and Tbet in these cells results in co-expression of the cytokines IL-17 and IFNγ [75]. Together, these cytokines triggering pro-inflammatory pathways in innate cell targets (e.g. intestinal epithelium, neutrophils, and macrophages) resulting in recruitment and amplification of anti-microbial defenses, some of which cause collateral damage to the mucosal barrier [92].

In a recent study published in PLOS Pathogens by Li and McSorley, mice deficient in B cells (uMT) exhibited systemic dissemination of bacteria following intra-vaginal infection with *Chlamydia muridarum* [93]. Pathology of the infection was attributed to altered priming of antigen-specific T cells and a reduction in anti-microbial IFNγ and IL-17 in lymphoid tissues draining the reproductive tract [93]. A corroborative study published by Andrew et al. [94] in *PLoS ONE* (2013) is consistent with these findings and suggest Th17 plasticity with Th1 cells is especially important for protection of the genital tract from infection. These results are consistent with the role B cells play in maintenance of the mucosal barrier through secretion of IgA and conversion of resident regulatory and effector cells (Th1 and Th17) to the follicular helper T cell phenotype necessary for maintenance of the firewall under homeostatic conditions.

Early studies suggested Tregs were responsible for the induction of IgA in the gut through their conversion to a follicular helper phenotype [95,96]. However, recent work suggests Treg are not necessary for this process and that adoptively transferred 'natural' (thymus-derived) Tregs do not convert to a follicular helper phenotype upon migration to the PP [67]. Rather, it is suggested plasticity between the Th17 and Tfh CD4 T cell phenotypes are responsible for induction of commensal-specific IgA in the gut. This disconnect may be explained by focus on study of the regulatory follicular helper T cell phenotype (discussed below), which may have been included in transfer populations of Foxp3+ cells. Th17 cells are responsible for producing the polymeric immunoglobulin receptor that transports IgA across the mucosal barrier. This production is dependent on exposure of these cells to a single species of commensal Firmicutes that adheres to the intestinal epithelium, the segmented filamentous bacteria (SFB) [97]. Germ-free and antibiotic-treated mice lacking intestinal Th17 are reconstituted upon mono-colonization with SFB. In complementary studies on this discovery published in Cell and Immunity in 2009, the authors note this reconstitution not only restores the steady-state balance of T-dependent cytokines, it also restores anti-microbial defenses essential to protection from intestinal pathogens [98,99]. Based on the role the follicular CD4 T cell phenotype plays in generation of high-affinity IgA by GC B cells, these findings suggested a link from the commensal microbiota to Th17/Tfh conversion.

This suggested link was confirmed in a seminal paper published in 2013 by Hirota et al. The authors used a fluorescent fate-reporter mouse model to demonstrate steady-state homing of Th17 cells to PPs and IL-23-independent plasticity with the Tfh phenotype. Importantly, employing bone marrow reconstitution and adoptive transfer of reporter cells into T cell-deficient mice, they demonstrated the absolute requirement of this conversion for GC B cell help to differentiate high-affinity antigen-specific IgA following immunization with cholera toxin B [67]. Potentially significant to the design of immune therapies to treat IBD, this study links cholera toxin B-induced plasticity of Th17 cells with the follicular phenotype.

Debate regarding the role of IL-21 in development and maintenance of Th17 and Tfh cells plays a large part in controversy surrounding the concept of Th17/Tfh functional plasticity [100]. Though not required for development of the phenotype, Th17 cells produce IL-21 and require signaling for maintenance. Differentiation of Th17 that occurs in the absence of IL-21 is likely due to redundancy provided by IL-6 signaling [65,101]. Differentiation of the Tfh phenotype is dependent on IL-21 signaling, but reportedly independent of expression of the Th17 master regulator RORγt [100]. Thus, the conversion of Th17 to Tfh in PP observed by Hirota et al. may be independent of IL-21, or IL-21-dependent Tfh phenotype commitment is regulated in an autocrine loop downstream of programming initiated by IL-6 signaling [101,102]. Consistent with this theory is the documented ability of mice genetically deficient in IL-21 receptor expression to differentiate Th17 cells in LP and MLN and to produce IgA [103]. Reconciling Tfh production of IL-21 with Th17 cells producing IL-21 suggests the helpful functions of IL-21 may require proper regulation imparted by interaction of ICOS on Tfh cells with ICOSL-expressing B cells [64,100,102,104].

