Regulatory T cells induced by B cells: a novel subpopulation of regulatory T cells
Chien-Hui Chien and Bor-Luen Chiang

Abstract
Regulatory T cells play a crucial role in the homeostasis of the immune response. In addition to CD4+Foxp3+ regulatory T cells, several subsets of Foxp3- regulatory T cells, such as T helper 3 (Th3) cells and type 1 regulatory T (Tr1) cells, have been described in mice and human. Accumulating evidence shows that na"ive B cells contribute to tolerance and are able to promote regulatory T cell differentiation. Na"ive B cells can convert CD4+CD25+ T cells into CD25-Foxp3+ regulatory T cells, named Treg-of-B cells by our group. Treg-of-B cells express LAG3, ICOS, GITR, OX40, PD1, and CTLA4 and secrete IL-10. Intriguingly, B-T cell-cell contact but not IL-10 is essential for Treg-of-B cells induction. Moreover, Treg-of-B cells possess both IL-10-dependent and IL-10-independent inhibitory functions. Treg-of-B cells exert suppressive activities in antigen-specific and non-antigen-specific manners in vitro and in vivo. Here, we review the phenotype and function of Foxp3+ regulatory T cells, Th3 cells, Tr1 cells, and Treg-of-B cells.

Keywords: Regulatory T cells, Lymphocyte-activation gene 3, Programmed cell death protein 1, Inducible T-cell co-stimulator, Interleukin 10, Cytotoxic T lymphocyte-associated antigen-4, Treg-of-B cells

Background
Regulatory T cells are a therapeutic strategy for immune dysregulated diseases and a potential target for cancer immunotherapy. In addition to CD4+Foxp3+ regulatory T (Treg) cells, studies have emphasized the roles of CD4+Foxp3- regulatory T cells, such as TGF-β-producing Th3 cells, IL-10-producing type 1 regulatory T (Tr1) cells, and others. Accumulating evidence demonstrates that naïve B cells possess the ability to promote naïve CD4+ T cells into CD25+ Foxp3+ regulatory T cells with the expression of lymphocyte activation gene-3 (LAG3, CD223), inducible co-stimulator (ICOS, CD278), programmed cell death protein 1 (PD1, CD279), and glucocorticoid-induced TNFR family-related protein (GITR). B-cell-induced CD4+Foxp3- regulatory T cells exert the inhibition through both IL-10-independent and cell-cell contact-dependent mechanisms, although they also show IL-10-mediated suppression. Furthermore, these B cell-induced regulatory T cells protect mice from several immune disorders, including graft-versus-host disease, experimental allergic asthma, collagen-induced arthritis, and inflammatory bowel disease. Here, we review the phenotypes and functional mechanisms of thymus-derived and peripherally derived CD4+Foxp3+ regulatory T cells, Th3 cells, Tr1 cells, B-cell-induced Foxp3- regulatory T cells, and B-cell-induced Foxp3+ regulatory T cells. The present article focuses on B-cell-induced CD4+Foxp3- regulatory T cells, which we have named Treg-of-B cells.

Main text
CD4+Foxp3+ regulatory T cells
Sakaguchi et al. demonstrated that CD4+CD25+ T cells contributed to maintaining self-tolerance in a non-antigen-specific manner [1]. Immune dysregulation, polyendocrinopathy, enteropathy X-linked (IPEX) syndrome is a recessive immune disorder. Reports showed that IPEX is caused by mutations of FOXP3 gene, which is orthologue of the Foxp3 gene mutated in scurfy mouse [2–4]. Further studies demonstrated that Foxp3 expressed predominantly in CD4+CD25+ T cells than CD4+CD25- T cells and CD19+ B cells. Moreover, retroviral transduction of Foxp3 in naïve CD4+CD25- T cells converted these cells toward Treg cells phenotype. Thus, Foxp3 has been identified as the master transcription factor of Treg cells [5].
Thymus-derived Foxp3+ regulatory T cells
In addition to Foxp3, thymus-derived CD4+CD25+Foxp3+ regulatory T (tTreg) cells highly expressed Helios, cytotoxic T lymphocyte-associated antigen-4 (CTLA4, CD152), neuropilin-1, GITR, galectin-1, IL-10, and granzyme B [6]. tTreg cells could be activated in an antigen-specific fashion and exerted suppressive activity in a non-antigen-specific fashion [7]. tTreg cells produced many inhibitory cytokines, including TGF-β1, IL-10, and IL-35, to downregulate immune responses [8]. Furthermore, tTreg cells exhibited cell-cell contact-dependent suppression via latency-associated peptide (LAP) [9], CD39 (ectonucleoside triphosphate diphosphohydrolase-1, ENTPD1) and CD73 (ecto-5′-nucleotidase) [10], and cytotoxic cyclic adenosine monophosphate (cAMP) [11]. Reports showed that tTreg cells induced effector T cell apoptosis via various pathways, including deprivation of IL-2 and IL-7 [12], disruption of effector cell membrane integrity by granzyme B [13], galectin-1-induced apoptosis [14], and the engagement of TNF-related apoptosis inducing ligand (TRAIL)-death receptor 5 (DR5) [15]. Additionally, tTreg cells inhibited effector T cell activation via downregulation of costimulatory molecules on DCs through CTLA4 [16] and LAG3 [17]. These studies indicate that tTreg cells are a polyclonal population, and the above mentioned complicated mechanisms result in maximal immunosuppression during homeostasis.

Peripherally derived Foxp3+ regulatory T cells
Foxp3+ regulatory T cells induced in vivo are called peripherally derived regulatory T cells (pTreg) cells and those generated in vitro are called in vitro-induced regulatory T cells (iTreg) cells [18]. Studies demonstrated that CD4+Foxp3+ T cells differentiated into Foxp3+CD25+CD45RBlow anergic T cells with suppressive functions in the presence of TGF-β1 in vitro as well as in vivo [19] and rescue Foxp3-deficient scurfy mice [20]. In the absence of iTreg cells, oral antigen administration induced the generation of CD4+CD25+Foxp3+ regulatory T cells in a TGF-β1-dependent manner [21]. Gut-associated lymphoid tissue CD103+ DCs played an important role in the de novo conversion of naïve T cells into pTreg cells, and retinoic acid facilitates that process [22]. Additionally, lung-resident tissue macrophages expressed retinal dehydrogenases, and TGF-β1 promoted pTreg cell induction under steady-state conditions [23]. Evidence has shown that the tumor environment induced pTreg cell generation to escape immune clearance [24]. One report demonstrated that tTreg and pTreg cells shared similar phenotypes, and neuropilin-1 serving as a surface marker to distinguish tTreg cells from pTreg cells [25].

CD4+Foxp3+ regulatory T cells
The most well-defined Foxp3+ regulatory T cells are Th3 cells and Tr1 cells. Th3 cells have been identified as TGF-β-producing CD4+LAP+ T cells exhibiting TGF-β-mediated suppression [26]. Tr1 cells have been characterized by the higher production of IL-10 and IL-10-mediated suppressive functions [27].

T helper 3 cells
Th3 cells were first found in mesenteric lymph node CD4+ T cells as single cell clones producing TGF-β1 after oral administration of self-antigen [28]. Oida et al. found that primary purified CD4+CD25 LAP+ regulatory T cells protected mice from T-cell-induced colitis in a TGF-β1-dependent manner [29]. Tumor environment CD4+CD25 CD69+Foxp3+ LAP+ T cells expressed IL-2 receptor β chain, produced TGF-β1, and exerted TGF-β1-mediated functional activity [30]. Gandhi et al. showed that human peripheral CD4+LAP+Foxp3+CD69+ T cells exhibited TGF-β1- and IL-10-dependent suppression in the periphery in healthy individuals [31]. Furthermore, human CD4+CD25+LAP+Foxp3+ T cells in colorectal tumors expressed LAG3 and exhibited inhibitory functions through TGF-β1 and IL-10 [32]. To date, the specific transcription factor for Th3 cells remains to be identified.

Type 1 regulatory T cells
The first study on Tr1 cells reported that naïve T cells repeated stimulation with peptide-pulsed splenocytes in the presence of IL-10 induced IL-10-producing CD4+Foxp3+ regulatory T cells with suppressive ability and hypoproliferative ability [33]. Akbari et al. demonstrated that bronchial DCs promoted Tr1 cells in vitro in an IL-10-and ICOS/ICOS ligand (ICOSL)-dependent manner in the context of nasal tolerance [34]. By microarray analysis Tr1 and Th0 cell clones, CD49b, LAG3, and CD226 have been identified as the surface markers of Tr1 cells [35].

It has been shown that c-Maf transactivated IL-10 expression under CD4+Th17 polarization conditions [36]. Aryl hydrocarbon receptor (AhR) and c-Maf facilitated IL-10 production in CD4+ T cells in an IL-27-dependent fashion [37, 38]. Another study reported that c-Maf, IL-21, and ICOS were essential for IL-27-induced Tr1 cell generation [39]. Consistent with these observations, Awasthi et al. showed that CD4+Foxp3+ regulatory T cell-educated DCs produced IL-27 and promoted Tr1 cell generation [38]. Nasal anti-CD3e antibody treatment induced the expression of IL-10, IL-27, and TGF-β in nasal tolerogenic DCs, which further facilitated Tr1 cell generation through c-Maf, IL-21, and AhR [40]. Orally antigen treated tolerogenic Peyer’s patch DCs increased the production of IL-10 and IL-27 and promoted the induction of Tr1 cells [41]. Carrier et al. reported that
constitutive ectopic expression of GITR ligand (GITRL) on MHCI^+ APCs increased IL-27 production and further upregulated the expression of c-Maf and IL-10 in T cells [42].

In addition to cytokines, reports have demonstrated that Tr1 cells could be induced by different proteins, different APCs, and different types of T cells. Galectin-1 promoted IL-10 expression in CD4^+ T cells in an APC-independent pathway by binding to CD45 on T cells and inducing the expression of c-Maf and AhR [43]. In vitro activation of CD4^+CD44^hiFoxp3^+ T cells through anti-CD3/CD28 antibodies and IL-2 generated CD49b^-, LAG3^-, c-Maf^-, and AhR-expressing Tr1 cells [44]. Nie et al. found that long-term stimulation of lipopolysaccharide (LPS) conferred ICOSL expression in bone marrow-derived mast cells through NF-κB, subsequently promoting Tr1 cell development [45]. These reports suggest that the generation mechanisms for Tr1 cells consist of a fine-tuning program.

**B cells in tolerance induction**

B cells have been shown to have a role in the fine equilibrium for immune tolerance. Genetically B-cell-deficient mice delayed recovery from experimental autoimmune encephalomyelitis and suggested B cells might contribute to immune modulation [46]. Collagen fragments expressed on B cell MHC class II sufficiently delayed the onset and decreased the severity of arthritis [47]. The role of B cells in oral tolerance has been investigated because B-cell-deficient mice exhibit a defective oral tolerogenic response characterized by lower levels of IL-10 and TGF-β in the spleen and gut-associated lymphoid tissues [48]. Gutgemann et al. showed that B cells interacted with T cells at the B-T border in the spleen after 4 h of oral administration of proteins [49]. Furthermore, orally antigen treated B cells have an enhanced ability to induce CD4^+ regulatory T cells in vitro [50]. Anterior chamber-associated immune deviation was characterized by antigen-specific downregulation of the immune response to antigen occurs in the anterior chamber of the eye [51], and this phenomenon was abrogated in the absence of B cells [52]. Studies suggested that splenic B cells presented antigens derived from ocular APCs and induced CD4^+CD25^+ regulatory T cells via IL-10 and MHC class II [52, 53]. These evidence emphasize the role of B cells in the induction and maintenance of self-tolerance.

There is accumulating evidence demonstrating that specific B cell subsets modulate immune responses named as regulatory B (Breg) cells by Mizoguchi et al. [54]. Breg cells dampened immune responses though the secretion of IL-10, TGF-β, directly interact with activated CD4^+ T cells, and the production of antibody that neutralized harmful soluble molecules [55]. Several Breg cells have been described in mice and IL-10-producing Breg cells are the most widely studied [56]. IL-10 produced by a variety of Breg cells suppressed inflammatory cytokines and promoted regulatory T cell differentiation [57, 58]. These indicate that B cells contribute to the maintenance of tolerance.

In addition, naïve B cells functioned as antigen-presenting cells presented antigen and resulted in T cell tolerance to antigen [59]. Raimondi et al. demonstrated that adoptive transfer of antigen-presenting B cells four times in a week lead to antigen-specific CD4^+ T cells tolerance independent of naïve or activated B cells [60, 61]. Antigen-presenting follicular B, marginal zone B, and B-1a cells rendered antigen-specific T cells hyporesponsiveness without Foxp3^+ Treg cells induction [62]. One study reported that B cells contributed to Treg cells homeostasis and cooperated with Treg cells to ameliorate inflammation [63]. These findings suggest that B cells play a role in immune modulation and might through the manipulation of CD4^+ Treg cells.

**B-cell-induced CD4^+Foxp3^+ Treg-of-B cells**

Naïve splenic B2 cells, peritoneal B-1a cells, and mucosal Peyer’s patch B cells have been shown to induce CD4^+CD25^+Foxp3^+ regulatory T cells, which named Treg-of-B cells by our group, without additional cytokines or molecules [50, 64]. Naïve splenic B cells and naïve splenic CD4^+CD25^+ T cells formed a stable immunological synapse and promoted CD62L^hiCD25^Foxp3^+ regulatory T cell generation [65]. In our reports, transferrin delivery during B-T coculture abrogated Treg-of-B cell induction suggesting that cell-cell contact between B and T cells was essential. By applying blocking antibodies during B-T coculture both CD80 and CD86 on splenic B cells were required to induce functional Treg-of-B [64]. In consistent with above, