REVIEW

TGF-β-secreting regulatory B cells: unsung players in immune regulation

Guoli Huai1,2, James F Markmann2, Shaoping Deng1 & Charles Gerard Rickert2

1Organ Transplantation Center, Sichuan Provincial People’s Hospital, School of Medicine, University of Electronic Science and Technology of China, Chengdu, China
2Center for Transplantation Sciences, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Correspondence
JF Markmann, Center for Transplantation Sciences, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA.
E-mail: jmarkmann@mgh.harvard.edu

Received 28 September 2020; Revised 25 December, 16 February and 5 March 2020; Accepted 9 March 2021
doi: 10.1002/cti2.1270

Clinical & Translational Immunology 2021; 10: e1270

Abstract
Regulatory B cells contribute to the regulation of immune responses in cancer, autoimmune disorders, allergic conditions and inflammatory diseases. Although most studies focus on regulatory B lymphocytes expressing interleukin-10, there is growing evidence that B cells producing transforming growth factor β (TGF-β) can also regulate T-cell immunity in inflammatory diseases and promote the emergence of regulatory T cells that contribute to the induction and maintenance of natural and induced immune tolerance. Most research on TGF-β+ regulatory B cells has been conducted in models of allergy, cancer and autoimmune diseases, but there has, as yet, been limited scrutiny of their role in the transplant setting. Herein, we review recent investigations seeking to understand how TGF-β+ producing B cells direct the immune response in various inflammatory diseases and whether these regulatory cells may have a role in fostering tolerance in transplantation.

Keywords: allergy, autoimmune diseases, cancer, TGF-β+ regulatory B cells, transplantation

INTRODUCTION

The immune system relies on a complex and intimately intertwined network of regulatory cells that restrain the response to self-antigens, prevent autoimmunity and temper physiological activation to promote resolution of immune responses. The regulatory cell population most thoroughly studied to date is the FOXP3-expressing CD4 T cell (Tregs).1,2 However, regulatory activity has been identified in varied immune cell lineages, including myeloid-derived suppressor cells (MDSCs), DC-regs and monocyte-derived regulatory macrophages, as well as NK and B cells.3 The anti-inflammatory and immunomodulatory functions of B cells were first described in the 1970s by Katz et al. and Neta et al., who showed that B cells with suppressive qualities play a role in preventing delayed hypersensitivity.4,5 In more recent studies, regulatory B cells (Bregs) have been found to modulate the immune response against tumors, autoimmune diseases, graft rejection and inflammatory diseases.6-8 In contrast to Tregs, which are predominately identified by the transcription factor, FOXP3, there are no lineage-specific markers or transcription factors that clearly define a discrete Breg subset.9 Therefore, the basic characterisation of Bregs, including identifiable surface markers and the mechanisms of immunosuppression, remains areas of active research.

© 2021 The Authors. Clinical & Translational Immunology published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.

2021 | Vol. 10 | e1270
Page 1
The regulatory actions of Bregs are exerted primarily via the elaboration of immunoregulatory cytokines, such as interleukin-10 (IL-10). In fact, in many studies, IL-10 expression is used as the best marker to define the subset of B cells with regulatory activity. However, other cytokines can also contribute to Breg function, including TGF-β and IL-35. In addition, TGF-β secretion by Bregs has been found to also play an important and independent role in immune regulation in various inflammatory diseases. Studies examining the suppression of IL-17+ and IFN-γ+ T cells by B cells indicate that both IL-10- and TGF-β-dependent processes are involved. In murine studies of Brucella infection, neutralisation of both IL-10 and TGF-β is more efficacious in promoting clearance of infection than either alone. These findings suggest that IL-10 and TGF-β can work in concert to dampen harmful immune responses.

Additional studies have demonstrated the singular importance of TGF-β1 in models of immune regulation. Bjarnadottir et al. showed that B-cell-specific deletion of TGF-β1 (B-TGF-β1−/−) in mice led to earlier onset of experimental autoimmune encephalitis (EAE), higher cumulative disease burden and higher T-cell production of GM-CSF and IFN-γ. Experiments utilising a coculture system of human CD19+CD25hi B cells and CD4+ T cells in vitro found TGF-β, not IL-10, to be the primary B-cell cytokine fostering the differentiation of Tregs. Furthermore, Bregs isolated from human blood suppressed the proliferation of CD4+ T cells and enhanced the expression of FOXP3 and CTLA-4 of Treg cells in TGF-β alone or with IDO, but not through IL-10-dependent ways. Collectively, these findings suggest that TGF-β secreted by B cells may not only partner with IL-10 but also possess a unique and, in some cases, an independent and dominant role in regulating the immune response.

This review examines the pathways that underlie B-cell production of TGF-β and how its function is both independent of and complementary to other Breg factors. We discuss how TGF-β has emerged as a key mediator in multiple Breg subsets with importance in cancer, allergy and autoimmune diseases. Finally, we examine the potential of TGF-β as an essential component of Breg-dependent transplant tolerance and how this cytokine might be utilised in concert with other immunomodulators to produce safer, more effective tools for clinical use.

TGF-β ACTIVATION, FUNCTION AND SIGNALLING PATHWAY

Transforming growth factor-β has three isoforms (TGF-β1, TGF-β2 and TGF-β3), with TGF-β1 as the prototypical TGF-β family member. TGF-β is secreted in a latent form, non-covalently associated with a homodimer of the latency-associated peptide (LAP). This LAP-TGF-β complex is either secreted or associated with another protein, LAP-TGF-β-binding protein (LTBP), to produce a larger latent form deposited onto the extracellular matrix. TGF-β can only function once separated from LAP and TGF-β is released through interactions between LAP and integrins, although integrin-independent pathways have been described, including alterations in pH, ROS and proteases. Once in its active form, TGF-β exerts its functions by binding to one of its cognate transmembrane signalling receptors, TGF-β type II receptor (TGFβRII), which then phosphorylates the accompanying TGF-β type I receptor (TGFβRI). TGFβRI will then activate Smothers against decapentaplegic homolog (SMAD)-dependent pathways, which transllocate to the nucleus and regulate the expression of several genes. In addition, TGF-β can activate several SMAD-independent signalling pathways, including the extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), p38, phosphatidylinositol 3-kinase (PI3K) and protein kinase B (AKT). These pathways may also contribute to regulating various cellular functions depending on cellular and tissue contexts.

B CELLS PRODUCE TGF-β IN MULTIPLE IMMUNOLOGICAL ENVIRONMENTS

Under physiological, healthy conditions, most B cells produce relatively low levels of both the precursor and active forms of TGF-β. Thus, the production and activation of TGF-β by a subset of B cells with suppressive functions mark a significant departure from typical B-cell activity. Given the complexity of Breg activity found in both murine and human systems, it is not surprising that TGF-β regulation is also complex, dependent on multiple signalling pathways and impacted greatly by the local environment. While there is overlap among the various mediators of TGF-β, three broad categories are found in B cells: (1) classic signalling pathway stimulation (e.g. TLR, BCR and CD40/CD40L), (2) growth
factor stimulation and (3) tumor-induced signalling.