4.3. Tregs: plasticity with or distinct from Tfh?

Compelling published evidence suggests cross-reactivity to self-antigens impacts positive selection in the thymus and TCR avidity of circulating pathogen-specific T cells [105]. Research has demonstrated that some foreign antigen-specific T cells selected in the thymus cross-react to commensal bacteria antigens, and that breach of the mucosal epithelial barrier and exposure of the commensal microbiota to the circulation can result in an anti-microbial immune response triggering autoimmunity and cancer [106,107]. Central tolerance induction in the thymus selects for T cells of intermediate affinity that will react strongly to foreign pathogens but maintain tolerance to self. The resulting repertoire that leaves the thymus and populates the periphery constitutes a mix of foreign and self antigen-specific naïve precursors, a percentage of which function to suppress the reactivity of the others. T cells selected in the thymus with reactivity to self that survive deletion adopt a regulatory phenotype characterized by expression of the repressive transcription factor Foxp3. Published evidence suggests some cells that react to commensal antigens are not deleted in the thymus, but instead are purposed for barrier maintenance in mucosal tissues. These mucosa-resident Foxp3+ regulatory T cells function to maintain gut homeostasis and prevent establishment

of commensal-specific adaptive immune memory through mechanisms we are just beginning to appreciate. A study published in Science in 2009 employed adoptive transfer of Foxp3+ CD4 T cells into CD3ε-deficient recipients to demonstrate the preferential conversion of these cells into follicular helpers, promoting induction of GC formation in PPs and IgA-producing cells in the gut [96]. Results suggested that, although down-regulation of Foxp3 expression does not require B cells, B cells and CD40/CD40L signaling are required for acquisition of the Tfh phenotype. The authors noted that IL-6 provided by mucosal DCs enhanced IL-21 expression in responding CD4 T cells, effectively reinforcing the follicular helper phenotype. This conversion of Foxp3+ cells into Tfh takes place preferentially in the PP, but Foxp3- T cells can convert to Tfh systemically in response to systemic immunization. The enrichment of plastic Tregs in the gut may be related to antigen availability and what antigens the T cells are responding to (i.e. commensal antigens) [96,108].

This is consistent with the role of Tfh cells in creating and maintaining the plasma cell/secretory IgA mucosal firewall, in that naïve CD4 T cells encountering high antigen doses and/or engaged in prolonged interaction with antigen-presenting cells (APCs) are more likely to adopt a follicular helper T cell rather than an anti-microbial effector fate [85]. This result was corroborated by a study published later the same year that suggested regulatory T cells were necessary for generation of protective intestinal IgA in the context of commensal-specific CD4 T cell-induced inflammation [95].

4.4. Regulatory Tfh cells: perhaps the best example of Treg/Tfh plasticity

Regulatory T cells resident to the mucosa typically express the Bcl-6 antagonist Blimp-1 and have high expression of IL-10. However, a small percentage of these cells can respond to T-dependent antigen and co-express Bcl-6, upregulate CXCR5, and migrate in to the GC to function as regulatory follicular helper T cells [109,110]. Plasticity in phenotypic differentiation between 'regulatory' and 'conventional' follicular helper T cells may be the key to establishing healthy/functional anti-commensal T and B cell immunity at mucosal barriers (gut/lung/skin/genital tract). In addition to the migration of thymus-derived Tregs to the follicle, regulatory follicular helper T cells may arise from the 'conventional' Tfh fraction in response to self-like/abbreviated antigen stimulation. In much the same way peripheral conversion of conventional CD4 T cells in MALT are thought to broaden the TCR repertoire beyond the thymus-derived regulatory pool, the conversion of follicular helpers to a regulatory phenotype may be necessary for tuning GC responses to a wide variety of antigenic stimuli, including those that are not overtly pathogenic [109]. Consistent with this theory, in the absence of strong activation resulting in the production of the homeostatic cytokine IL-21, a higher proportion of follicular helper T cells adopt a regulatory phenotype than a conventional one. They are characterized by the expression of all the Tfh phenotypic markers, exceptionally high PD-1, and Foxp3. Of research interest is the role these particular Tfh-regs play in autoimmune diseases when present.

