



# Interaction between Tfh/Tfr Ratio and Regulatory B Cell in Autoimmune Diseases

Chunhong Zhu<sup>1,2†</sup>, Xiaoying Ni<sup>3†</sup>, Jiangming Xu<sup>2</sup>, Hao Wang<sup>1\*</sup>, Hongqiang Shen<sup>1\*</sup>

<sup>1</sup>Department of Clinical Laboratory, Children's Hospital, Zhejiang University School of Medicine, National Clinical Research Center for Child Health, Hangzhou, China; <sup>2</sup>Wangjiang Subdistrict Community Healthcare Center, Shangcheng District, Hangzhou, China; <sup>3</sup>Department of pediatrics, No.903 Hospital of PLA Joint Logistic Support Force, Xi Hu Affiliated Hospital of Hangzhou Medical College, Hangzhou, China

<sup>†</sup>Contributed equally to this work.

## ABSTRACT

The balance between follicular helper T cells (Tfh) and follicular regulatory T cells (Tfr) is crucial for maintaining immune tolerance. Tfh cells are key in producing autoantibodies by providing essential help to germinal center (GC) B cells, while Tfr cells prevent autoimmune inflammatory processes by controlling Tfh responses. However, the signals that regulate Tfh and Tfr cells are largely unknown. Due to dysregulated Tfr/Tfh balance and autoantibody production, regulatory B cells (Bregs) have emerged as a key checkpoint in the GC response. Bregs are B cells with immunosuppressive capabilities. Significant advancements have been made in understanding the roles of Bregs, particularly their capacity to produce cytokines with anti-inflammatory properties and regulate Th17, Th1, and regulatory T cells (Tregs) in the context of autoimmune conditions. Bregs also play a pivotal role in shaping the development, regulation, and localization of Tfh and Tfr cells within the immune environment. Consequently, gaining mechanistic knowledge about the interactions between Tfh-Bregs and Tfr-Bregs has the potential to establish homeostasis and suppress the development of autoantibodies in a various disorders. Within the context of autoimmune disorders, this article provides a concise summary of the dysregulation of Tfh/Tfr, highlighting the critical role of Bregs in regulating this balance. The previously unrecognized interplay between Bregs and Tfh/Tfr cells will serve as an essential basis for the comprehension and management of autoimmune illnesses. It also promises to offer invaluable knowledge of the biological mechanisms of autoantibody synthesis.

**Keywords:** Autoantibody, Autoimmune disease, Follicular helper T cell, Follicular regulatory T cell, Regulatory B cell

\*Corresponding author:

Wang Hao,  
Department of Clinical  
Laboratory, Children's Hospital,  
Zhejiang University School  
of Medicine, National Clinical  
Research Center for Child  
Health, Hangzhou, China  
Email: wang\_hao@zju.edu.cn  
Hongqiang Shen,  
Department of Clinical  
Laboratory, Children's Hospital,  
Zhejiang University School  
of Medicine, National Clinical  
Research Center for Child  
Health, Hangzhou, China  
Email: shenhq@zju.edu.cn

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## INTRODUCTION

In autoimmune diseases (ADs), immunological tolerance becomes impaired, causing the immune system to mistakenly target the body's own tissues and result in damage either locally or throughout the body. Autoimmune-related conditions encompass a wide variety of ailments that can be categorized into several groups. Autoreactive lymphocytes and pathogenic autoantibodies are two key factors that contribute significantly to most autoimmune diseases, which are characterized by inflammation-mediated damage to organs and tissues. The production of autoantibodies is primarily linked to B cells in the GC of lymphoid follicles, a microenvironmental structure formed by the multi-stage differentiation of antigen-specific B cells. In response to antigen stimulation, naive B cells residing in the GC differentiate into plasma cells that produce antibodies and memory B cells. This process involves somatic hypermutation, antibody affinity maturation, and class switch recombination (1). Under normal circumstances, the GC reaction is tightly regulated by the body, allowing for the targeted production of antibodies against foreign pathogens. This process is regulated by follicular dendritic cells (FDC), Tregs, and the microbiome. In particular, Tfh and Tfr are immune cells with unique functions, playing a crucial role in defending against autoimmune reactions and maintaining immune tolerance. Tfh cells, distinguished by their expression of CXCR5 chemokine receptor 5 (CXCR5) in addition to B-cell lymphoma 6 (BCL6), develop from primary CD4<sup>+</sup> T cells following activation by antigen-presenting cells (APC) and migrate to lymphoid follicles primarily in a CXCR5-dependent manner (2, 3). During various stages of the GC reaction, Tfh cells act as essential helpers, delivering proliferation, selection, and survival signals to cognate B cells, thereby driving antibody production. Subsequently, Tfr cells, which regulate immune function, have received significant

attention. Tfr cells constitutively express forkhead box protein 3 (Foxp3) and CXCR5, and their phenotype is partially similar to Tregs and Tfh cells, constitutively expressing Foxp3 and CXCR5, respectively. Tfr cells also express the key transcription factor Bcl-6, which is involved in the regulation of Tfr cell differentiation, migration, and interaction with B cells. It is essential for regulating the formation and function of Tfr cells and further contributes to their differentiation (4). Clinical investigations on ADs have shown that circulating Tfr cell frequency is decreased, Tfh cell frequency is raised, and the Tfh/Tfr ratio is disturbed in conditions such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and myasthenia gravis. Furthermore, the ratio of Tfh/Tfr was positively correlated with the serum anti-DAS28-CRP antibody level in SLE patients ( $r=0.510$ ) (5). Hence, the disruption of the balance between Tfh and Tfr cells can contribute to autoantibody production in ADs. Furthermore, Bregs play a pivotal role in autoantibody generation by intricately interacting with diverse lymphocytes during the GC reaction. Bregs comprise a heterogeneous group of B lymphocytes that exert immunosuppressive effects through distinct mechanisms. Currently, several Breg subsets have been identified and classified, including CD1d<sup>hi</sup>CD5<sup>+</sup>Bregs, and Tim-1<sup>+</sup>Bregs, each with unique characteristics and functions (6). Numerous studies have consistently demonstrated a compelling link between aberrant levels or dysfunctional states of Bregs and the development of ADs, notably including SLE, RA, and multiple sclerosis (MS) (7).

This review succinctly outlines the intricate interplay between Bregs and Tfh/Tfr cells, elucidating how this dynamic interaction regulates autoantibody production in the context of ADs. Furthermore, it delves into the regulatory influence of the Breg-mediated Tfh/Tfr ratio on these disorders, highlighting the therapeutic implications and challenges that arise. This comprehensive

analysis offers valuable insights for the development of targeted treatments and monitoring strategies for ADs.

### *1. Phenotype of Bregs and Their Associated Inducible Regulatory Mechanisms*

Bregs are primarily defined by their immunosuppressive functions, both in laboratory settings and in living organisms. A key feature of Bregs is their production of the anti-inflammatory cytokine IL-10, which is the most important characteristic that defines them. Despite the high heterogeneity of Bregs, the specific phenotypic characteristics that distinguish them from effector B cells have not been fully understood. However, distinct subpopulations of B cells with inhibitory activities have been identified in both model organisms and humans. Research conducted on rodents has shown that transitional 2 marginal zone precursor (T2-MZP) B cells, which are CD1d<sup>+</sup>CD21<sup>+</sup>CD23<sup>+</sup> have the ability to produce IL-10 and display immunosuppressive actions both in vivo and in vitro. Another study in mice demonstrated that both CD21<sup>+</sup>CD23<sup>-</sup>CD24<sup>hi</sup>MZ Bregs and CD1d<sup>hi</sup>CD5<sup>+</sup>Bregs produced IL-10 (8). The phenotypes of Bregs show varying degrees of overlap or divergence across different species, organs, and disease models. This variability is likely attributed to their adaptive nature, enabling them to respond and function effectively within specific immune microenvironments. Consequently, there is a pressing need for further research to elucidate whether the immunomodulatory function of Bregs is inherently tied to their phenotypic characteristics. Current evidence suggests that microenvironmental factors, particularly those involved in inflammatory regulation, play a pivotal role in the induction and maintenance of Bregs. In addition to the well-known triggers of Bregs production of IL-10 such as canonical BCR recognition, CD40 signaling, and TLR engagement, a variety of inflammatory factors have been identified as potent inducers of Bregs function. These factors include cytokines like IL-6, IL-21,

and IFN- $\alpha$ , as well as the B cell-activating factor (BAFF). Together, these factors form a complex network of signals that regulate Bregs. Understanding the intricate interplay between Breg phenotypes, their microenvironments, and the diverse array of inducers that shape their function represents a critical frontier in immunology. Such insights will not only advance our fundamental knowledge of immune regulation but also have the potential to inform the development of novel therapeutic strategies targeting Bregs for the treatment of ADs and other immune-mediated conditions (9). The primary regulatory mechanism of Bregs relies on their ability to produce IL-10, a potent anti-inflammatory cytokine. In mice with B-cell-specific IL-10 knockout, the lack of this cytokine results in exacerbated manifestations of arthritis. This is accompanied by increased antibody secretion and a skewed immune response characterized by increased inflammation mediated by Th1 and Th17 cell. This underscores the essential role of IL-10 in immune regulation mediated by B cells (10). However, Bregs have regulatory mechanisms that go beyond just relying on IL-10. Specifically, TGF- $\beta$  and granzyme B (GZMB) are two key factors in IL-10-independent Bregs function. TGF- $\beta$  exerts its immunosuppressive effects by promoting the differentiation of Tregs, thereby enhancing immune tolerance. Meanwhile, GZMB contributes to Bregs-mediated immunosuppression by impairing the activation and function of effector T cells. Collectively, these mechanisms demonstrate the versatility and complexity of Bregs-mediated immune regulation, which encompasses both IL-10-dependent and IL-10-independent pathways.

The intricate interplay between Bregs and various other immune cell populations forms the cornerstone of their ability to regulate immune responses and maintain homeostasis. A fascinating facet of Bregs biology lies in their potential to modulate Tfh and Tfr cells, two specialized T cell subsets crucial for humoral immunity and immune regulation,

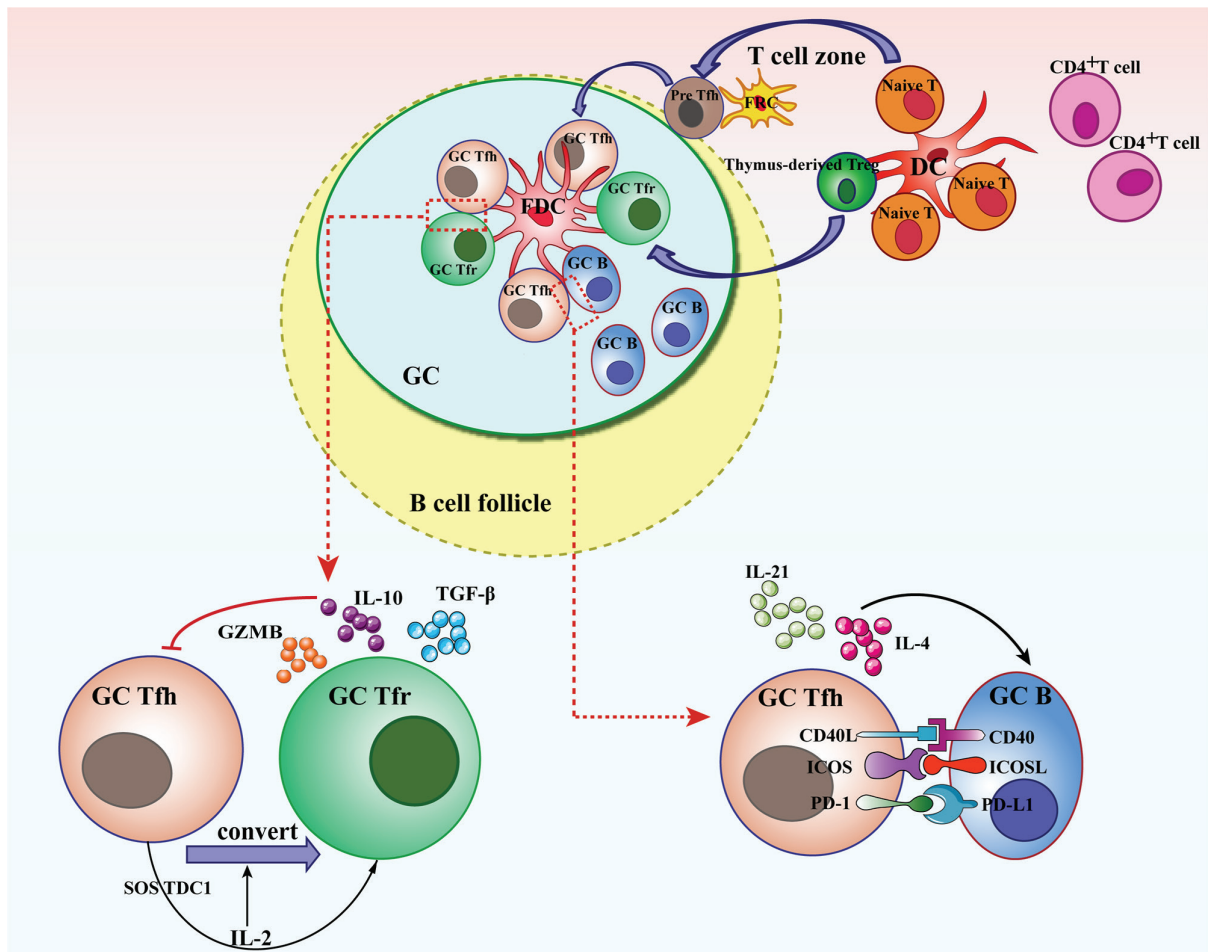
respectively. Tfh cells are indispensable for generating high-affinity antibodies by B cells in germinal centers, while Tfr cells serve as counter-regulators, reducing excessive Tfh-driven B cell activation and autoantibody production. By maintaining a balance between Tfh and Tfr cells, Bregs could play a pivotal role in reducing inflammatory responses and preserving immune tolerance. Yet, the precise mechanisms underlying the interactions among Bregs, Tfh, and Tfr cells remain largely enigmatic. Further investigation is imperative to unravel the molecular and cellular pathways that orchestrate these complex relationships.

## 2. Autoantibody Production Due to Dysregulated Tfh/Tfr Ratios in ADs

Autoantibodies serve as the serological hallmark and pathological catalyst for numerous ADs, triggering the deposition of immune complexes in various organs. This sets off the immune cascade and activates immune effectors, ultimately causing widespread tissue inflammation. After detecting antigens mature naïve B cells move to the germinal center through chemotaxis, where they interact with T cells to mature into antibody-secreting plasma cells. A crucial stage in the development of high-affinity antibodies involves the somatic hypermutation of BCRs. This process, while enhancing affinity, can also generate autoreactive BCRs, leading to the production of anti-autotissue antibodies (11). With the identification of Tfh and Tfr subsets, the immune mechanisms responsible for autoantibody production in ADs have been increasingly understood. Tfh cells, as CD4<sup>+</sup> T lymphocytes, initiate the GC response within the humoral immune system. Their functional markers include CXCR5, BCL6, and IL-21 (12). Within lymphoid follicles, Tfh cells play a pivotal role in regulating the survival, antibody affinity maturation, proliferation, and differentiation of GC-B cells. These signals include cytokines such as IL-4 and IL-21, as well as intercellular interactions mediated by surface molecules like CD40L,

ICOSL, and PD-1 (13). Excessive Tfh signaling may support autoreactive B cells in breaking immune tolerance, leading to the production of autoantibody. In contrast, Tfr cells act as negative regulators of autoantibody production, predominantly developing from Treg precursors and expressing Foxp3 and CXCR5, in addition to other markers like ICOS and CTLA-4 (14). Tfr cells play a pivotal role in modulating the GC response. They inhibit the proliferation and function of Tfh and B cells within the GC in a CTLA-4-dependent manner. Notably, the removal of CTLA-4 on Tfr cells disrupts antibody class switching, leading to uncontrolled Tfh proliferation (15). Recent studies have shown that Tfh cells can also differentiate into Tfr cells in response to IL-2 signalling. This discovery challenges conventional wisdom and advocates for regulating the balance between Tfh and Tfr as a new approach for treating autoimmune-related conditions. For instance, in the treatment of RA, low-dose IL-2 can specifically expand Tregs, leading to a targeted conversion to Tfr, suppression of Tfh, and correction of the Tfh/Tfr imbalance. This helps in maintaining immune tolerance and controlling the progression of RA disease (16, 17). In addition, other studies have revealed a new mechanism by which Tfh cells promote Tfr cell differentiation through the secretion of SOSTDC1. Tfh cells can differentiate into a new subgroup called SOSTDC1<sup>+</sup>Tfh cells. Unlike traditional Tfh cells, the novel SOSTDC1<sup>+</sup>Tfh cells lose the ability to assist B cells in producing antibodies but instead promote Tfr cell differentiation by secreting the protein SOSTDC1 and negatively regulating GC responses (18). Additionally, Tfr cells secrete immunosuppressive factors such as IL-10, TGF- $\beta$ , and GZMB, acting as inhibitors in this context (Fig. 1).

Studies conducted using infection models have shown that Tfr cells suppress autoreactive B cells during the germinal center response, highlighting their essential role in maintaining B-cell immunological tolerance (19).



**Fig. 1.** Tfh and Tfr dynamics in germinal center (GC) response. T cells and Tregs differentiate into Tfh and Tfr, migrating to lymphoid follicles via CXCR5. Tfh promotes B cell differentiation via cytokines (IL-4, IL-21) and co-stimulatory molecules (PD-1, ICOS, CD40L), while Tfr suppresses GC activity with IL-10, TGF- $\beta$ , and GZMB. IL-2 induces Tfh-to-Tfr conversion, and SOSTDC1-producing Tfh cells can promote Tfr development.

GC constitutes transient microanatomical structures that promote clonal expansion and affinity maturation of B cells. B cells enter these centers based on their inherent affinity for antigens (20, 21). Once inside the germinal center, B cells undergo rapid and repetitive cell division, which is followed by mutations in antibody genes and rigorous affinity selection (22, 23). This complex selection process is controlled by the availability of GC-Tfh cells, which provide vital nutritional signals to B cells contingent based on their ability to capture and present antigens in the form of peptide-major histocompatibility complex II (p-MHCII). The proportion of Tfh assistance rendered is closely linked to the amount of p-MHC presented by B cells (24-26). Therefore, B cells with the highest

affinity antigen receptors are skilled at capturing and presenting a larger number of antigens, ultimately gaining the favor from Tfh cells for clonal expansion.

In addition to clonal growth, Tfh cells also regulate the development of GC B cells into plasma cells and memory B cells (11, 27). Similar to B cells, Tfh cells themselves undergo clonal expansion and selection within the GC microenvironment. GC is dynamic structures that actively recruit new B cells during the immune response, while also facilitating the migration of differentiated Tfh cells between established GCs (17, 28). However, the question of whether GCs remain permeable to the influx of naive T cells throughout the immune response and how this continued recruitment might disrupt

the delicate balance between Tfh and Tfr cells remains an open area of investigation (4).

The complex interaction between Tfh and Tfr cells is crucial for preserving the equilibrium of the GC reaction. Disruption of this balance has become a key focus in understanding the pathogenesis of ADs, leading to research efforts aimed at uncovering its specific role. In various commonly used mouse models of ADs, the coordination between Tfh and Tfr cells is disturbed. For example BXD2 mice show a decreased frequency of Tfr cells in the spleen, along with a significant rise in Tfh cell frequency that correlates with an increase in the GC B cell numbers ( $r^2=0.4456$ ). Notably, the infusion of Tfr cells into BXD2 mice effectively inhibits GC formation, underscoring their crucial role in immune tolerance (29). Translating these findings to human ADs, research primarily focuses on circulating Tfh (cTfh) and circulating Tfr (cTfr) cells. Imbalances in the cTfr/cTfh ratio are observed across a spectrum of ADs, including RA, SLE, myasthenia gravis, primary cholangitis, and antineutrophil cytoplasmic antibody-associated vasculitis. These patients commonly exhibit an overproliferation of cTfh cells accompanied by a reduction in cTfr numbers (30). In MS patients, cTfr function is significantly compromised, resulting in a reduced cTfr/cTfh ratio that is negatively associated with IgG production (31). These observations suggest that Tfr impairment and Tfh hyperactivity may contribute to the development of ADs. Intriguingly, while some studies in RA patients report increased cTfh and cTfr counts, the cTfr/cTfh ratio is diminished. Alternative explanations suggest that the increased numbers of cTfh may be linked to a decrease in the percentage of activated cTfr subsets. This ultimately results in an exaggerated GC response despite an overall increase in cTfr cell counts (32). Conversely, studies on SLE consistently demonstrate phenotypic changes in cTfh and cTfr that trigger autoimmune reactions. However, there are no significant

differences in the overall numbers of cTfh and cTfr between patients with SLE and healthy controls (33). These data suggest that variations in frequency, function, and subpopulation disparities within the Tfr and Tfh compartments contribute to dysregulated GC responses in ADs. Therefore, isolating and characterizing Tfh and Tfr from human tissues show great promise for understanding their functions and underlying mechanisms in autoimmune pathogenesis, ultimately leading to targeted therapeutic interventions.

### 3. *Tfh and Tfr Regulation of Bregs Function*

The intricate relationship between the formation and function of Bregs is believed to be regulated by Tfh cells. Research has shown that Tfh cells stimulate the proliferation of  $CD19^+CD5^+CD1d^{hi}$  Bregs in individuals with SLE by producing IL-21 (34). This increased in Tfh activity on Bregs suggests a possible regulatory feedback mechanism to control pro-inflammatory responses. It is hypothesized that disruptions in this pathway could contribute to the development of ADs (35). While studies examining the influence of Tfr cells on Bregs in autoimmune conditions are limited, a recent study on arteriosclerosis revealed Tfr's capacity to promote the expansion of  $B220^+CD43-CD1d^{hi}CD5^+$  Bregs both within the organism and in cultured cells, particularly when Tfr cells were transferred into atherosclerosis-prone mice. This led to a significant increase in this Bregs subset ( $P<0.05$ ) (36). Notably, the Tfr-mediated expansion of  $B220^+CD43-CD1d^{hi}CD5^+$  Bregs requires direct cellular contact, highlighting the complexity of this interaction.

Moreover, this regulatory effect depends on the presence of Tfh, as increased Tfh activity can trigger a series of Tfr-mediated anti-inflammatory reactions, including a significant increase in  $B220^+CD43-CD1d^{hi}CD5^+$  Bregs ( $P<0.05$ ). However, it remains unclear whether this augmentation is solely attributed to Tfr-mediated mechanisms or if Tfh also directly contributes to Bregs expansion. Thus,

further investigation is imperative to unravel the precise mechanisms underpinning Tfr's regulation of Bregs and to ascertain whether impairments in this regulatory network are tied to the pathogenesis of ADs.

#### 4. Breg Regulates Tfh and Tfr Differentiation and Function

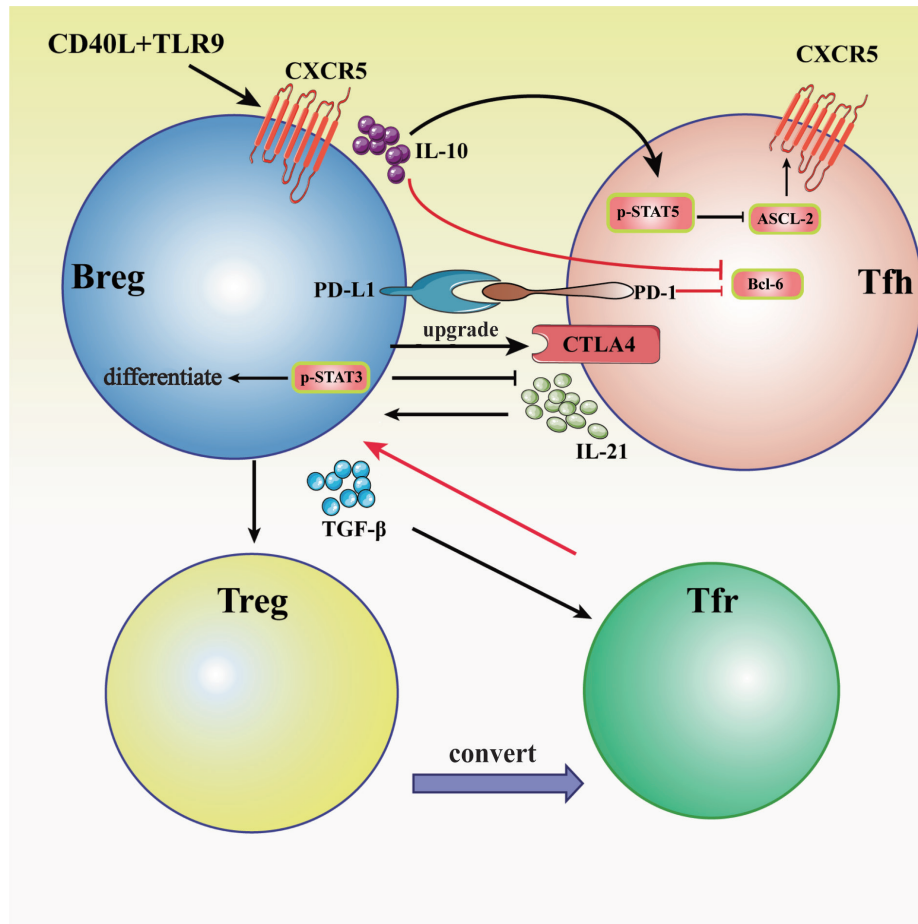
Bregs affect several immune cell types as strong regulators in the complex immunological microenvironment. A co-culture experiment involving Tfh and B cells has illuminated a pivotal role for human CD19<sup>hi</sup>IgD<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup>CD40<sup>hi</sup>PD-L1<sup>+</sup>IL-21R<sup>+</sup>Bregs, which significantly inhibits the maturation of Tfh while concurrently expanding Tfr cells (37). Furthermore, Bregs inhibit Tfh cell-mediated antibody synthesis while simultaneously enhancing Tfr cells. These novel findings highlight the function of Bregs in the GC response and imply that their dysfunction can lead to an aberrant autoimmune response by reducing cell-dependent regulation of Tfh humoral immunity (38).

Despite its crucial role, the intricate regulatory mechanism underlying Breg's influence on Tfh differentiation and function remains largely elusive. Nevertheless, research has shed light on one potential pathway: Bregs-derived IL-10 is capable of downregulating the expression of ASCL2, a key developmental transcription factor in Tfh cells. This downregulation, in turn, hinders the expression of CXCR5, a crucial mediator of Tfh differentiation and function, ultimately leading to impaired Tfh development and performance (39).

Furthermore, the suppression of p-STAT5 has been shown to abolish the IL-10-mediated inhibition of Tfh cells, reinforcing the notion that the IL-10-p-STAT5-ASCL2-CXCR5 axis is a pivotal regulatory pathway in Tfh cell development and activity (40). Additionally, the PD-1/PD-L1 signaling pathway emerges as another critical component in Bregs-mediated inhibition of Tfh cells. Blocking PD-L1 on B cells has

been shown to have a stimulatory effect on spleen B cells, enhancing the expression of the functional transcription factor Bcl-6 in Tfh cells (41). This is significant because there is a correlation between a decrease in circulating CD3-CD19<sup>+</sup>CD24<sup>hi</sup>CD27<sup>+</sup>Bregs and changes in Tfh subpopulations in patients with idiopathic pulmonary fibrosis. These alterations are characterized by increasing levels of Tfh2 and activated PD-1+ICOS<sup>+</sup>Tfh, as well as a decrease in Tfh17 cells (38). These discoveries necessitate further examination of the precise impacts of Bregs on various Tfh subsets, especially Tfh17 and Tfh2, which are associated with antibody production and ADs, including vasculitis and Sjogren's Syndrome. In vitro co-culture studies with Tfh and B cells have shown that human-derived CD19<sup>hi</sup>IgD<sup>+</sup>CD38<sup>hi</sup>CD24<sup>hi</sup>CD40<sup>hi</sup>PD-L1<sup>+</sup>IL-21R<sup>+</sup>Bregs significantly restrict Tfh maturation while promoting the expansion of Tfr cells. This finding underscores the potential of this specific Bregs subset to regulate Tfh and Tfr populations, with implications for immune regulation and the treatment of autoimmune conditions.

Indeed, the multifaceted nature of Bregs is exemplified by their ability to induce Tregs production through IL-10 secretion, as demonstrated by human CD19<sup>+</sup>CD24<sup>hi</sup>CD38<sup>hi</sup>Bregs, CD19<sup>+</sup>CD25<sup>hi</sup>Bregs, IL-10<sup>+</sup>CD1d<sup>hi</sup>CD5<sup>+</sup>Bregs, and mouse B220<sup>+</sup>CD23<sup>+</sup>Bregs. This induction of Tregs production could subsequently facilitate Tfr development (42). This highlights a potential link between Bregs, Tregs, and Tfrs in maintaining immune homeostasis. Research emphasizes the essential function of IL-10 in this process by demonstrating that the absence of IL-10 in B cells impairs Tfr differentiation and disrupts allograft tolerance. This underscores the significance of IL-10 in B-cell-mediated Tfr activation. Overall, these data support a complex regulatory network involving multiple Breg subsets that modulate the function and differentiation of Tfh and Tfr cells. This network promotes central tolerance and inhibits autoantibody production (Fig. 2).



**Fig. 2.** Mutual regulatory effects of Tfh, Tfr and Bregs: CD40L and TLR9 assist Bregs migration to germinal center (GC) by promoting CXCR5 expression. IL-10 secreted by Bregs inhibits ASCL2, the positive regulator of CXCR5, thereby down-regulating the level of CXCR5 in Tfh, promoting Tfh maturation and limiting the production of IL-21. Tfh activates p-STAT3 through IL-21, which drives the differentiation of Bregs. In response to TGF- $\beta$ , Bregs promotes Tfr differentiation.

