The multifaceted functions of follicular regulatory T cells

Peter T Sage[1] and Arlene H Sharpe[2,3,4]

The immune system is capable of generating robust antibody responses to foreign antigens during infection and vaccination, while simultaneously limiting antibodies to self-antigens. T follicular regulatory (Tfr) cells are a subset of follicular T cell with specialized roles in regulating humoral immunity. Although Tfr cells have been studied for the past 10 years, their roles have remained elusive. In this review we discuss the current understanding of Tfr cell functions in autoimmunity and how Tfr cells simultaneously control foreign and autoantigen specific antibody responses. We highlight new tools that enable in-depth study of Tfr cells *in vivo* and recent data suggesting an important role for Tfr cells in limiting participation of autoreactive B cells in germinal centers.

Addresses
[1] Transplantation Research Center, Renal Division, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, United States
[2] Department of Immunology, Blavatnik Institute, Harvard Medical School, Boston, MA 02115, United States
[3] Evergrande Center for Immunologic Diseases, Harvard Medical School and Brigham and Women's Hospital, Boston, MA 02115, United States
[4] Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, United States

Corresponding authors:
Sage, Peter T (psage@bwh.harvard.edu), Sharpe, Arlene H (arlene_sharpe@hms.harvard.edu)

Introduction

Antibody responses to most foreign antigens result from interactions between T follicular helper (Tfh) cells and B cells in the B cell follicle and germinal centers (GCs) [1]. Tfh cells promote class switch recombination, somatic hypermutation and affinity maturation of B cells which enhance the effector functions of antibodies. After interaction with Tfh cells, B cells either differentiate into memory B cells which respond after antigen re-exposure, or into plasma cells that produce high affinity antibody [2]. Dysregulation of Tfh cells can result in autoreactive antibodies and systemic autoimmune disease. The immune system therefore must ensure Tfh and B cell responses are appropriate to eliminate foreign pathogens while simultaneously preventing autoimmune disease. Although central tolerance can limit most autoreactive T and B cells from making it into the periphery, it does not prevent this completely. Additional peripheral immunoregulatory mechanisms are required to ensure antibody responses are appropriate in terms of specificity, strength and duration. Mechanisms that control antibody responses after antigenic exposure are still being elucidated.

Tfr cells are a specialized T regulatory (Treg) cell subset that can gain access to B cell follicles and regulate Tfh-mediated B cell responses after antigenic exposure [3–8]. Although other pathways likely contribute, Tfr cells are thought to be an important mechanism that regulates antibody responses following antigen exposure. Tfr cells have a unique transcriptional program compared to Treg cells in which the transcription factor FoxP3 coopts a Tfh cell transcriptional program to elicit Tfr cell identity and suppressive function [6,9•]. Tfr cells regulate B cell responses through a number of mechanisms, including CTLA-4-mediated inhibition of B cells and inhibition of proinflammatory cytokine production by Tfh cells [10–12]. Although some mechanisms of Tfr cell differentiation and function have been elucidated, the *in vivo* functions of Tfr cells are an active area of investigation. Early adoptive transfer work suggested an important role for Tfr cells in controlling antibody responses. Recently, studies using an inducible Tfr-deleter mouse demonstrate that Tfr cells regulate early, but not late, GC responses to limit autoreactive and antigen-specific antibody responses [13••]. However, non-inducible models of Bcl6 deficiency in Treg cells have suggested either subtle, or positive, roles for Tfr cells in controlling antibody responses [14,15••,16•]. These divergent results have led to ambiguity in the roles of Tfr cells *in vivo*. In this review we discuss the current understanding of the *in vivo* functions of Tfr cells in settings of autoimmunity and suggest a new paradigm to rectify inconsistencies and elucidate the complex immunoregulatory roles of Tfr cells.

Tfr cell identity
Brief history of Tfr cells

Tfr cells were discovered in 2011 when three groups simultaneously identified a population of FoxP3+ Treg cells that expressed CXCR5 and migrated to B cell follicles [5–7]. Although precise functions of Tfr cells were not fully elucidated at that time due to a lack of

specific tools, Tfr cells were identified as a distinct population based on phenotype, positioning and/or transcriptional program. Since these first reports, the precise definition of a Tfr cell has been somewhat inconsistent. Currently Tfr cells are defined as FoxP3+ CD4+ T cells that express at least some levels of the chemokine receptor CXCR5, have a distinct transcriptional program consistent with a Tfr cell, can gain access to B cell follicles, and are capable of modulating B cell responses. The most common way to identify Tfr cells is through flow cytometric analysis of CXCR5 expression on FoxP3+ cell populations. However, inconsistent gating strategies and the difficulty of measuring CXCR5 expression have led to variability in Tfr cell identification in the literature, leading to confusion. In addition, costaining with a second receptor or a transcription factor (PD-1, ICOS or Bcl6) has added another dimension of variability, since some of these strategies weigh expression of PD-1, ICOS or Bcl6 more than CXCR5. Since Tfr cell function is likely most potent in B cell follicles, immunofluorescence of FoxP3+ cells in the B cell follicle also has been used as a secondary approach to identify Tfr cells. In addition, some previous studies, including ours, have assessed the presence of FoxP3+ cells directly in GCs to identify Tfr cells. However, these strategies were based on the assumption that Tfr cells predominantly controlled antibody responses within GCs, an assumption that is not quite accurate, as discussed below. Tfr cells likely also control responses at the T-B border, but delineating differences between Treg and Tfr cells at the T-B border can be extremely difficult using immunofluorescence approaches.

Direct comparisons of Tfr cells to Tfh cells also have contributed to inconsistencies in Tfr cell identification and function. Although Tfh and Tfr cells share similar expression of a number of cell surface receptors and transcription factors, these cells are quite distinct. In particular, gating strategies for Tfh cells are not always valid for Tfr cells. For instance, some studies that simultaneously analyze Tfh and Tfr cells use typical Tfh gating strategies of CXCR5^{high}Bcl6^{high} cells and then subdivide these cells based on FoxP3 to delineate Tfh and Tfr cells. This approach assumes that the only difference between Tfh and Tfr cells is FoxP3 expression; however, this is an oversimplification. Tfr cells typically have lower expression of CXCR5 and Bcl6, compared to Tfh cells [9[•],17–19]. Therefore, using typical Tfh gating strategies may 'gate out' and fail to identify some Tfr cell populations. As a result, Tfr cells are likely present but missing when such gating strategies are used, leading to misinterpretation of the presence of Tfr cells as rare in some settings. In addition, the tissue microenvironment can impact expression of receptors used to identify Tfh and Tfr cells. For example, we have shown that Tfh (and Tfr) cells express higher levels of PD-1 in the spleen versus skin draining lymph nodes, whereas the opposite is true for ICOS [9[•]].

Thus, caution must be used in overinterpreting data based on Tfh cell gating strategies. We recommend a flow cytometric gating strategy to identify Tfr cells where CXCR5+ FoxP3+ cells are identified using strict staining controls. Gating strategies should be validated with functional assays and/or transcriptional analyses, when available. If costimulatory and/or transcription factors are used for costaining, multiple costaining strategies should be employed. Gating strategies for human Tfr cells are more complex than in murine systems because of variable immunological histories between patients, less definitive identification of Treg cells using FoxP3 staining, and the limited availability of healthy lymphoid tissue. Nevertheless, the phenotype of Tfr cells in humans seems to match murine Tfr cells. It is possible that human Tfr cells may express lower levels of PD-1 and Bcl6 than murine Tfr cells, but it is not possible to make direct comparisons [19]. More definitive studies of human Tfr cells, including transcriptomic analysis in a number of tissues, need to be performed.

Differentiation of Tfr cells

Tfr cells differentiate from natural Treg precursors upon interaction with dendritic cells, following similar cues as Tfh cells, although with slightly delayed kinetics [7,18]. While some Tfr cells may originate from induced Treg populations, this is infrequent even in the most permissive circumstances [20]. Tfr cell differentiation begins with dendritic cell interactions, which initiate the Tfr cell program [18]. Circulating blood Tfr cell development requires priming by DCs but these circulating Tfr cells do not require interactions with B cells for their generation [9[•],18]. Within the B cell follicle Tfr cells likely receive full effector differentiation signals through interactions with B cells, strengthening the Tfr cell transcriptional program and facilitating suppression, although this has yet to be proven experimentally [6,21,22]. A recently published study found that Tfh (and fibroblastic reticular cells) cells induce Tfr cell generation through secretion of sclerostin domain-containing protein 1 (SOSTDC1) which inhibits the β-catenin pathway in Tfr cells [23]. The Tfr cell transcriptional program encodes both Tfr cell identity and functionality. Although Tfr cells are fairly stable, we recently found that a small percentage of Tfr cells (∼10−20%) can lose FoxP3 and become 'ex-Tfr' cells with diminished suppressive capacity [9[•]]. Importantly, without fate mapping alleles, these ex-Tfr cells would be contained in typical Tfh cell gating strategies.

Interestingly, the transcription factor FoxP3 is necessary, but not sufficient, for the Tfr cell transcriptional program. Tfr cells do not have a distinct lineage transcription factor, but instead rely on multiple transcription factors. In a recent study we showed that loss of FoxP3 results in an altered transcriptional program and increased features of Tfh cells [9[•]]. When FoxP3 expression was enforced in

Tfh cells, a Tfr-like cell phenotype and suppressive potential resulted. These studies suggest that FoxP3 alters the Tfh transcriptional program to induce Tfr cell suppressive function and transcriptional identity. This is similar to tissue Treg cell subsets in which FoxP3 may alter tissue-specific programs to facilitate unique features of suppression [24]. For instance, adipose tissue Treg cells require FoxP3 to alter tissue-specific programs within visceral fat [25]. The overlap of transcriptional programming between tissue Treg and Tfr cells further suggests that the B cell follicle is similar to a peripheral tissue regarding how microenvironment may direct and strengthen Treg transcriptional identity [26]. The role of ex-Tfr cells in autoimmune disease has yet to be established, but the altered functionality of these cells suggests a putative role in altered autoantibody production.

Strategies to study Tfr cells
Models to study Tfr cells
The lack of specific tools for studying Tfr cells has impeded our understanding of the role of Tfr cells in controlling antibody responses. *In vitro* suppression assays have been developed to study Tfr cell functions. In these assays Tfr cells are cultured with Tfh and B cells. These *in vitro* assays have demonstrated that Tfr cells regulate class switch recombination, antibody secretion and somatic hypermutation of B cells [9•,13••,17,27], and shown that Tfr cells alter metabolic reprogramming and suppress IL-21 production by Tfh cells. The ability of Tfr cells to suppress IL-21 production by Tfh is critical for Tfr cell function, as IL-21 can inhibit Tfr cells [12,28,29].

Although these studies have been useful for mechanistic interrogation of B, Tfh and Tfr communication, they do not recapitulate all components of the B cell follicle. Early *in vivo* studies used adoptive transfer of Tfr cells into lymphopenic mice to show that Tfr cells suppress Tfh-mediated B cell responses including antibody secretion, GC and plasma cells [17,7,10]. However, such transfer approaches have limitations because they do not reconstitute the entire Tfr cell compartment, Tfr cells are lost over time, and *de novo* Tfr cells are unable to form. Therefore, only short-term Tfr cell functionality can be assessed by these adoptive transfer assays. Additional strategies using mixed bone marrow chimeras of *Sh2d1a*/SAP deficient and *Foxp3*-DTR cells have achieved attenuated Tfr cell frequencies, resulting in enhanced germinal center B cell responses, but not antigen-specific antibody [6]. Although these strategies achieved diminished Tfr cell frequencies, the complexities of performing these chimeras have limited their broad use.

Newer strategies have been employed to study the immunoregulatory roles of Tfr cells in intact mice *in vivo*. One approach utilized mice in which Bcl6 was selectively deleted in Treg cells with a FoxP3^{Cre} system [14,15••,16•,30–32]. The rationale behind this strategy was that since Bcl6 is required for follicular T cell populations, loss of Bcl6 would result in elimination of all Tfr cells from birth. However, although there is an essential requirement for Bcl6 in Tfh cell development, the absolute requirement for Bcl6 in Tfr cells has been suggested, but not tested rigorously [5,6]. The Bcl6-Treg cell deletion approach strongly attenuates CXCR5+PD1+ Treg cells, but CXCR5+PD1− Treg cells are less affected and may remain in some settings [15••,16•,30,31]. Since PD-1^{high} Tfr cells are likely the most activated Tfr cells, and Bcl6 seems to be enriched in Tfr cells in GCs, the Bcl6-Treg Tfr cell deficiency model may only attenuate highly activated, or 'GC', Tfr cells. In addition, recent studies in which CXCR5 was deleted in an inducible manner from Treg cells using a cre-lox system suggest that CXCR5 may not be absolutely required for Tfr cell positioning [33]. Since Bcl6 controls optimal CXCR5 expression, Bcl6 deficiency may result in Tfr cells without CXCR5 expression (and these Tfr cells are therefore lost in flow cytometric gating strategies), but nevertheless may gain access to B cell follicles to suppress B cell responses. Moreover, Bcl6 is expressed (at least at the transcriptional level) in other effector Treg subsets and may have important roles in these Treg subsets, complicating interpretation of these data [9•,24,34]. Studies using the Bcl6-Treg Tfr cell deletion approach have suggested Tfr cells may have minor or positive roles in regulating foreign antigen-specific antibody responses [15••,16•,30–32]. Lack of a strong phenotype was also found when the CXCR5 gene was eliminated from Treg cells [33]. These findings have raised questions about whether Tfr cells have a less dominant and more complex role in regulating humoral immune response than initially thought.

To overcome limitations of existing tools to perturb Tfr cells, a Tfr-DTR mouse was developed to delete Tfr cells and assess Tfr cell function *in vivo*. This mouse uses a dual gene-driven system (requiring FoxP3 *and* CXCR5 expression) to induce diphtheria toxin receptor (DTR) on the surface of Tfr cells, making them susceptible to deletion by diphtheria toxin administration. Importantly, this system allows temporal deletion studies. These studies are distinct from CXCR5 floxed FoxP3-creERT2 studies because the CXCR5 floxed FoxP3-creERT2 strategy only eliminates the CXCR5 gene from Tfr cells so CXCR5 cannot be expressed on the surface of Tfr cells, whereas the Tfr-DTR strategy eliminates the Tfr cells all together. Studies with the Tfr-DTR mouse demonstrated that Tfr cells potently regulate antibody responses elicited by a foreign antigen vaccine *before* GC formation, but have less of a role *after* GC formation [13••]. Interestingly, although vaccine-specific antibody was increased when Tfr cells were deleted, the antibody was of lower affinity. Therefore, although Tfr cells are thought to broadly 'inhibit' B cell responses, the end result of Tfr cell suppression may be more or less protective antibody responses, depending upon whether low amounts of high affinity antibody, or large amounts of low affinity antibody, can mediate protection from a particular pathogen.