**Classic signalling pathway stimulation**

Established B-cell signalling pathways, such as TLR, BCR and CD40/CD40L signalling, which are known to stimulate IL-10 production, can also regulate the production and activation of TGF-β. Moreover, Mishima et al. explored the effect of TLR stimulation on murine B cells by examining the differential production of IL-10 and TGF-β1 when B cells are cultured with lipopolysaccharide (LPS, a TLR-4 agonist) or unmethylated bacterial DNA (CpG, a TLR-9 agonist). The results showed that B cells upregulate the expression of both IL-10 and TGF-β when stimulated by these TLR ligands, with some differences in the degree of TGF-β expression. Of note, the level of TGF-β production in CpG-stimulated B cells was significantly higher than in B cells stimulated with LPS. Additionally, Parekh et al. have demonstrated that concurrent *in vitro* stimulation through the BCR and CD40 pathways can upregulate TGF-β expression in murine splenic B cells; however, when compared to the LPS-activated B cells, the level of TGF-β in the LPS-activated B cells was significantly higher.

Beyond the impact on gene and protein expression, multiple lines of research have revealed important signalling pathways regulating the activation of TGF-β in B cells. Recently, a type I transmembrane docking receptor, glycoprotein A repetitions predominant (GARP), was identified as the key cellular membrane attachment of the LAP-TGF-β complex and as a mediator of TGF-β activation and availability. Additionally, the overexpression of GARP on B lymphocytes was demonstrated to reduce B-cell proliferation, decrease T-cell-independent antibody production and increase class-switch recombination (CSR) to IgA. Stimulation through various Toll-like receptors, including TLR4, TLR7/8 and TLR9, can drive surface GARP expression on Peyer’s patch B cells. Using murine splenic B cells and human peripheral B cells, Wallace et al. have shown that *in vitro* stimulation of TLRs and the BCR results in upregulation of surface GARP and latent TGF-β. In these studies, stimulation of TLR4, TLR7/8 or TLR9 resulted in higher levels of surface GARP-TGF-β than in anti-IgM stimulation. In earlier studies, Dedobbeleer et al. also demonstrated that these pathways can upregulate GARP. In addition to

upregulation of surface GARP through TLR and BCR signalling, they demonstrated that CD40L stimulation can induce GARP at a low level on human B cells. In contrast to the results from Wallace et al., they found stimulation of the BCR to be more potent than either TLR signalling or CD40 stimulation in the induction of surface GARP. Dedobbeleer et al. also demonstrated that signalling through TLR, BCR, CD40L and cytokines together results in even higher levels of surface GARP and TGF-β, than in stimulation through only one pathway. While these two studies demonstrate the potential of these pathways to regulate TGF-β, the differences with regard to the relative potency of TLR and BCR signalling highlight the complexity of TGF-β regulation and the need for further research to fully understand how best to control B-cell-derived TGF-β.

**Thrombospondin 1 (TSP1)** is another important factor for TGF-β signalling by converting the LAP-TGF-β to active TGF-β. Some studies report that activated B cells, expressing TSP1, can convert DCs to tolerogenic dendritic cells (TolDCs), which can secrete TGF-β. TSP1-producing CD35+ B cells generated after specific immunotherapy (SIT) can attenuate ongoing allergic reactions in the intestine through Treg induction in a TGF-β-dependent fashion. *In vitro* studies using human B cells, stimulation by integrins αvβ6 plus anti-IgM antibody can upregulate TSP1.

**Growth factor stimulation**

Published data indicate that multiple growth factors can induce immune Bregs and upregulate TGF-β. Under the right stimulatory conditions, glioma cells can produce placenta growth factor (PIGF), a key molecule in angiogenesis and vasculogenesis, which can induce differentiation of naive B cells into TGF-β-producing Bregs. Therefore, *in vitro* co-culture of B cells with glioma-derived exosomes, anti-CD40 antibody and anti-IgM antibody could promote TGF-β Breg development. A study of patients with advanced stages of breast cancer showed that epidermal growth factor receptor 2 (HER2) is amplified as the proportion of circulating Tregs increases, correlating with high expression of TGF-β1 from CD19+CD24+CD38hi B cells. This correlation suggests that TGF-β from B cells may contribute to Treg induction. Other growth factors, such as insulin-like growth factor-2, have been associated

© 2021 The Authors. Clinical & Translational Immunology published by John Wiley & Sons Australia, Ltd on behalf of Australian and New Zealand Society for Immunology, Inc.
with the generation of Bregs.\textsuperscript{41} The direct impact of such growth factors on TGF-\(\beta\)-expression and activation in B cells remains to be fully explored.

### Tumor-induced signalling

Several studies suggest that B cells can be converted to TGF-\(\beta\)-producing B cells by exposure to various human cancer cell lines, including breast, ovarian and colon carcinomas. Importantly, the ability of cancer cells to generate Bregs may differ \textit{in vivo} versus \textit{in vitro}. \textit{In vivo} examination of tumor-infiltrating B cells (TIL-B) isolated from tumor tissue from mice injected subcutaneously with EMT-6 tumor cells noted that approximately 30\% of recovered TIL-B cells expressed TGF-\(\beta\) and 5\% expressed IL-10. The majority (~75\%) of the IL-10\(^+\) TIL-B cells also expressed LAP/TGF-\(\beta\). \textit{In vitro}, B cells co-cultured with EMT-6 cells showed similarly increased levels of LAP/TGF-\(\beta\)1 to TIL-B. Of note, these Bregs induced by EMT-6 cells require contact between B cells and tumor cells.\textsuperscript{42} \textit{In vivo}, CD19\(^+\) B cells isolated from mice bearing transplanted 4T1 mammary adenocarcinoma cells or infused with 4T1 cell-conditioned media were found to suppress T-cell proliferation. \textit{In vitro} treatment of B cells with cultured media from the 4T1 cell line results in a specific type of tumor-evoked Bregs (tBregs) that express high levels of TGF-\(\beta\). These tBregs were found to be functionally and phenotypically different from TLR- or BCR-activated suppressive B cells and other Bregs involved in autoimmune responses.\textsuperscript{43} While the underlying signalling that produces tBregs is not fully understood, their existence highlights a treatment strategy to promote TGF-\(\beta\) distinct from the earlier mentioned signalling.

### Additional signalling pathways involved TGF-\(\beta\) regulation

While the three categories of stimuli mentioned above constitute the best understood regulators of TGF-\(\beta\) in B cells, several other mechanisms have been shown to be important. These mechanisms include allergen stimulation, cytokines and products from microbes. Among allergens, the mixture of purified a-, b- and k-casein has been shown to stimulate PBMC from milk-tolerant individuals to produce TGF-\(\beta\)-producing CD19\^-CD5\^+ Bregs.\textsuperscript{44} For cytokines, B-cell-activating factor (BAFF, TNF family) stimulation has been shown to stimulate B-cell production of TGF-\(\beta\).\textsuperscript{43} Also, the cytokine IL-1\(\beta\) combined with the TLR3 immunostimulant, poly(I:C), can induce surface expression of GARP on B cells.\textsuperscript{34} Additionally, treatment with IL-4 in conjunction with CpG and CD40L enhances B-cell-dependent induction of Tregs through TGF-\(\beta\).\textsuperscript{45} One final promoter of B cell that has been studied in somewhat limited series is microbial factors, such as phorbol myristate acetate (PMA) from Penicillium and ionomycin from Streptomycetes, which facilitate TGF-\(\beta\) production from B cells.\textsuperscript{21} Moreover, stimulation by the bacterium \textit{Staphylococcus aureus} Cowan (SAC) complements CD40 and BCR signalling in B cells, resulting in suppression of T-cell stimulation through and IL-10 and PD-L1, and induction of Tregs by TGF-\(\beta\).\textsuperscript{46}

In sum, several stimuli can act alone or in combination to stimulate high expression levels and activation of TGF-\(\beta\) in B cells. Thus, optimising future production of TGF-\(\beta\)-producing Bregs requires further study and will likely utilise more than one of these various signalling pathways.