5. miRNA-dependent Tfh differentiation: potential for resolution of controversy

The commensal microbiome establishes the miRNA 'landscape' in T cells important for confirmation and maintenance of early differentiation fate decisions [111]. As mentioned with the introduction of Bcl-6 in Section 3, the action of miRNAs in Tfh cells reinforces the differentiation program. Published studies linking early transcriptional profiles dictating CD4 helper T cell phenotype to the influence of the microbiota focus on the activity of the miR-17–92 family of miRNA clusters important for immune homeostasis. Though questions regarding plasticity between Tfh and other effector phenotypes were not addressed, results published by Kang et al. in 2013 suggest divergence of fates and control of chronic viral infection is dependent on the ability of miR-17–92-expressing Tfh cells to migrate into GCs. In a complementary study published by Baumjohann et al. in the same issue of Nature Immunology, the authors demonstrate a requirement for the same miRNA family for suppression of 'Tfh-inappropriate' gene programs inducing the Th17 and Th22 phenotypes in developing Tfh cells. These studies nicely complement published work characterizing Roquin$^{san/san}$ and combined Roquin-1 and -2 mutant mice. Disruption of the Roquin gene in mutant mice results in unregulated ICOS expression, uncontrolled Tfh differentiation, and development of lupus-like systemic autoimmunity and IBD due to spontaneous GC reactions [112,113]. One would presume conversion of conventional Tfh cells in these genetically modified mice to a regulatory phenotype is either not taking place or is not contributing significantly to progression of disease. It might be interesting to study Tfh differentiation, both conventional and regulatory, in Roquin$^{san/san}$ mice housed in a germ-free or gnotobiotic environment, given that the barrier-protective Th17 phenotype does not develop in germ-free mice.

6. Potential therapeutics to treat Tfh-related intestinal disease

There is clinical interest in Treg adoptive T cell therapy to treat autoimmunity. Published studies discussed above suggest the transfer of cholera toxin-specific Tregs into autoimmune patients may result in conversion of some of those Tregs into Tfh cells and potential restoration of the high-affinity, gut commensal specific IgA production and epithelial barrier in the gut. This, in theory, would be especially helpful in a patient suffering from reconstitution syndrome – i.e. a patient that has a full repertoire of T cells. Patients that are lymphopenic would still, in theory, run the risk of systemic autoimmunity, should those transferred cells convert and aberrantly induce B cell affinity maturation and antibody secretion.

Published data suggest older mice have decreased GC responses and that the decrease is dependent on the age of T cell, B cells, and the environment in which they are activated and differentiate. There is suggestion that aged humans have a skewed/altered microbiome composition and that this contributes to proclivity for IBD and Crohn's disease. This skewing of the microbiota may have to do, in part, with changes in diet as mammals age. All-trans retinoic acid (ATRA) is thought to link diet to microbiome homeostasis to mucosal immunity through its role in establishment of lymphoid follicles in gut-draining lymphoid tissue [114,115]. Recently published work suggests intraperitoneal delivery of ATRA to rats alleviated their autoimmunity by balancing their conventional versus regulatory T cell phenotypes (Tfh:Tfr and Th1/Th17/Treg) [116].

7. Concluding remarks

Research characterizing CD4 T cell phenotypic plasticity has significantly expanded our understanding of adaptive immunity as a whole. As a crucial link between T cell and humoral immunity, we are beginning to appreciate the central role of Tfh cells play in the mutualistic relationship we maintain with our commensal microbiota.

Much of the work done to characterize the Tfh cell phenotype has been done in mice – a research model wherein context-dependent anatomical measures are feasible. The larger

challenge is translation to humans, where identification through surface phenotyping of cells is the only currently feasible means of tracking the phenotype and drawing correlations to health and disease [41]. Studies focused on defining the Tfh subset in human and non-human primate health and disease are beginning to appear [117–120]. Recent advances with MHCII tetramer use to track antigen-specific CD4 T cells from human blood have led to the discovery of circulating pathogen-specific cells that are cross-reactive to commensal antigens, so the potential to correlate presence of these cells with phenotypes essential for homeostasis of immune barriers to the microbiome is on the verge of realization [107].

Acknowledgements

The author would like to thank Alexander Khoruts, MD and members of the UAB Program in Immunology for helpful discussions in preparation of this manuscript.

References

[1] Honda K, Littman DR. The microbiome in infectious disease and inflammation. Annu Rev Immunol 2012;30:759–95.
[2] Chow J, Tang H, Mazmanian SK. Pathobionts of the gastrointestinal microbiota and inflammatory disease. Curr Opin Immunol 2011;23:473–80.
[3] Guy-Grand D, Griscelli C, Vassalli P. Peyer's patches, gut IgA plasma cells and thymic function: study in nude mice bearing thymic grafts. J Immunol 1975;115:361–4.
[4] Faith JJ, Ahern PP, Ridaura VK, Cheng J, Gordon JI. Identifying gut microbe-host phenotype relationships using combinatorial communities in gnotobiotic mice. Sci Transl Med 2014;6:220ra11.
[5] Cannons JL, Lu KT, Schwartzberg PL. T follicular helper cell diversity and plasticity. Trends Immunol 2013:1–8.
[6] Gowthaman U, Chodisetti SB, Agrewala JN. T cell help to B cells in germinal centers: putting the jigsaw together. Int Rev Immunol 2010;29:403–20.
[7] Blumberg R, Powrie F. Microbiota, disease, and back to health: a metastable journey. Sci Transl Med 2012;4:137rv7.
[8] Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489:220–30.
[9] Rook GA. Regulation of the immune system by biodiversity from the natural environment: an ecosystem service essential to health. PNAS 2013;110:18360–7.
[10] Buffie CG, Pamer EG. Microbiota-mediated colonization resistance against intestinal pathogens. Nat Rev Immunol 2013;13:790–801.
[11] Hand TW, Santos Dos LM, Bouladoux N, Molloy MJ, Pagán AJ, Pepper M, et al. Acute gastrointestinal infection induces long-lived microbiota-specific T cell responses. Science 2012;337:1553–6.
[12] Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. Nature 2012;489:231–41.
[13] Peterson LW, Artis D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. Nat Rev Immunol 2014;14:141–53.
[14] Brandtzaeg P, Kiyono H, Pabst R, Russell MW. Terminology: nomenclature of mucosa-associated lymphoid tissue. Mucosal Immunol 2008;1:31–7.
[15] Farstad IN, Halstensen TS, Fausa O, Brandtzaeg P. Heterogeneity of M-cell-associated B and T cells in human Peyer's patches. Immunology 1994;83:457–64.
[16] Yamanaka T, Straumfors A, Morton H, Fausa O, Brandtzaeg P, Farstad I. M cell pockets of human Peyer's patches are specialized extensions of germinal centers. Eur J Immunol 2001;31:107–17.
[17] Vinuesa CG, Cyster JG. How T cells earn the follicular rite of passage. Immunity 2011;35:671–80.
[18] Hsu M-C, Toellner K-M, Vinuesa CG, MacLennan ICM. B cell clones that sustain long-term plasmablast growth in T-independent extrafollicular antibody responses. PNAS 2006;103:5905–10.
[19] Fagarasan S, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science 2002;298:1424–7.
[20] Fagarasan S, Kinoshita K, Muramatsu M, Ikuta K, Honjo T. In situ class switching and differentiation to IgA-producing cells in the gut lamina propria. Nature 2001;413:639–43.
[21] Fagarasan S, Honjo T. Intestinal IgA synthesis: regulation of front-line body defences. Nat Rev Immunol 2003;3:63–72.
[22] Pabst O. New concepts in the generation and functions of IgA. Nat Rev Immunol 2012;12:821–32.
[23] Brandtzaeg P. Mucosal immunity: induction, dissemination, and effector functions. Scand J Immunol 2009;70:505–15.
[24] Suzuki K, Ha S-A, Tsuji M, Fagarasan S. Intestinal IgA synthesis: a primitive form of adaptive immunity that regulates microbial communities in the gut. Semin Immunol 2007;19:127–35.
[25] Cullender TC, Chassaing B, Janzon A, Kumar K, Muller CE, Werner JJ, et al. Innate and adaptive immunity interact to quench microbiome flagellar motility in the gut. Cell Host Microbe 2013;14:571–81.
[26] Rogier EW, Frantz AL, Bruno MEC, Wedlund L, Cohen DA, Stromberg AJ, et al. Secretory antibodies in breast milk promote long-term intestinal homeostasis by regulating the gut microbiota and host gene expression. PNAS 2014;111:3074–9.
[27] Elgueta R, Benson MJ, de Vries VC, Wasiuk A, Guo Y, Noelle RJ. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev 2009;229:152–72.