Tfr cells can regulate antibody isotypes beyond IgG. We co-discovered a population of IL-13 producing Tfh cells, Tfh13 cells, which have important roles in mediating IgE responses [13[••],35]. In the Tfr-DTR model, Tfr cells potently suppressed Tfh13 cell mediated IgE responses as well as allergen-specific IgE *in vivo* to limit allergic airway disease. Recent studies in the Bcl6-Treg model of Tfr deficiency have also demonstrated a role for Tfr cells in controlling allergen specific IgE and lung inflammation [36[•]]. In humans, Tfr cell frequencies have been suggested to contribute to allergic rhinitis, and allergen immunotherapy may correct this defect to limit disease [37[•]]. However, Tfr cells may have a stimulatory role in food allergy through production of IL-10 [38]. This broad role of Tfr cells in regulating antibody isotypes has implications for a variety of protective and pathogenic antibody responses.

Roles of Tfr cells in autoimmunity

Since the discovery of Tfr cells, a number of groups have assessed Tfr cell frequencies in human autoimmune diseases. Although initial studies suggested that the frequency of Tfr cells inversely correlates with autoimmune disease, more recent studies with improved gating strategies have demonstrated more complex relationships and inconsistencies in findings [4,21]. In settings of systemic lupus erythematosus (SLE) recent studies have shown inverse, positive, or no correlation of Tfr cells (or the Tfr:Tfh ratio) with disease severity [39–41]. In primary Sjogren's syndrome, a higher Tfr:Tfh ratio positively correlates with disease as well as tertiary lymphoid structures in tissues [42[•],43]. In rheumatoid arthritis circulating Tfr cells have been found to have either positive or negative correlations with disease [44–48]. Increased Tfr frequencies (or Tfr:Tfh ratios) negatively correlate with disease in multiple sclerosis, autoimmune hepatitis, and ulcerative colitis [49–51]. Inconsistencies in findings regarding alterations in Tfr cells are likely due to variability in gating strategies, complexities in treatment of patients, and the relatively late stages of analysis. We hypothesize that Tfr cells have key functional roles in mitigating autoimmune disease, but in clinical settings where the disease has progressed substantially, Tfr cells may be indicators of enhanced inflammation and not suppressive state. For this reason, elucidating the roles of Tfr cells in autoimmunity in pre-clinical models is essential.

Studies using both the Bcl6-Treg knockout and the Tfr-DTR models suggest Tfr cells control autoreactive antibody responses. Bcl6-Treg deficiency resulted in substantial increases in autoreactive antibodies to salivary glands, kidney antigens and dsDNA, but these occurred only after 30 weeks in spontaneous settings [15[••]]. Antinuclear antibodies (ANA) were not increased in Bcl6-Treg deficient mice in the basal state in another study, but were increased 30 days after influenza infection compared to controls [32]. In a lupus-like pristane injection model Bcl6-Treg deficient mice had small increases in anti-dsDNA IgA, but not IgG,

four months after injection [16[•]]. Also using the Bcl6-Treg knockout model, experimental Sjogren's syndrome was exacerbated, but only 5 weeks after initiation [15[••]]. Therefore, in the Bcl6-Treg deficiency model autoantibodies develop relatively slowly in unmanipulated mice, but are accelerated in settings of induced inflammation.

In the Tfr-DTR model autoreactive IgG was found as early as 21 days after Tfr cell deletion, suggesting that Tfr cells consistently control autoreactive responses [13[••]]. However, it is important to note that these mice had been vaccinated with a foreign antigen and strong adjuvant. The autoreactive IgG reacted with a diverse array of autoantigens found in many different diseases. Interestingly, there also were substantial amounts of autoreactive IgE in the vaccinated Tfr-DTR mice. Although autoreactive IgE has been suggested to have roles in SLE nephritis, the function of autoreactive IgE after Tfr cell deletion is unclear [52,53]. When Tfr cells were deleted after GC formation in Tfr-DTR mice, although the vaccine-specific IgG was not altered, total IgG, IgA and IgE was increased, suggesting that Tfr cells may have roles in controlling autoreactive antibodies after GC formation. These studies illustrate the key function for Tfr cells in restraining autoreactive antibody responses. Although the temporal roles for Tfr cells in controlling autoreactive antibody responses are not yet clear, *in vitro* functional assays suggest Tfr cells can suppress initial activation of autoreactive B cells [13[••]].

Integrated paradigm for regulation of antibody responses by Tfr cells

Based on current data, a new paradigm of how Tfr cells function is emerging (Figure 1). Outside of germinal centers in the B cell follicle (for instance, early during vaccination, infection or allergen exposure, before GCs form), Tfr cells prevent autoreactive B cells from being activated by Tfh, most likely by attenuating cytokine production (e.g. IL-21 and IL-4) and/or costimulation. At the same time, Tfr cells prevent GCs elicited by foreign antigen (vaccine, microbe or allergen) from forming by inhibiting cytokine production by Tfh cells (such as IL-21 and IL-4) and dampening B cell metabolic flux. However, in some settings B and Tfh cells can overcome this suppression due to lack of local Tfr cells, high affinity BCR driving high signals, high metabolic flux, unusual concentration of Tfh cells, and/or other mechanisms. In settings where Tfh and B cells are able to escape Tfr cell suppression, GCs start to form. Within developing GCs, Tfr cells may be present but are suppressed by IL-21 and other cytokines produced by Tfh cells. The high concentration of cytokines simultaneously activates GC B cells to expand and enhances their stimulatory capacity (through CD80/86, antigen presentation, e.g.). This creates a stimulatory positive feedback loop, allowing more cytokine-producing Tfh cells to become activated and more GC B cell activation/expansion. The stimulatory

Figure 1
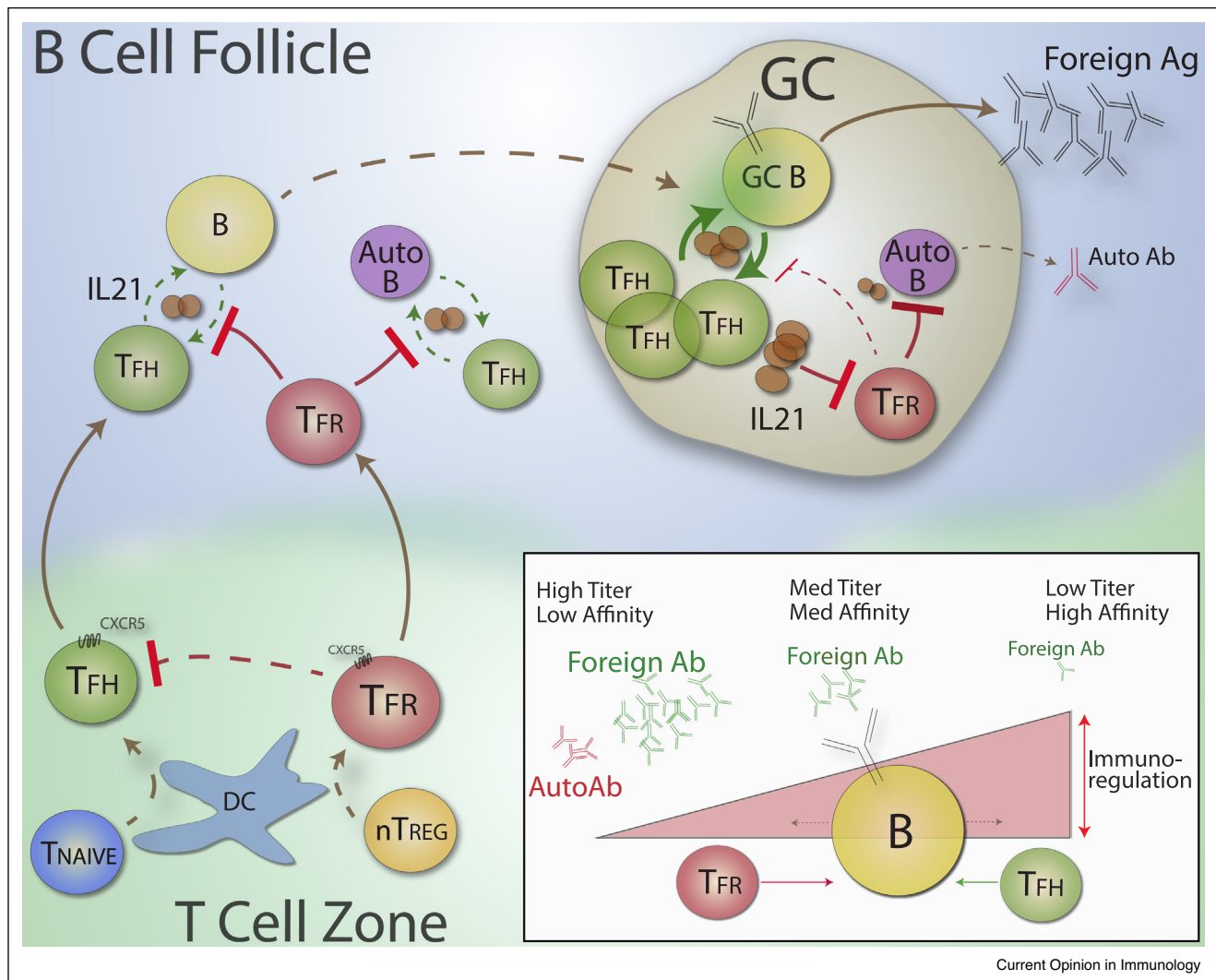
Integrated paradigm for regulation of antibody responses by Tfr cells.

Tfr cell differentiation is initiated by DCs upon interaction with nTreg cells. The Tfr transcriptional program forms, including expression of CXCR5 which, in part, directs Tfr cells to the B cell follicle. While crossing the T cell zone Tfr cells may inhibit Tfh cell differentiation. In the B cell follicle Tfr cells interact with B cells which strengthens the Tfr phenotype, enhancing and strengthening the Tfr transcriptional program. In the B cell follicle, Tfr cells can attenuate foreign vaccine-specific Tfh cell cytokine production and B cell responses, thereby preventing initial GC formation. At the same time, Tfr cells prevent autoreactive B cells from being activated through similar mechanisms. If Tfh and B cells are able to escape suppression in the B cell follicle and form a GC, Tfh and B cells in GCs become activated which induces heightened IL-21 production by Tfh cells and enhanced costimulation/antigenic signals from B cells. IL-21 production by Tfh cells inhibits Tfr cells, preventing Tfr cell expansion. The lower concentration of Tfr cells results in more substantial Tfh and GC B cell interactions and expansion, resulting in more IL-21 and more suppressed Tfr cells, and leading to a positive feedback loop in which Tfr cells are no longer able to suppress foreign antigen-specific GC B cells. In situations where autoreactive B cells stray into GCs, Tfr cells may be able to suppress autoreactive GC B cells because of lower BCR signaling in these cells and/or less substantial Tfh cognate recognition, even in the presence of high IL-21 levels. (inset) Tfr cells control activation thresholds in B cells. In settings of high Tfr cells, small amounts of foreign antigen-specific antibody can be made, but it will be high affinity since only B cells with the highest affinity BCR can escape Tfr cell suppression. In settings with intermediate levels of Tfr cells, moderate amounts of antibody with moderate affinity can be made because Tfr cells impose a moderate activation threshold allowing foreign-antigen specific antibody to be made but preventing autoreactive B cells from being activated. In settings of low Tfr cells, high amounts of low affinity foreign-specific antibody can be made since signaling thresholds to overcome Tfr suppression are extremely low and even low affinity BCRs can overcome suppression. In this setting, even autoreactive antibody can be made even with relatively low affinity BCRs and lack of Tfh help.

positive feedback loop also suppresses Tfr cells, facilitating more Tfh and GC B cell responses. Within GCs, Tfr cells are present (although at lower concentrations) but have difficulty suppressing GC B cells responding to a foreign antigen vaccine or pathogen, but can still suppress autoreactive B cells that may wander into GCs and become activated by high levels of cytokines present there. Tfr cells may be able to suppress autoreactive B cells in GCs because cytokines alone cannot overcome Tfr cell suppression. Cytokines in combination with strong BCR signaling and close interaction with antigen-specific Tfh cell may be needed to overcome Tfr cell suppression. If this is the case, Tfr cells are the 'guardians' of the GC and ensure antibody responses are appropriate and targeted. Although it has not yet been demonstrated, Tfr cells may have additional roles in existing GCs beyond restraining autoreactive B cells. For instance, Tfr cells may participate in altering the breadth of antibody responses, in GC resolution after antigen exposure ends, or by preventing hyperproliferation of effector cells.

Conclusions
Tfr cells appear to have critical roles in controlling both foreign antigen-specific and autoreactive B cells. In order to modulate Tfr cells to enhance vaccine-specific responses and promote protective antibody responses to pathogens, methods are needed to uncouple Tfr cell suppression of foreign antigen-specific and autoreactive antibody responses. Such approaches could make possible selective targeting of Tfr cells to enhance vaccine-specific responses. Likewise, if Tfr cells can be augmented to limit autoreactive antibody while still allowing responses to vaccines or pathogens, this may provide new strategies for treating autoimmune diseases. The tools developed by the field will be instrumental in enabling a deeper understanding of Tfr cells in health and disease, and their therapeutic potential.

Conflict of interest statement
Nothing declared.

Acknowledgement

References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Victora GD, Nussenzweig MC: **Germinal centers**. *Annu Rev Immunol* 2012, **30**:429-457.

2. McHeyzer-Williams M, Okitsu S, Wang N, McHeyzer-Williams L: **Molecular programming of B cell memory**. *Nat Rev Immunol* 2012, **12**:24-34.

3. Sage PT, Sharpe AH: **T follicular regulatory cells in the regulation of B cell responses**. *Trends Immunol* 2015, **36**:410-418.

4. Sage PT, Sharpe AH: **T follicular regulatory cells**. *Immunol Rev* 2016, **271**:246-259.

5. Chung Y *et al.*: **Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions**. *Nat Med* 2011, **17**:983-988.

6. Linterman MA *et al.*: **Foxp3+ follicular regulatory T cells control the germinal center response**. *Nat Med* 2011, **17**:975-982.

7. Wollenberg I *et al.*: **Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells**. *J Immunol* 2011, **187**:4553-4560.

8. Wu H, Xie MM, Liu H, Dent AL: **Stat3 is important for follicular regulatory T cell differentiation**. *PLoS One* 2016, **11**:e0155040.

9. Hou S *et al.*: **FoxP3 and Ezh2 regulate Tfr cell suppressive function and transcriptional program**. *J Exp Med* 2019, **216**:605-620.
•
In this study we assessed the Tfr cell transcriptional program in various tissues, identified a population of ex-Tfr cells, and assessed the roles of FoxP3 and Ezh2 in suppression.