### TGF-\(\beta\)-DEPENDENT REGULATORY B CELLS FUNCTION IN INFLAMMATORY DISEASES

Several studies have demonstrated that TGF-\(\beta\)-producing B cells can regulate the immune response in various inflammatory diseases,\textsuperscript{47} including autoimmune diseases, cancer and allergic reactions (Table 1).\textsuperscript{7} From these studies, it is apparent that multiple mechanisms exist whereby TGF-\(\beta\)-producing B cells suppress immune responses that are distinct from IL-10-producing B cells (summarised in Figure 1). This diversity in functional capacity is likely impacted by differences in inflammatory environments and specific stimuli, resulting in TGF-\(\beta\)\(^+\) Breg generation.

### TGF-\(\beta\) PRODUCING B CELLS IN AUTOIMMUNE DISEASE

Studies in experimental autoimmune disease models reveal that Bregs modulate the course of the disease via the production of suppressive cytokines, principally IL-10, but also through the generation of TGF-\(\beta\). In fact, the first definitive evidence for the existence of Bregs was found in the observation that mice selectively lacking TGF-\(\beta\) in B cells (B-TGF-\(\beta\)\(^{-/-}\)) developed an exacerbated form of EAE, compared to wild-type controls.\textsuperscript{16}
Table 1. Different TGF-β-producing B cells regulate the immune response in various inflammatory diseases

| Species | Study | Designation/phenotype | Mechanism | Mediator | Induction | Diseases | Refs |
|---------|-------|-----------------------|-----------|----------|-----------|----------|------|
| Mice    | In vitro/in vivo | CD5⁺CD19⁺CX3CR1⁺ Tol B | Th2↓; Tregs↑; suppress T-cell activation | TGF-β | αvβ6 + anti-IgM | Food allergy | 38   |
| In vitro | | | CD8⁺ T-cell proliferation; CD8⁺ cell energy; IL-2, IFN-γ, TNF-α, IL-6, IL-13↑ | TGF-β/-fas-L | LPS; anti-Ig + anti-CD40 | Healthy | 31   |
| In vitro/vivo | | LAP⁺GARP⁺ B | GARP⁺; B-cell proliferation and activation; | TGF-β1, TGF-β2 | Anti-IAb + anti-C57BL/6J | SLE, oral-tolerance | 34   |
| In vitro/vivo | | | LAP⁺GARP⁺ T | T and B apoptosis; Th1↓ | TGF-β/Fas-L | LPS | T1DM | 50   |
| In vitro/vivo | | | LAP⁺GARP⁺ B | CD4⁺ Treg⁺; MDSCs (CD11b⁺Gr-1⁺)↑; CD8⁺ Tregs↑ | IL-10/TGF-β | CPG | Lung carcinoma | 55   |
| In vitro/vivo | | | CD19⁺CD25⁺B7H1⁺CD81⁺CD40⁺CD62L⁺IgM⁺IgG⁺ | Tregs↑; T-cell proliferation; GrzB-expressing CD8⁺ T cells; CD8⁺ INF-γ↓ | TGF-β | CM-4T1 PE | Breast cancer | 43   |
| In vivo | | | CD19⁺CD4⁺CD45⁺ | Th1/Th2/Th1 balance; Tregs↑ | TGF-β | | |   |
| In vivo | | | CD19⁺CD4⁺ | TSP1↓; Tregs↑; CD80/CD86 of DC↑; Th2↑ | TGF-β | | |   |
| In vivo | | | CD19⁺CD22⁺CD21⁺IgD⁺IgM⁺CD19⁺CD5⁺ | Tregs↑; CCR7 and CCR5↑ | TGF-β | LIT | Asthma | 61   |
| In vivo | | | TIM-1⁺LAP⁺ | Tregs↑; CC68; CCR7↑; INF-α, INF-γ, INF-γ; CD80/CD86⁺ | TGF-β | Anti-CD45RB + anti-Tim-1 | Islet Tx | 67   |
| In vivo | | | Tim-1⁺Transitional 2 B | Tregs↑; INF-γ; CD80⁺CD86⁺ | TGF-β | DST + MRI | Skin Tx | 70   |
| In vivo | | | CD19⁺IgM⁺IgA⁺PD-L1⁺CD4⁺CD21⁺CD23⁺ | CD4⁺ T-cell proliferation↑ | TGF-β | Anti-CD45RB | Skin Tx | 71   |
| Human   | In vitro | CD19⁺CD5⁺ | B-cell apoptosis | TGF-β | Casein | Allergy | 44   |
| In vitro | | | IL-10⁺; TGF-β⁺; CCR4↑; CXCL12↑; CD40⁺HLA-DR⁺ICAM-1⁺ B↑ | TGF-β | Fingolimod | MS | 51,52 |
| In vitro | | | CD4⁺ T-cell proliferation; CD4⁺ INF-γ↑; IL-4/IL-10⁺; TGF-β⁺ | TGF-β | Laquinimod | RRMS | 53   |
| In vitro | | | CD19⁺CD24⁺CD38⁺ | Tregs↑; TGF-β↑ | TGF-β | HER2 | Breast cancer | 40   |
| In vitro | | | CD19⁺CD24⁺CD38⁺ | Treg↑; TGF-β↑ | TGF-β | CD40L + anti-Ig + SAC | End plate inflammation | healthy | 46   |

(Continued)
Also noteworthy is the observation of reduced levels of CD19^Foxp3^ and CD19^TGFβ^ Bregs in blood PBMC of patients with a severe, accelerated form of rheumatoid arthritis (RA) associated with the development of interstitial lung disease (ILD). This observation suggests that TGF-β^ Bregs may contribute to the prevention of RA and ILD in a manner discrete from IL-10^ Bregs. TGF-β-producing B cells were demonstrated that primarily exert their anti-inflammatory effects early in the course of EAE through TGF-β-dependent signaling and that Bregs downregulate the surface expression levels of MHC class II and CD86 molecules of DCs. This may impede the initiation of a replete T-cell response, thus abrogating the encephalitogenic Th1/17 responses and pro-inflammatory cytokines, such as GM-CSF and IFN-γ, suggesting TGF-β may be relevant for B-cell-targeted therapies.

TGF-β-producing B cells can regulate autoimmune responses in both mice and humans. In mice, adoptive transfer of LPS-activated splenic B cells from NOD mice into prediabetic NOD mice inhibited spontaneous autoimmunity. Further experiments in mice suggest that these activated B cells can trigger apoptosis of T and B cells through TGF-β expression and/or by secretion of Fas ligand (Fas-L), preventing immune-mediated tissue destruction. Moreover, TGF-β secreted from LPS-activated B cells can induce CD8^ T-cell anergy. In patients with relapsing multiple sclerosis (MS), treatment with the immunomodulatory drug, fingolimod, increases TGF-β expression on Bregs and induces an exhausted-like phenotype in T cells, characterised by the reduction in IL-17 and IFN-γ expression, elevation of IL-10 and TGF-β, and expression of exhaustion markers such as PD-1 and Tim-3. Similarly, the MS treatment, laquinimod, is capable of modulating B-cell surface markers and increasing IL-10 and TGF-β in both B and T cells, in a B-cell-mediated manner.