[28] Shekhar S, Yang X. The darker side of follicular helper T cells: from autoimmunity to immunodeficiency. Cell Mol Immunol 2012;9:380–5.
[29] Taylor JJ, Pape KA, Jenkins MK. A germinal center-independent pathway generates unswitched memory B cells early in the primary response. J Exp Med 2012;209:597–606.
[30] Gloudemans AK, Plantinga M, Guilliams M, Willart MA, Ozir-Fazalalikhan A, van der Ham A, et al. The mucosal adjuvant cholera toxin B instructs non-mucosal dendritic cells to promote IgA production via retinoic acid and TGF-β. PLoS ONE 2013;8:e59822.
[31] Tsuji M, Suzuki K, Kitamura H, Maruya M, Kinoshita K, Ivanov II, et al. Requirement for lymphoid tissue-inducer cells in isolated follicle formation and T cell-independent immunoglobulin A generation in the gut. Immunity 2008;29:261–71.
[32] Bergqvist P, Gärdby E, Stensson A, Bemark M, Lycke NY. Gut IgA class switch recombination in the absence of CD40 does not occur in the lamina propria and is independent of germinal centers. J Immunol 2006;177:7772–83.
[33] Boehmer von H, Kisielow P. Self-nonself discrimination by T cells. Science 1990;248:1369–73.
[34] Blackman M, Kappler J, Marrack P. The role of the T cell receptor in positive and negative selection of developing T cells. Science 1990;248:1335–41.
[35] Hataye J. Naive and memory CD4+ T Cell survival controlled by clonal abundance. Science 2006;312:114–6.
[36] Cader MZ, Kaser A. Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. Gut 2013;62:1653–64.
[37] Schaerli P, Moser B. Chemokines: control of primary and memory T-cell traffic. Immunol Res 2005;31:57–74.
[38] 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;192:1545–52.
[39] Kim CH, Rott LS, Clark-Lewis I, Campbell DJ, Wu L, Butcher EC. Subspecialization of Cxcr5+ T cells. J Exp Med 2001;193:1373–81.
[40] Casamayor-Palleja M, Feuillard J, Ball J, Drew M, MacLennan IC. Centrocytes rapidly adopt a memory B cell phenotype on co-culture with autologous germinal centre T cell-enriched preparations. Int Immunol 1996;8:737–44.
[41] Tangye SG, Ma CS, Brink R, Deenick EK. The good, the bad and the ugly—TFH cells in human health and disease. Nat Rev Immunol 2013;13:412–26.
[42] Shulman Z, Gitlin AD, Targ S, Jankovic M, Pasqual G, Nussenzweig MC, et al. T follicular helper cell dynamics in germinal centers. Science 2013;341:673–7.
[43] 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;325:1001–5.
[44] Ye BH, Cattoretti G, Shen Q, Zhang J, Hawe N, de Waard R, et al. The BCL-6 proto-oncogene controls germinal-centre formation and Th2-type inflammation. Nat Genet 1997;16:161–70.
[45] Dent AL, Shaffer AL, Yu X, Allman D, Staudt LM. Control of inflammation, cytokine expression, and germinal center formation by BCL-6. Science 1997;276:589–92.
[46] 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;31:457–68.
[47] 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;325:1006–10.
[48] Okazaki T, Chikuma S, Iwai Y, Fagarasan S, Honjo T. A rheostat for immune responses: the unique properties of PD-1 and their advantages for clinical application. Nat Immunol 2013;14:1212–8.
[49] 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 2012;14:152–61.
[50] Maruya M, Kawamoto S, Kato LM, Fagarasan S. Impaired selection of IgA and intestinal dysbiosis associated with PD-1-deficiency. Gut Microbes 2013;4:165–71.
[51] Kawamoto S, Tran TH, Maruya M, Suzuki K, Doi Y, Tsutsui Y, et al. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. Science 2012;336:485–9.
[52] Rui Y, Honjo T, Chikuma S. Programmed cell death 1 inhibits inflammatory helper T-cell development through controlling the innate immune response. PNAS 2013;110:16073–8.
[53] Haynes NM, Allen CDC, Lesley R, Ansel KM, Killeen N, Cyster JG. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1 high germinal center-associated subpopulation. J Immunol 2007;179:5099–108.
[54] Odegard JM, Marks BR, DiPlacido LD, Poholek AC, Kono DH, Dong C, et al. ICOS-dependent extrafollicular helper T cells elicit IgG production via IL-21 in systemic autoimmunity. J Exp Med 2008;205:2873–86.