10. Sage PT, Paterson AM, Lovitch SB, Sharpe AH: **The coinhibitory receptor ctla-4 controls B cell responses by modulating T follicular helper, T follicular regulatory, and T regulatory cells**. *Immunity* 2014, **41**:1026-1039.

11. Wing JB, Ise W, Kurosaki T, Sakaguchi S: **Regulatory T cells control antigen-specific expansion of Tfh cell number and humoral immune responses via the coreceptor CTLA-4**. *Immunity* 2014, **41**:1013-1025.

12. Sage PT *et al.*: **Suppression by TFR cells leads to durable and selective inhibition of B cell effector function**. *Nat Immunol* 2016, **17**:1436-1446.

13. Clement RL *et al.*: **Follicular regulatory T cells control humoral and allergic immunity by restraining early B cell responses**. *Nat Immunol* 2019, **20**:1360-1371.
••
In this study we created an inducible Tfr-DTR mouse model and showed that Tfr cells control vaccine-elicited antibody and B cell memory before GC formation, as well as control autoreactive B cells. We also co-discovered Tfh13 cells and showed that Tfr cells regulate Tfh13-mediated IgE responses to control allergic airway disease.

14. Laidlaw BJ *et al.*: **Interleukin-10 from CD4(+) follicular regulatory T cells promotes the germinal center response**. *Sci Immunol* 2017, **2**.

15. Fu W *et al.*: **Deficiency in T follicular regulatory cells promotes autoimmunity**. *J Exp Med* 2018, **215**:815-825.
••
In this study, the authors use a Bcl6-Treg model of Tfr deficiency and show worsening of experimental Sjogren's syndrome.

16. Wu H *et al.*: **Follicular regulatory T cells repress cytokine production by follicular helper T cells and optimize IgG responses in mice**. *Eur J Immunol* 2016, **46**:1152-1161.
•
In this study, the authors develop a Bcl6-Treg model of Tfr cell deficiency but find only minor roles for Tfr cells in controlling foreign antigen specific antibody, and small increases in autoreactive antibodies in induced lupus-like disease.

17. 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**:152-161.

18. Sage PT, Alvarez D, Godec J, von Andrian UH, Sharpe AH: **Circulating T follicular regulatory and helper cells have memory-like properties**. *J Clin Invest* 2014, **124**:5191-5204.

19. Sayin I *et al.*: **Spatial distribution and function of T follicular regulatory cells in human lymph nodes**. *J Exp Med* 2018, **215**:1531-1542.

20. Aloulou M *et al.*: **Follicular regulatory T cells can be specific for the immunizing antigen and derive from naive T cells**. *Nat Commun* 2016, **7**:10579.

21. Deng J, Wei Y, Fonseca VR, Graca L, Yu D: **T follicular helper cells and T follicular regulatory cells in rheumatic diseases**. *Nat Rev Rheumatol* 2019, **15**:475-490.

22. Wing JB, Lim EL, Sakaguchi S: **Control of foreign Ag-specific Ab responses by Treg and Tfr**. *Immunol Rev* 2020, **296**:104-119.

23. Wu X *et al.*: **SOSTDC1-producing follicular helper T cells promote regulatory follicular T cell differentiation**. *Science* 2020, **369**:984-988.

24. Panduro M, Benoist C, Mathis D: **Tissue Tregs**. *Annu Rev Immunol* 2016, **34**:609-633.

25. Li C *et al.*: **TCR transgenic mice reveal stepwise, multi-site acquisition of the distinctive Fat-Treg phenotype**. *Cell* 2018, **174**:285-299 e212.

26. Zemmour D *et al.*: **Single-cell gene expression reveals a landscape of regulatory T cell phenotypes shaped by the TCR**. *Nat Immunol* 2018, **19**:291-301.

27. Sage PT, Sharpe AH: **In vitro assay to sensitively measure TFR suppressive capacity and TFH stimulation of B cell responses**. *Methods Mol Biol* 2015, **1291**:151-160.

28. Jandl C *et al.*: **IL-21 restricts T follicular regulatory T cell proliferation through Bcl-6 mediated inhibition of responsiveness to IL-2**. *Nat Commun* 2017, **8**:14647.

29. Ding Y *et al.*: **Interleukin-21 promotes germinal center reaction by skewing the follicular regulatory T cell to follicular helper T cell balance in autoimmune BXD2 mice**. *Arthritis Rheumatol* 2014, **66**:2601-2612.

30. Xie MM *et al.*: **Follicular regulatory T cells inhibit the development of granzyme B-expressing follicular helper T cells**. *JCI Insight* 2019, **4**.

31. Xie MM *et al.*: **Roles of T follicular helper cells and T follicular regulatory cells in autoantibody production in IL-2-deficient mice**. *Immunohorizons* 2019, **3**:306-316.

32. Botta D *et al.*: **Dynamic regulation of T follicular regulatory cell responses by interleukin 2 during influenza infection**. *Nat Immunol* 2017, **18**:1249-1260.