Collectively, these data from multiple experimental settings substantiate a role for TGF-β-producing Bregs in preventing autoimmune disease. Furthermore, the action of some clinically used therapies may involve increasing TGF-β expression from B cells to regulate autoimmunity. Based on in vitro experiments showing increased TGF-β expression in B cells under various stimulatory conditions, future efforts may capitalise on this function of B cells to design novel therapies for autoimmune diseases.

| Species | Study | Designation/phenotype | Mechanism | Induction | Refs |
|---------|-------|-----------------------|-----------|-----------|------|
| In vitro | CD19^CD24^CD38^- | Tregs; CD4^Foxp3^- | CPG + CD40L + IL-4 | TGF-β | 39 |
| In vitro | CD25^CD27^ | CD1d^IL-10^ | CPG | TGF-β | 45 |
| In vitro | Transitional 2 B | TGF-β-producing B cells | CPG | CPG | 75 |
| In vitro | CD41^CD11c^- | TGF-β-producing B cells | CPG | CPG | 18 |
| In vitro | CD19^ | TGF-β expression in B cells under various stimulatory conditions | CPG | CPG | 39 |

↑ increase; ↓ decrease.
TGF-β-PRODUCING B CELLS IN CANCER

It is widely understood that cancer immunosurveillance and immunoediting are complex processes. Numerous regulatory components have been identified to limit an effective antitumor immune response. Regulatory effects of B cells and TGF-β produced by them have been implicated in various neoplastic settings. In B-cell-deficient mice, the growth rate of certain tumors is lower than in wild-type mice, and tumor cell growth increases markedly after the adoptive transfer of B cells, suggesting that the transferred B cells negatively modulate the anticancer immune response underway. This phenomenon has been demonstrated in cancer models using EL-4 thymoma, MC38 colon carcinoma, EMT-6 breast carcinoma and D5 mouse melanoma. These studies also found convincing evidence that Bregs aid in mediating tumor escape. However, the exact mechanism of Breg-mediated immune escape remains elusive, and how different subsets of Bregs, including TGF-β+ Bregs, contribute to protecting cancer cells is yet to be determined.

Multiple studies have demonstrated that TGF-β+ Bregs can be induced in vitro and in vivo directly by tumor cells or by various pro-tumorigenic growth stimuli, depending on the specific tumor environment. These Bregs express a high level of TGF-β and regulate the tumor immune response through the induction of Tregs. In both breast and gastric cancer, an increase in CD19+CD24+CD38 Bregs correlates with higher levels of CD4+FOXP3+ Tregs. TGF-β produced by these Bregs plays a significant immunosuppressive role in these cancer settings by inhibiting CD4+ effector T-cell cytokine production and converting CD4+CD25+ T cells to CD4+FOXP3+ Tregs, in a TGF-β-dependent fashion. TLR9-activated splenic B cells promote lung tumor growth by producing an increased immune-suppressive environment.
because of enhanced recruitment of Tregs, MDSCs and CD8+ Tregs cells together with higher levels of suppressive cytokines such as IL-10 and TGF-β. As mentioned earlier, incubation with 4T1 cancer cell-conditioned media for 2 days induces tBregs in vitro. These tBregs can mediate TGF-β-dependent conversion of non-Treg CD4+ T cells into metastasis-promoting FOXP3+ Tregs, which subsequently inactivate antitumor NK cells and effector CD8+ T cells, thereby protecting metastasising cancer cells. Some human cancer cell lines induce the generation of TGF-β+ Bregs and Tregs, which collaborate to increase the tumor’s potential to escape immunosurveillance and metastasise. Tregs, induced with B-cell-derived TGF-β, is an attractive therapeutic for future clinical application. For example, the natural-occurring phenol, resveratrol, has been shown, at high doses, to suppress cancer progression in mice via direct induction of apoptosis of malignant cells and indirect blockade of the generation of tumor escape-promoting FOXP3+ Tregs. Interestingly, Bodegai et al. found that in vivo treatment with CpG delivered via a modified form of CXXCL13 could, through inactivating Bregs and bolstering an antitumor T-cell response, block lung metastasis.

These findings collectively demonstrate that TGF-β+ Bregs are present in various tumor environments and can play a role in the growth and metastasis of the tumor. In many of these models, the induced Bregs increase the level of Tregs in a TGF-β-dependent manner, which assists in tumor escape from immunity; however, there are many other mechanisms that could also be B-cell-mediated. Understanding the role of TGF-B and Bregs in tumorigenesis and tumor immune escape will be of great importance for the rapidly advancing field of anticancer immunotherapeutics.

**TGF-β-PRODUCING B CELLS IN ALLERGY**

A key component of an allergy-prone immune environment is the failure of antigen-specific immune tolerance, this has been linked in some studies to TGF-β+ Bregs-induced regulation. Lee et al. studied human eczematous allergic reactions to cow’s milk and found that the proportion of TGF-β-producing CD19+CD5+ B cells increased and proliferated in the milk-tolerant group but not in the milk-allergic group. The disruption of TGF-β receptor signalling predisposes patients to develop allergic pathology, including asthma, food allergy, eczema and allergic rhinitis. Therefore, TGF-β-producing Bregs may factor in the generation of antigen-specific immune tolerance.

Some studies have demonstrated that TGF-β+ Bregs can regulate the allergic immune response through various mechanisms such as altering the balance of T-cell subtypes, inducing Treg development and enhancing apoptosis of effector inflammatory cells. In an OVA-based allergic airway inflammation model, the resulting allergic response manifested an increase in Th17 cells and skewed the balance of Th1/Th2 cells towards Th2. Interestingly, when monitored over time, the proportion of TGF-β+ Bregs and Tregs increased, and the expression of Th2-associated cytokines was inhibited. Meanwhile, the ratio of Th1/Th2 and the functioning of Th17 returned to normal. TSP1-producing CD35+ B cells, which increased after immunotherapy, can decrease the levels of CD80/CD86 on dendritic cells, convert naive CD4+ T cells to Tregs and suppress the Th2 response. TGF-β+ Bregs, isolated in vivo or produced from in vitro stimulation, can suppress the allergic reaction after adoptive transfer to recipients known to be allergic to specific stimuli. B cells isolated from hilar lymph nodes (HLNs) in mice with local inhalational tolerance (LIT) contained fivefold more TGF-β+ cells than IL-10+ cells and could convert naive CD4+CD25+ T cells into functionally suppressive CD4+CD25+FOXP3+ Tregs. After adoptive transfer of the LIT HLN CD5+ B cells into OVA-sensitised recipients, TGF-β-expressing CD5+ B cells and Tregs co-localised in B-cell zones, and the chemokines, CXCR4 and CXCR5, were upregulated. In the setting of this local immune-suppressed microenvironment, airway eosinophilia was inhibited. Another study on using a TGF-β-producing suppressive B cells, termed TolBCs (CD5+CD19+CX3CR1+ B), found that these cells can convert Th0 cells to CD4+CD25+FOXP3+ Tregs through TGF-β-dependent but not IL-10-dependent signalling. Adoptive transfer of TolBCs markedly suppressed the food allergy-induced intestinal Th2 inflammation pattern in mice.
therapies could be a promising treatment for allergic inflammation.

**THE PROSPECT OF TGF-β-PRODUCING B CELLS IN TRANSPLANTATION TOLERANCE**

In transplantation, B cells impact the response to a foreign graft in diverse ways, including a role in both the immune system’s attack on the graft and, paradoxically, the promotion of tolerance. The former action is tied to anti-allograft antibody generation and B-cell-mediated presentation of allograft antigens, resulting in allo-aggressive responses to organ transplants. The catastrophic impact of anti-allograft antibodies has been well-studied. In contrast, the graft-protective functions of B cells are much less understood and linked to the suppressive effects of a Breg subset that can constrain an immune response and prevent rejection. Bregs may be a novel and untapped candidate as an immunotherapeutic to curtail allo-aggressive T- and B-cell-mediated alloimmunity. As noted above for autoimmunity, cancer and allergy, the regulatory mechanism of Bregs is most often ascribed to IL-10. However, secreted TGF-β has the powerful ability to convert effector T cells to Tregs, which are an attractive weapon in the fight for long-lasting immune tolerance. The mechanisms of Breg-mediated TGF-β in multiple immune-privileged environments provide significant insight into the underpinnings of the role Bregs play in graft acceptance and transplantation tolerance induction.

A murine model of B-cell-dependent transplantation tolerance was first reported in 2007. This first demonstration utilised tolerance induction through *in vivo* treatment of wild-type and B-cell-deficient mice with anti-CD45RB. Mice lacking B cells failed to produce durable tolerance to islet allograft transplants, establishing B cells as a requisite mediator of tolerance in the model. The contribution of IL-10 was explored using the model of anti-CD45RB-induced allograft tolerance, through the administration of anti-IL-10 antibodies or by repopulating B-cell-deficient mice with B cells from IL-10−/− mice. Surprisingly, IL-10 neutralisation or deficiency failed to prevent tolerance and even accelerated rejection in some circumstances. In contrast, antibody neutralisation of TGF-β consistently prevented tolerance development. In additional studies, TGF-β−/− B cells were evaluated for their ability to promote tolerance in the same model. Adoptive transfer of B cells from B-cell-specific TGF-β−/− mice were unable to support tolerance development, whereas their littermate controls, TGF-β−/− heterozygotes, did so, providing strong evidence that TGF-β specifically from B cells is integral in the formation of allograft tolerance.

Parallel studies using a modified tolerance protocol consisting of anti-CD45RB and anti-TIM-1 antibody treatment (‘dual antibody treatment’) result in 100% long-term islet allograft survival that is dependent on the production of IL-10 by Tim-1+ Bregs. Interestingly, these dual antibody-treated islet transplant animals coexpress a significantly higher percentage of TIM-1+ LAP+ B cells versus no treatment, and these B cells can convert CD4+CD25+ T cells to CD4+CD25+FOXP3+ Tregs. As expected by this finding of LAP+ Bregs, tolerance induction using this protocol relies on TGF-β, in addition to the aforementioned IL-10. Tolerance via dual antibody treatment was abrogated when TGF-β activity was neutralised by anti-TGF-β antibody treatment. Tolerance through the adoptive transfer of B cells isolated from long-term survival mice was also abrogated by treatment with anti-TGF-β antibody, resulting promptly in rejection of allo-islets. These data demonstrate that the dual antibody treatment can induce Bregs that rely on the expression of both IL-10 and TGF-β to promote islet transplantation tolerance.

Similar to those experiments demonstrating TGF-β-dependent tolerance, transitional-2 (T2) splenic B cells from mice tolerant to MHC class I-mismatched skin grafts express high levels of Tim-1 and are capable of prolonging skin allograft survival and suppressing T-cell activation. The mechanism of tolerised T2 B cells may rely on direct infiltration of the graft and upregulated expression of TGF-β to alter Treg/T effector ratios. Recently, B cells isolated from OVA-specific B-cell receptor (OB1) mice, which underwent OVA skin graft and anti-CD45RB treatment to induce tolerance, were found to express almost 10 times higher LAP than IL-10. Furthermore, these tolerant mice failed to develop long-term graft survival after receiving the anti-TGF-β antibody. Adoptive transfer of the antigen-specific tolerant OB1 B cells is more potent than wild-type B cells at conferring tolerance to recipients undergoing OVA+ skin grafting. These experiments in mice demonstrate...
that Breg-dependent tolerance relies on the function of TGF-β and that this process is, at least in some circumstances, antigen-specific. 71

In NHP studies and human clinical transplants, there is mounting evidence that Bregs play a key role in transplant outcome. 72 In an interesting clinical trial for kidney transplants using B-cell depletion as the only induction therapy, there appeared to be an increased incidence of early rejection, possibly because of the elimination of the Breg subset 73. Also, a study of spontaneously tolerant recipients of kidney grafts identified a molecular B-cell signature associated with renal transplant tolerance through comparison of three groups: (1) tolerant patients who had stable graft function at least 1 year off immunosuppression, (2) patients with stable graft function while on immunosuppression and (3) healthy (non-transplanted) control subjects. Unexpectedly, these studies discovered that T1 and T2 transitional B-cell populations were found to express high levels of IL-10 in tolerant patients, but failed to show differences in the number of TGF-β-expressing B cells. 74 However, others have reported that in vitro stimulation of B cells, isolated from the peripheral blood of tolerant patients, possess increased expression of TGF-β, but no significant differences in IL-10 production. 75 These observations foster speculation that renal transplant tolerance may be associated with alterations in both T-cell- and B-cell-mediated functions. Whether IL-10 or TGF-β produced by B cells is required for tolerance in these human patients remains an open question.

Collectively, the data from mouse and human investigations indicate both B-cell-derived IL-10 and TGF-β contribute to the regulation of cellular immunity in the transplant setting. Based on established models of autoimmune disease, cancer and allergy, IL-10 and TGF-β can play different and complementary roles in regulation. The relative expression and importance of these two tolerogenic mediators may be dictated by the environment that provokes their expression in B cells.
cells. For example, in the tumor environment, high levels of Tregs are needed for tumor cell growth, migration and immune escape. TGF-β produced by Bregs has the potential to convert the CD4+CD25+ effector T cells to CD4+CD25-FOXP3+ Tregs and, to some extent, can induce CD8+ Tregs and tolerogenic DCs and macrophages, whereas IL-10 can suppress the generation of pro-inflammatory cytokines by T cells. Therefore, TGF-β from Bregs may be readily produced in the tumor environment through different mechanisms compared to those that promote IL-10 production.

Induction of donor-specific Tregs in vivo in transplant recipients is a theoretically attractive approach to limit acute and chronic allograft rejection and to avoid the toxicities of prolonged immunosuppressive therapy. Thus, the TGF-β+ Bregs may possess attributes directly relevant to developing innovative strategies for inducing transplant tolerance. Theoretically, to produce Breg-based cell therapies for clinical use, (Figure 2), B cells isolated from donor PBMCs would be stimulated by one or more signalling pathways to generate TGF-β+ Bregs. Next, these Bregs would be adoptively transferred to a recipient of the donor’s organ to promote durable tolerance. As mentioned earlier, the exact combination of stimuli to optimally produce TGF-β+ Bregs requires further research, but likely include factors that have been previously identified in various Breg environments, such as CpG, PMA, ionomycin, glioma-derived exosomes, anti-CD40, anti-IgM and IL-4. To some extent, the potency of TGF-β+ Bregs stimulated by a combination of stimuli may be stronger than if stimulated through a single signalling pathway, which would be expected based on the work from Dedobbeleer et al. Currently, our team at MGH is investigating the tolerogenic potential of B-cell activation by combination treatment with CpG, LPS, PMA and ionomycin. This protocol is a potent inducer of TGF-β+ B cells from naïve B cells in vitro. Adoptive transfer of B cells stimulated in this manner to murine transplant recipients results in long-term survival in a majority of recipients, in both islet and skin graft models. The results to date show promise in induced TGF-β+ B cells to suppress the T-cell immune response and prolong allograft survival.

Some key issues have to be addressed in order to establish TGF-β+ Bregs as a cell-based treatment for clinical transplantation tolerance induction, including (1) safe stimulation that avoids pro-inflammatory B-cell development, (2) understanding long-term in vivo survival and stability and (3) appropriate dosing depending on recipient and transplant factors. (1) The stimulation strategy to generate TGF-β+ Bregs in vitro must ensure that a safe product is produced. Multiple stimulation strategies can activate B cells to produce TGF-β in vitro, but different combinations of stimuli result in the different functions of TGF-β+ Bregs in various inflammatory responses and sometimes may lead to the opposite function. For example, after stimulation with BCR and anti-CD40 antibody, B cells showed markedly higher IgG expression than LPS-activated B cells. When B cells are stimulated with CpG and BCR, the result showed lower levels of TGF-β1 and high expression of IL-6 and TNF. These data suggest that some stimuli may promote excessive immune responses and favor the pro-inflammatory cytokine secretion from activated B cells. These different findings may be attributed to distinct stimulatory conditions of the B cells. For instance, human B cells activated by T-cell-dependent stimuli such as CD40/CD40L and BCR can confer strong CD8+ T-cell stimulation and response, while stimulation by T-independent pathways such as LPS may induce Breg-mediated anergy of CD8+ T cells, the results owing to distinct levels of surface TGF-β1 produced by B cells. The function of TGF-β from B cells is dependent not just on expression, but also on conversion from its latent to active form. The activation depends on the immunology circumstances and the target cell type. Therefore, identifying and understanding the regulatory function of Bregs from different stimuli are essential to their effective and safe application. (2) The stability of TGF-β+ Bregs in vivo is unclear, and the impact on long-term graft survival is not completely determined. Theoretically, if transferred Bregs could revert from their regulatory function and develop anti-allograft or anti-host properties, the outcome for the patient could be catastrophic. Thus, the plasticity and overall functional stability of Bregs in vivo require further investigation. (3) The optimal number of Bregs to be delivered will need to be ascertained to ensure both safety and efficacy of the therapy. Furthermore, the migration of Bregs to the target transplantation site may impact the number of
cells needed to achieve tolerance. How best to target the Bregs will be an important future direction to achieve a functional treatment. To address these important concerns for developing clinically valuable Breg-mediated transplant tolerance, it will be vital to utilise murine models, large animal transplant models and careful clinical observation.

CONCLUSION

B cells exhibit an array of powerful immunological functions including antigen presentation, antibody secretion and pro-inflammatory cytokine production. The nature and scope of B cells as regulators of immune responses in transplant are just now coming into focus. Bregs have demonstrated T regulatory functions through secretion of cytokines including IL-10, TGF-β and IL-35, as well as other immunomodulatory molecules such as granzyme B and the expression of negative co-stimulatory molecules such as PD-L1. IL-10 production is the most commonly studied and best understood suppressive phenotype, yet the role for TGF-β+ B cells in the regulatory network continues to grow and to be better explicated. Its role is now widely accepted in cancer immunity, allergy, autoimmune disease and other inflammatory diseases. The mechanisms by which TGF-β-producing B cells regulate immunity in the setting of various inflammatory diseases should be thoroughly considered for relevant application to the field of transplantation. A robust, mechanistic understanding of Breg-mediated immune regulation will enable further work to establish TGF-β+ Bregs as effective mediators of transplantation tolerance. Consequently, work towards the therapeutic administration of TGF-β+ Bregs may progress quickly given the straightforward manufacturing protocols used to produce large numbers of these cells in vitro alone or combined with Tregs. Bregs have enticing therapeutic potential for application in future transplant tolerance-inducing regimens.

ACKNOWLEDGMENTS

Our gratitude goes to Kevin Deng, who revised and corrected the grammar of the manuscript carefully and patiently. This work was supported by NIH Grant No. S01AI057851 (JFM), and the Science and the National Natural Science Foundation of China (No. 81571565).

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Guoli Huai: Data curation; Investigation; Project administration; Writing-original draft; Writing-review & editing. James F Markmann: Funding acquisition; Project administration; Supervision; Validation. Shaoping Deng: Funding acquisition; Validation. Charles Gerard Rickert: Validation; Writing-review & editing.

REFERENCES

1. Allos H, Al Dulaijan BS, Choi J, Azzi J. Regulatory T cells for more targeted immunosuppressive therapies. Clin Lab Med 2019; 39: 1–13.
2. Romano M, Fanelli G, Albany CJ, Giganti G, Lombardi G. Past, Present, and future of regulatory T cell therapy in transplantation and autoimmunity. Front Immunol 2019; 10: 43.
3. Najar M, Raicevic G, Fayyad-Kazan H, Bron D, Tourougou M, Lagneaux L. Mesenchymal stromal cells and immunomodulation: a gathering of regulatory immune cells. Cytotherapy 2016; 18: 160–171.
4. Katz SI, Parker D, Turk JL. B-cell suppression of delayed hypersensitivity reactions. Nature 1974; 251: 550–551.
5. Neta R, Salvin SB. Specific suppression of delayed hypersensitivity: the possible presence of a suppressor B cell in the regulation of delayed hypersensitivity. J Immunol 1974; 113: 1716–1725.
6. Cai X, Zhang L, Wei W. Regulatory B cells in inflammatory diseases and tumor. Int Immunopharmacol 2019; 67: 281–286.
7. van de Veen W. The role of regulatory B cells in allergen immunotherapy. Curr Opin Allergy Clin Immunol 2017; 17: 447–452.
8. Sarvaria A, Madrigal JA, Saudemont A. B cell regulation in cancer and anti-tumor immunity. Cell Mol Immunol 2017; 14: 662–674.
9. Rincon-Arevalo H, Sanchez-Parra CC, Castano D, Yassin L, Vasquez G. Regulatory B cells and mechanisms. Int Rev Immunol 2016; 35: 156–176.
10. Cerqueira C, Manfroi B, Fillatreau S. IL-10-producing regulatory B cells and plasmocytes: molecular mechanisms and disease relevance. Semin Immunol 2019; 44: 101323.
11. Molnarfi N, Bjarnadottir K, Benkhoucha M, Juillard C, Lalive PH. Activation of human B cells negatively regulates TGF-β1 production. J Neuroinflammation 2017; 14: 13.
12. Wang K, Liu J, Li J. IL-35-producing B cells in gastric cancer patients. Medicine (Baltimore) 2018; 97: e0710.
13. Ray A, Wang L, Dittel BN. IL-10-independent regulatory B-cell subsets and mechanisms of action. Int Immunol 2015; 27: 531–536.
14. Goenka R, Parent MA, Elzer PH, Baldwin CL. B cell-deficient mice display markedly enhanced resistance to the intracellular bacterium Brucella abortus. J Infect Dis 2011; 203: 1136–1146.
et al
15. Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol 2006; 176: 705–710.

16. Bjarnadottir K, Benkhoucha M, Merkler D et al. B cell-derived transforming growth factor-β1 expression limits the induction phase of autoimmune neuroinflammation. Sci Rep 2016; 6: 34594.

17. Hong M, Liao Y, Liang J et al. Immunomodulation of human CD19+CD25hi regulatory B cells via Th17/Foxp3 regulatory T cells and Th1/Th2 cytokines. Hum Immunol 2019; 80: 863–870.

18. Nouel A, Pochard P, Simon Q et al. B-cells induce regulatory T cells through TGF-β/IDO production in a CTLA-4 dependent manner. J Autoimmun 2015; 59: 53–60.

19. Coomes SM, Moore BB. Pleiotropic effects of transforming growth factor-β in hematopoietic stem-cell transplantation. Transplantation 2010; 90: 1139–1144.

20. Ungefroren H. Blockade of TGF-β signaling: a potential target for cancer immunotherapy? Expert Opin Ther Targets 2019; 23: 679–693.

21. Mizushima Y, Ishihara S, Hansen JJ, Kinoshita Y. TGF-β detection and measurement in murine B cells: pros and cons of the different techniques. Methods Mol Biol 2014; 1190: 71–80.

22. Wipff PJ, Hinz B. Integrins and the activation of latent transforming growth factor β1 - an intimate relationship. Eur J Cell Biol 2008; 87: 601–615.

23. Tamayo E, Alvarez P, Merino R. TGFβ superfamily members as regulators of B cell development and function-implications for autoimmunity. Int J Mol Sci 2018; 19: 3928.

24. Kehrl JH, Roberts AB, Wakefield LM, Jakowlew S, Sporn MB, Fauci AS. Transforming growth factor β is an important immunomodulatory protein for human B lymphocytes. J Immunol 1986; 137: 3855–3860.

25. Zan H, Cerutti A, Dramitinos P, Schaffer A, Casali P. CD40 engagement triggers switching to IgA1 and IgA2 in human B cells through induction of endogenous TGF-β: evidence for TGF-β but not IL-10-dependent direct Sm–Sx and sequential Sm–Sγ1 α–Sx DNA recombination. J Immunol 1998; 161: 5217–5225.

26. Saulep-Easton D, Vincent FB, Quah PS et al. The BAFF receptor TACI controls IL-10 production by regulatory B cells and CLL B cells. Leukemia 2016; 30: 163–172.

27. Nova-Lamperti E, Fanelli G, Becker PD et al. IL-10 produced by human transitional B-cells down-regulates CD86 expression on B-cells leading to inhibition of CD4+ T-cell responses. Sci Rep 2016; 6: 20044.

28. Matsushita T, Horikawa M, Iwata Y, Tedder TF. Regulatory B cells (B10 cells) and regulatory T cells have independent roles in controlling experimental autoimmune encephalomyelitis initiation and late-phase immunopathogenesis. J Immunol 2010; 185: 2240–2252.

29. Mishima Y, Ishihara S, Aziz MM et al. Decreased production of interleukin-10 and transforming growth factor-β1 in Toll-like receptor-activated intestinal B cells in SAMP1/Yit mice. Immunology 2010; 131: 473–487.

30. McWilliam Q, Sellebjerg F, Marquart HV, van Essen MR. B cells from patients with multiple sclerosis have a pathogenic phenotype and increased LTα and TGFβ1 response. J Neuroimmunol 2018; 324: 157–164.

31. Parekh JV, Prasad DV, Banerjee PP, Joshi BN, Kumar A, Mishra GC. B cells activated by lipopolysaccharide, but not by anti-lg and anti-CD40 antibody, induce anergy in CD8+ T cells: role of TGF-β1. J Immunol 2003; 170: 5897–5911.

32. Zhou AX, Kozhaya L, Fujii H, Unutmaz D. GARP-TGF-β1 complexes negatively regulate regulatory T cell development and maintenance of peripheral CD4+ T cells in vivo. J Immunol 2013; 190: 5057–5064.

33. Edwards JP, Fujii H, Zhou AX, Creemers J, Unutmaz D, Shevach EM. Regulation of the expression of GARP/latent TGF-β1 complexes on mouse T cells and their role in regulatory T cell and Th17 differentiation. J Immunol 2013; 190: 5506–5515.

34. Wallace CH, Wu BX, Salem M et al. Lymphocytes confer immune tolerance via cell surface GARP-TGF-β1 complex. JCI Insight 2018; 3: e98963. https://doi.org/10.1172/jci.insight.98963. PMID: 29618665; PMCID: PMC5928869.

35. Dedobbeleer O, Stockis J, van der Woning B, Coulie PG, Lucas S. Cutting edge: active TGF-β1–producing B cells restore antigen (Ag)-specific immune tolerance in an allergic environment. J Biol Chem 2015; 290: 12858–12867.

36. Yang G, Geng XR, Liu ZQ et al. Thrombospondin-1 (TSP1)-producing B cells restore antigen (Ag)-specific immune tolerance in an allergic environment. J Biol Chem 2015; 290: 12858–12867.

37. Tabib A, Krispin A, Trahtemberg U et al. Thrombospondin-1-N-terminal domain induces a phagocytic state and thrombospondin-1-C-terminal domain induces a tolerizing phenotype in dendritic cells. PLoS One 2009; 4: e6840.

38. Liu ZQ, Wu Y, Song JP et al. Tolerogenic CX3CR1+ B cells suppress food allergy-induced intestinal inflammation in mice. Allergy 2013; 68: 1241–1248.

39. Han S, Feng S, Ren M et al. Glioma cell-derived placental growth factor induces regulatory B cells. Int J Biochem Cell Biol 2014; 57: 63–68.

40. Gheybi MK, Farrokhhi S, Ravanbod MR, Ostovar A, Mehrzad V, Nematiollahi P. The correlation of CD19+CD24+CD38- B cells and other clinicopathological variables with the proportion of circulating Tregs in breast cancer patients. Breast Cancer 2017; 24: 756–764.

41. Geng XR, Yang G, Li M et al. Insulin-like growth factor-2 enhances functions of antigen (Ag)-specific regulatory B cells. J Biol Chem 2014; 289: 17941–17950.

42. Zhang Y, Morgan R, Chen C et al. Mammary-tumor-educated B cells acquire LAP/TGF-β and PD-L1 expression and suppress anti-tumor immune responses. Int Immunol 2016; 28: 423–433.

43. Olkhanud PB, Damdsuren B, Bodgai M et al. Tumor-evoked regulatory B cells promote breast cancer metastasis by converting resting CD4+ T cells to T regulatory cells. Cancer Res 2011; 71: 3505–3515.

44. Lee JH, Noh J, Noh G, Choi WS, Cho S, Lee SS. Allergen-specific transforming growth factor-β1-producing CD19+CD5+ regulatory B-cell (Br 3) responses in human late eczematous allergic reactions to cow's milk. J Interferon Cytokine Res 2011; 31: 441–449.

45. Kessel A, Haj T, Peri R et al. Human CD19+CD25hi B regulatory cells suppress proliferation of CD4+ T cells and enhance Foxp3 and CTLA-4 expression in T regulatory cells. Autoimmun Rev 2012; 11: 670–677.
46. Xu C, Zhang M, Li K et al. CD24hiCD38hi B regulatory cells from patients with end plate inflammation presented reduced functional potency. *Int Immunopharmacol* 2019; 70: 295–301.
47. Dai YC, Zhong J, Xu JF. Regulatory B cells in infectious disease (Review). *Mol Med Rep* 2017; 16: 3–10.
48. Guo Y, Zhang X, Qin M, Wang X. Changes in peripheral CD19 Foxp3+ and CD19 TGFβ+ regulatory B cell populations in rheumatoid arthritis patients with interstitial lung disease. *J Thorac Dis* 2015; 7: 471–477.
49. Li MO, Wan YY, Sanjabi S, Robertson AK, Flavell RA. Transforming growth factor-β regulation of immune responses. *Annu Rev Immunol* 2006; 24: 99–146.
50. Tian J, Zekzer D, Hansen L, Lu Y, Olcott A, Kaufman DL. Lipopolysaccharide-activated B cells down-regulate Th1 immunity and prevent autoimmune diabetes in nonobese diabetic mice. *J Immunol* 2001; 167: 1081–1089.
51. Blumenfeld S, Staun-Ram E, Miller A. Fingolimod therapy modulates circulating B cell composition, increases B regulatory subsets and production of IL-10 and TGFβ in patients with Multiple Sclerosis. *J Autoimmun* 2016; 70: 40–51.
52. Blumenfeld-Kan S, Staun-Ram E, Miller A. Fingolimod reduces CXCR4-mediated B cell migration and induces regulatory B cells-mediated anti-inflammatory immune repertoire. *Mult Scler Relat Disord* 2019; 34: 29–37.
53. Toubi E, Nussbaum S, Staun-Ram E et al. Laquinimod modulates B cells and their regulatory effects on T cells in multiple sclerosis. *J Neuroimmunol* 2012; 251: 45–54.
54. Wang WW, Yuan XL, Chen H et al. CD19+CD24hiCD38hi Bregs involved in downregulate helper T cells and upregulate regulatory T cells in gastric cancer. *Oncotarget* 2015; 6: 33486–33499.
55. Sorrentino R, Morello S, Forte G et al. B cells contribute to the antitumor activity of CpG-oligodeoxynucleotide in a mouse model of metastatic lung carcinoma. *Am J Respir Crit Care Med* 2011; 183: 1369–1379.
56. Lee-Chang C, Bodogai M, Martin-Montalvo A et al. Inhibition of breast cancer metastasis by resveratrol-mediated inactivation of tumor-evoked regulatory B cells. *J Immunol* 2013; 191: 4141–4151.
57. Bodogai M, Lee Chang C, Wejksza K et al. Anti-CD20 antibody promotes cancer escape via enrichment of tumor-evoked regulatory B cells expressing low levels of CD20 and CD137L. *Cancer Res* 2013; 73: 2127–2138.
58. Braza F, Chesne J, Castagnet S, Magnan A, Brouard S. Regulatory functions of B cells in allergic diseases. *Allergy* 2014; 69: 1454–1463.
59. Wang M, Gu Z, Yang J, Zhao H, Cao Z. Changes among TGF-β1+ Breg cells and helper T cell subsets in a murine model of allergic rhinitis with prolonged OVA challenge. *Int Immunopharmacol* 2019; 69: 347–357.
60. Zhang HP, Wu Y, Liu J et al. TSP1-producing B cells show immune regulatory property and suppress allergy-related mucosal inflammation. *Sci Rep* 2013; 3: 3345.
61. Natarajan P, Singh A, McNamara JT et al. Regulatory B cells from hilar lymph nodes of tolerant mice in a murine model of allergic airway disease are CD5+, express TGF-β1, and co-localize with CD4+Foxp3+ T cells. *Mucosal Immunol* 2012; 5: 691–701.
62. Faust SM, Lu G, Marini BL et al. Role of T cell TGFβ1 signaling and IL-17 in allograft acceptance and fibrosis associated with chronic rejection. *J Immunol* 2009; 183: 7297–7306.
63. Regateiro FS, Howie D, Cobbold SP, Waldmann H. TGF-β in transplantation tolerance. *Curr Opin Immunol* 2011; 23: 660–669.
64. Deng S, Moore DJ, Huang X et al. Cutting edge: transplant tolerance induced by anti-CD45RB requires B lymphocytes. *J Immunol* 2007; 178: 6028–6032.
65. Huang X, Moore DJ, Moliuddin M et al. Inhibition of ICAM-1/FA-1 interactions prevents B-cell-dependent anti-CD45RB-induced transplantation tolerance. *Transplantation* 2008; 85: 675–680.
66. Zhao G, Moore DJ, Lee KM et al. An unexpected counter-regulatory role of IL-10 in B-lymphocyte-mediated transplantation tolerance. *Am J Transplant* 2010; 10: 796–801.
67. Lee KM, Kim JJ, Stott R et al. Anti-CD45RB/anti-TIM-1-induced tolerance requires regulatory B cells. *Am J Transplant* 2012; 12: 2072–2078.
68. Ding Q, Yeung M, Camirand G et al. Regulatory B cells are identified by expression of TIM-1 and can be induced through TIM-1 ligation to promote tolerance in mice. *J Clin Invest* 2011; 121: 3645–3656.
69. Lee KM, Stott RT, Zhao G et al. TGF-β-producing regulatory B cells induce regulatory T cells and promote transplantation tolerance. *Eur J Immunol* 2014; 44: 1728–1736.
70. Moreau A, Blair PA, Chai JG et al. Translational-B E cells acquire regulatory function during tolerance induction and contribute to allograft survival. *Eur J Immunol* 2015; 45: 843–853.
71. Kimura S, Rickert CG, Kojima L et al. Regulatory B cells require antigen recognition for effective allograft tolerance induction. *Am J Transplant* 2020; 20: 977–987.
72. Liu C, Noorhashm H, Sutter JA et al. B lymphocyte-directed immunotherapy promotes long-term islet allograft survival in nonhuman primates. *Nat Med* 2007; 13: 1295–1298.
73. Clatworthy MR, Watson CJ, Plotnek G et al. B-cell-depleting induction therapy and acute cellular rejection. *N Engl J Med* 2009; 360: 2683–2685.
74. Newell KA, Asare A, Kirk AD et al. Identification of a B cell signature associated with renal transplant tolerance in humans. *J Clin Invest* 2010; 120: 1836–1847.
75. Sagoo P, Perucha E, Sawitzki B et al. Development of a cross-platform biomarker signature to detect renal transplant tolerance in humans. *J Clin Invest* 2010; 120: 1848–1861.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.