[55] Veerman KM, Williams MJ, Uchimura K, Singer MS, Merzaban JS, Naus S, et al. Interaction of the selectin ligand PSGL-1 with chemokines CCL21 and CCL19 facilitates efficient homing of T cells to secondary lymphoid organs. Nat Immunol 2007;8:532–9.
[56] 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;35:633–46.
[57] Poholek AC, Hansen K, Hernandez SG, Eto D, Chandele A, Weinstein JS, et al. In vivo regulation of Bcl6 and T follicular helper cell development. J Immunol 2010;185:313–26.
[58] McCausland MM, Yusuf I, Tran H, Ono N, Yanagi Y, Crotty S. SAP regulation of follicular helper CD4 T cell development and humoral immunity is independent of SLAM and Fyn kinase. J Immunol 2007;178:817–28.
[59] Chtanova T, Tangye SG, Newton R, Frank N, Hodge MR, Rolph MS, et al. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. J Immunol 2004;173:68–78.
[60] Elsner RA, Ernst DN, Baumgarth N. Single and coexpression of CXCR4 and CXCR5 identifies CD4 T helper cells in distinct lymph node niches during influenza virus infection. J Virol 2012;86:7146–57.
[61] Ozaki K, Spolski R, Feng CG, Qi C-F, Cheng J, Sher A, et al. A critical role for IL-21 in regulating immunoglobulin production. Science 2002;298:1630–4.
[62] Bryant VL, Ma CS, Avery DT, Li Y, Good KL, Corcoran LM, et al. Cytokine-mediated regulation of human B cell differentiation into Ig-secreting cells: predominant role of IL-21 produced by CXCR5+ T follicular helper cells. J Immunol 2007;179:8180–90.
[63] Lee SK, Rigby RJ, Zotos D, Tsai LM, Kawamoto S, Marshall JL, et al. B cell priming for extrafollicular antibody responses requires Bcl-6 expression by T cells. J Exp Med 2011;208:1377–88.
[64] Zotos D, Coquet JM, Zhang Y, Light A, D'Costa K, Kallies A, et al. IL-21 regulates germinal center B cell differentiation and proliferation through a B cell-intrinsic mechanism. J Exp Med 2010;207:365–78.
[65] Linterman MA, Beaton L, Yu D, Ramiscal RR, Srivastava M, Hogan JJ, et al. IL-21 acts directly on B cells to regulate Bcl-6 expression and germinal center responses. J Exp Med 2010;207:353–63.
[66] Eto D, Lao C, DiToro D, Barnett B, Escobar TC, Kageyama R, et al. IL-21 and IL-6 are critical for different aspects of B cell immunity and redundantly induce optimal follicular helper CD4 T cell (Tfh) differentiation. PLoS ONE 2011;6:e17739.
[67] Hirota K, Turner J-E, Villa M, Duarte JH, Demengeot J, Steinmetz OM, et al. Plasticity of TH17 cells in Peyer's patches is responsible for the induction of T cell-dependent IgA responses. Nat Immunol 2013;14:372–9.
[68] M'Hidi H, Thibult M-L, Chetaille B, Rey F, Bouadallah R, Nicollas R, et al. High expression of the inhibitory receptor BTLA in T-follicular helper cells and in B-cell small lymphocytic lymphoma/chronic lymphocytic leukemia. Am J Clin Pathol 2009;132:589–96.
[69] Kashiwakuma D, Suto A, Hiramatsu Y, Ikeda K, Takatori H, Suzuki K, et al. B and T lymphocyte attenuator suppresses IL-21 production from follicular Th cells and subsequent humoral immune responses. J Immunol 2010;185:2730–6.
[70] O'Shea JJ, Paul WE. Mechanisms underlying lineage commitment and plasticity of helper CD4+ T cells. Science 2010;327:1098–102.
[71] Belkaid Y, Bouladoux N, Hand TW. Effector and memory T cell responsesto commensal bacteria. Trends Immunol 2013;34:299–306.
[72] Crotty S. Follicular helper CD4 T cells (TFH). Annu Rev Immunol 2011;29:621–63.
[73] Lu KT, Kanno Y, Cannons JL, Handon R, Bible P, Elkahloun AG, et al. Functional and epigenetic studies reveal multistep differentiation and plasticity of in vitro-generated and in vivo-derived follicular T helper cells. Immunity 2011;35:622–32.
[74] Lee YK, Turner H, Maynard CL, Oliver JR, Chen D, Elson CO, et al. Late developmental plasticity in the T helper 17 lineage. Immunity 2009;30:92–107.
[75] Wang Y, Godec J, Ben-Aissa K, Cui K, Zhao K. The transcription factors T-bet and Runx are required for the ontogeny of pathogenic interferon-γ-producing T helper 17 cells. Immunity 2014.
[76] De Nitto D, Sarra M, Pallone F, Monteleone G. Interleukin-21 triggers effector cell responses in the gut. World J Gastroenterol 2010;16:3638–41.
[77] Choi YS, Kageyama R, Eto D, Escobar TC, Johnston RJ, Monticelli L, et al. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. Immunity 2011;34:932–46.
[78] Kitano M, Moriyama S, Ando Y, Hikida M, Mori Y, Kurosaki T, et al. Bcl6 protein expression shapes pre-germinal center B cell dynamics and follicular helper T cell heterogeneity. Immunity 2011;34:961–72.
[79] Kerfoot SM, Yaari G, Patel JR, Johnson KL, Gonzalez DG, Kleinstein SH, et al. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. Immunity 2011;34:947–60.
[80] Choi YS, Yang JA, Yusuf I, Johnston RJ, Greenbaum J, Peters B, et al. Bcl6 expressing follicular helper CD4 T cells are fate committed early and have the capacity to form memory. J Immunol 2013;190:4014–26.
[81] Nakayamada S, Kanno Y, Takahashi H, Jankovic D, Lu KT, Johnson TA, et al. Early Th1 cell differentiation is marked by a Tfh cell-like transition. Immunity 2011;35:919–31.
[82] Johnston RJ, Choi YS, Diamond JA, Yang JA, Crotty S. STAT5 is a potent negative regulator of TFH cell differentiation. J Exp Med 2012;209:243–50.
[83] Oestreich KJ, Mohn SE, Weinmann AS. Molecular mechanisms that control the expression and activity of Bcl-6 in TH1 cells to regulate flexibility with a TFH-like gene profile. Nat Immunol 2012;13:405–11.
[84] Ballesteros-Tato A, León B, Graf BA, Moquin A, Adams PS, Lund FE, et al. Interleukin-2 inhibits germinal center formation by limiting T follicular helper cell differentiation. Immunity 2012;36:847–56.
[85] Tubo NJ, Pagán AJ, Taylor JJ, Nelson RW, Linehan JL, Ertelt JM, et al. Single naive CD4(+) T cells from a diverse repertoire produce different effector cell types during infection. Cell 2013;153:785–96.
[86] Fahey LM, Wilson EB, Elsaesser H, Fistonich CD, McGavern DB, Brooks DG. Viral persistence redirects CD4 T cell differentiation toward T follicular helper cells. J Exp Med 2011;208:987–99.
[87] Perez-Shibayama C, Gil-Cruz C, Pastelin-Palacios R, Cervantes-Barragan L, Hisaki E, Chai Q, et al. IFN-γ-producing CD4+ T cells promote generation of protective germinal center-derived IgM+ B cell memory against *Salmonella typhi*. J Immunol 2014;192:5192–200.
[88] Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 2005;6:1123–32.
[89] Ivanov II, McKenzie BS, Zhou L, Tadokoro CE, Lepelley A, Lafaille JJ, et al. The orphan nuclear receptor RORγt directs the differentiation program of proinflammatory IL-17+ T helper cells. Cell 2006;126:1121–33.
[90] Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, et al. Transforming growth factor-β induces development of the TH17 lineage. Nature 2006;441:231–4.
[91] Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B. TGFβ in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. Immunity 2006;24:179–89.
[92] Chappert P, Bouladoux N, Naik S, Schwartz RH. Specific gut commensal flora locally alters T cell tuning to endogenous ligands. Immunity 2013;38:1198–210.
[93] Li L-X, McSorley SJ. B cells enhance antigen-specific CD4 T cell priming and prevent bacteria dissemination following *Chlamydia muridarum* genital tract infection. PLoS Pathog 2013;9:e1003707.
[94] Andrew DW, Cochrane M, Schripsema JH, Ramsey KH, Dando SJ, O'Meara CP, et al. The duration of *Chlamydia muridarum* genital tract infection and associated chronic pathological changes are reduced in IL-17 knockout mice but protection is not increased further by immunization. PLoS ONE 2013;8:e76664.
[95] Cong Y, Feng T, Fujihashi K, Schoeb TR, Elson CO. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. PNAS 2009;106:19256–61.
[96] 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;323:1488–92.
[97] Suzuki K, Meek B, Doi Y, Muramatsu M, Chiba T, Honjo T, et al. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. PNAS 2004;101:1981–6.
[98] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009;139:485–98.
[99] Gaboriau-Routhiau V, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, et al. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. Immunity 2009;31:677–89.
[100] Nurieva RI, Chung Y, Hwang D, Yang XO, Kang HS, Ma L, et al. Generation of T follicular helper cells is mediated by interleukin-21 but independent of T helper 1, 2, or 17 cell lineages. Immunity 2008;29:138–49.
[101] Zhou L, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, et al. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. Nat Immunol 2007;8:967–74.
[102] Vogelzang A, McGuire HM, Yu D, Sprent J, Mackay CR, King C. A fundamental role for interleukin-21 in the generation of T follicular helper cells. Immunity 2008;29:127–37.
[103] Vogelzang A, McGuire HM, Liu SM, Gloss B, Mercado K, Earls P, et al. IL-21 contributes to fatal inflammatory disease in the absence of Foxp3+ T regulatory cells. J Immunol 2014;192:1–11.
[104] King IL, Mohrs K, Mohrs M. A nonredundant role for IL-21 receptor signaling in plasma cell differentiation and protective type 2 immunity against gastrointestinal helminth infection. J Immunol 2010;185:6138–45.
[105] Persaud SP, Parker CR, Lo W-L, Weber KS, Allen PM. Intrinsic CD4+ T cell sensitivity and response to a pathogen are set and sustained by avidity for thymic and peripheral complexes of self peptide and MHC. Nat Immunol 2014;15:266–74.
[106] Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio C-W, Santacruz N, et al. Peripheral education of the immune system by colonic commensal microbiota. Nature 2011;478:250–4.
[107] Su LF, Kidd BA, Han A, Kotzin JJ, Davis MM. Virus-specific CD4(+) memory-phenotype T cells are abundant in unexposed adults. Immunity 2013.
[108] Gottschalk RA, Corse E, Allison JP. TCR ligand density and affinity determine peripheral induction of Foxp3 in vivo. J Exp Med 2010;207:1701–11.
[109] Linterman MA, Pierson W, Lee SK, Kallies A, Kawamoto S, Rayner TF, et al. Foxp3+ follicular regulatory T cells control the germinal center response. Nat Med 2011;17:975–82.
[110] Wollenberg I, Agua-Doce A, Hernández A, Almeida C, Oliveira VG, Faro J, et al. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. J Immunol 2011;187:4553–60.
[111] Dalmasso G, Nguyen HTT, Yan Y, Laroui H, Srinivasan S, Sitaraman SV, et al. MicroRNAs determine human intestinal epithelial cell fate. Differentiation 2010;80:147–54.

[112] Pratama A, Ramiscal RR, Silva DG, Das SK, Athanasopoulos V, Fitch J, et al. Roquin-2 shares functions with its paralog Roquin-1 in the repression of mRNAs controlling T follicular helper cells and systemic inflammation. Immunity 2013;38:669–80.
[113] Vogel KU, Edelmann SL, Jeltsch KM, Bertossi A, Heger K, Heinz GA, et al. Roquin paralogs 1 and 2 redundantly repress the Icos and Ox40 cCostimulator mRNAs and control follicular helper T cell differentiation. Immunity 2013;38:655–68.
[114] Uematsu S, Fujimoto K, Jang MH, Yang B-G, Jung Y-J, Nishiyama M, et al. Regulation of humoral and cellular gut immunity by lamina propria dendritic cells expressing Toll-like receptor 5. Nat Immunol 2008;9:769–76.
[115] Hall JA, Cannons JL, Grainger JR, Santos Dos LM, Hand TW, Naik S, et al. Essential role for retinoic acid in the promotion of CD4(+) T cell effector responses via retinoic acid receptor alpha. Immunity 2011;34:435–47.
[116] Xie X, Mu L, Yao X, Li N, Sun B, Li Y, et al. ATRA alters humoral responses associated with amelioration of EAMG symptoms by balancing Tfh/Tfr helper cell profiles. Clin Immunol 2013;148:162–76.
[117] Kroenke MA, Eto D, Locci M, Cho M, Davidson T, Haddad EK, et al. Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. J Immunol 2012;188:3734–44.
[118] Petrovas C, Yamamoto T, Gerner MY, Boswell KL, Wloka K, Smith EC, et al. CD4 T follicular helper cell dynamics during SIV infection. J Clin Invest 2012;122:3281–94.
[119] Ma CS, Deenick EK. Human T follicular helper (Tfh) cells and disease. Immunol Cell Biol 2014;92:64–71.
[120] Vinuesa CG, Fagarasan S, Dong C. New territory for T follicular helper cells. Immunity 2013;39:417–20.