33. Vanderleyden I *et al.*: **Follicular regulatory T cells can access the germinal center independently of CXCR5**. *Cell Rep* 2020, **30**:611-619 e614.

34. Li Y *et al.*: **Bcl6 preserves the suppressive function of regulatory T cells during tumorigenesis**. *Front Immunol* 2020, **11**:806.

35. Gowthaman U *et al.*: **Identification of a T follicular helper cell subset that drives anaphylactic IgE**. *Science* 2019, **365**.

36. Koh B *et al.*: **Bcl6 and Blimp1 reciprocally regulate ST2(+) Treg-
• cell development in the context of allergic airway inflammation**. *J Allergy Clin Immunol* 2020 http://dx.doi.org/ 10.1016/j.jaci.2020.03.002. In Press.
In this study the authors use the Bcl6-Treg model to show that Tfr cells regulate allergic airway inflammation.

37. Yao Y *et al.*: **Allergen immunotherapy improves defective
• follicular regulatory T cells in patients with allergic rhinitis**. *J Allergy Clin Immunol* 2019, **144**:118-128.
In this study, the authors find that defects in Tfr cells are associated with allergic rhinitis in patients. Allergen immunotherapy normalizes Tfr cell defects and alleviates disease suggesting function of Tfr cells in human allergy.

38. Xie MM *et al.*: **T follicular regulatory cells and IL-10 promote food antigen-specific IgE**. *J Clin Invest* 2020, **130**:3820-3832.

39. Xu B *et al.*: **The ratio of circulating follicular T helper cell to follicular T regulatory cell is correlated with disease activity in systemic lupus erythematosus**. *Clin Immunol* 2017, **183**:46-53.

40. Liu C *et al.*: **Increased circulating CD4(+)CXCR5(+)FoxP3(+) follicular regulatory T cells correlated with severity of systemic lupus erythematosus patients**. *Int Immunopharmacol* 2018, **56**:261-268.

41. Jacquemin C *et al.*: **OX40L/OX40 axis impairs follicular and natural Treg function in human SLE**. *JCI Insight* 2018, **3**.

42. Fonseca VR *et al.*: **The ratio of T follicular regulatory cells to T
• follicular helper cells marks ectopic lymphoid structure formation and PD-1(+) ICOS(+) T follicular helper cells indicate disease activity in primary Sjogren's syndrome**. *Arthritis Rheumatol* 2018, **70**:774-784.
In this study, the authors use rigorous flow cytometric analysis to show that Tfr cells may be a biomarker of disease as well as ectopic lymphoid structures.

43. Kim JW *et al.*: **Circulating CCR7(lo)PD-1(hi) follicular helper T cells indicate disease activity and glandular inflammation in patients with primary Sjogren's syndrome**. *Immune Netw* 2019, **19**:e26.

44. Liu C *et al.*: **Increased circulating follicular Treg cells are associated with lower levels of autoantibodies in patients with rheumatoid arthritis in stable remission**. *Arthritis Rheumatol* 2018, **70**:711-721.

45. Wang X *et al.*: **Imbalance of circulating Tfr/Tfh ratio in patients with rheumatoid arthritis**. *Clin Exp Med* 2019, **19**:55-64.

46. Niu Q *et al.*: **Enhanced IL-6/phosphorylated STAT3 signaling is related to the imbalance of circulating T follicular helper/T follicular regulatory cells in patients with rheumatoid arthritis**. *Arthritis Res Ther* 2018, **20**:200.

47. Romao VC, Fonseca JE, Agua-Doce A, Graca L: **T follicular regulatory cells are decreased in patients with established treated rheumatoid arthritis with active disease: comment on the article by Liu et al**. *Arthritis Rheumatol* 2018, **70**:1893-1895.

48. Cao G *et al.*: **An imbalance between blood CD4(+)CXCR5(+) Foxp3(+) Tfr cells and CD4(+)CXCR5(+)Tfh cells may contribute to the immunopathogenesis of rheumatoid arthritis**. *Mol Immunol* 2020, **125**:1-8.

49. Puthenparampil M *et al.*: **Peripheral imbalanced TFH/TFR ratio correlates with intrathecal IgG synthesis in multiple sclerosis at clinical onset**. *Mult Scler* 2019, **25**:918-926.

50. Liang M *et al.*: **Dysregulated TFR and TFH cells correlate with B-cell differentiation and antibody production in autoimmune hepatitis**. *J Cell Mol Med* 2020, **24**:3948-3957.

51. Long Y *et al.*: **The imbalance of circulating follicular helper T cells and follicular regulatory T cells is associated with disease activity in patients with ulcerative colitis**. *Front Immunol* 2020, **11**:104.

52. Dema B *et al.*: **Immunoglobulin E plays an immunoregulatory role in lupus**. *J Exp Med* 2014, **211**:2159-2168.

53. Dema B *et al.*: **Autoreactive IgE is prevalent in systemic lupus erythematosus and is associated with increased disease activity and nephritis**. *PLoS One* 2014, **9**:e90424.