Moreover, the localization of Tfh and Tfr within the immune microenvironment, particularly within the GC of secondary lymphoid organs, is a critical determinant of their function. The dynamic interplay between activated FDC and CXCR5-expressing T and B cells, guided by CXCL13, underscores the importance of stromal cell-mediated guidance signals in shaping GC architecture and cell interactions (43). The observed differences in Tfr migration patterns and intercellular interactions between the outer and central regions of the GC suggest that positional changes may significantly impact Tfh and Tfr function.

Given the pivotal role that autoantibodies play in the onset and progression of ADs, current therapeutic strategies are primarily

aimed at reducing autoantibody production or tightly inhibiting their functional activity. Among these, therapies that specifically target the key cellular players responsible for autoantibody generation have gained widespread popularity due to their remarkable efficacy and minimal adverse effects. Notably, a decrease in the number of Bregs, specifically subsets such as CD1d<sup>hi</sup>CD5<sup>+</sup>Bregs and CD19<sup>+</sup>CD25<sup>+</sup>CD1d<sup>hi</sup>IgM<sup>hi</sup>Bregs, can disrupt their interactions with relevant target cells, thereby influencing immune regulation. Consequently, strategies aimed at inducing the expansion of these Bregs have emerged as therapeutic options in mouse models of ADs, successfully restoring the delicate balance of immune homeostasis (44). This underscores the potential of targeting



autoantibody-producing cells as a therapeutic approach for ADs. Furthermore, Bregs may serve as a valuable biomarker, providing insights into the efficacy of treatment and disease prognosis. However, the complex interaction between Bregs, Tfh, and Tfr cells in both healthy individuals and AD patients are still not understood. This gap in understanding poses a significant obstacle in achieving precise modulation of Tfh and Tfr populations through Bregs manipulation. Therefore it highlights the need for further research to unravel these complex mechanisms and translate them into effective clinical interventions.

## DISCUSSION

Indeed, considering the crucial role of the Tfh/Tfr balance and Bregs in ADs, future research efforts should focus on several key aspects. Firstly, the development of new immunomodulatory drugs that specifically target Tfh, Tfr, and Bregs shows great potential for restoring immune balance by fine-tuning the distribution and function of these cells. These drugs could potentially modulate the abnormal interactions among these cell populations, thus reducing autoimmune responses. Secondly, the utilization of biomarkers, including specific cytokines, surface molecules, and other molecular signatures, represents a vital strategy for monitoring disease progression and assessing the efficacy of therapeutic interventions. These biomarkers can provide a real-time window into the underlying immune dynamics, enabling clinicians to tailor treatments more precisely and evaluate their impact in a timely manner. Thirdly, the exploration of cell-based therapies, particularly those involving the infusion of immunomodulatory cells such as Bregs, offers a promising avenue for reestablishing immune homeostasis in autoimmune patients. By replenishing or enhancing the numbers and functions of Bregs, these therapies aim to rebalance the immune system and suppress autoimmune attacks. Collectively, these research avenues have

the potential to not only unravel the intricate pathogenesis of ADs but also facilitate the creation of innovative, targeted therapeutic approaches. By advancing our understanding of the intricate interplay between Tfh, Tfr, and Bregs, we can harness these insights to devise more effective and personalized therapeutic approaches for patients suffering from autoimmune conditions.

The extensive literature emphasizes the pivotal role of the Tfh/Tfr balance in producing appropriate antibodies and its complex connection with Bregs. Additionally, Bregs not only influence the strategic positioning of Tfr and Tfh cells within the immunological environment, but also regulate the differentiation and function of these cells. By fine-tuning the levels of Tfh and Tfr subpopulations, Bregs help restore the delicate balance between these two cell types, which is often disrupted in ADs. Theoretically, targeting Bregs represents a feasible therapeutic strategy with promising clinical applications for modulating Tfh and Tfr functions and controlling autoimmunity in ADs. However, to fully harness the potential of this approach, several critical questions pertaining to the differentiation, phenotype, and functional characteristics of Bregs must be addressed. A comprehensive understanding of the intricate interplay between Bregs, Tfh, and Tfr in both healthy and autoimmune conditions is essential. This includes elucidating the specific signaling pathways, cytokines, and other molecular mechanisms that govern their interactions and influence their functional outcomes. Such insights will provide a solid foundation for designing more targeted and effective immunotherapies aimed at restoring immune balance and alleviating autoimmune pathology. In summary, while the role of Tfh/Tfr balance and Bregs in AD is well-established, further research is needed to unravel the complex web of interactions among these cells. This knowledge gap must be bridged to enable the creation of novel and effective therapeutic approaches for managing ADs.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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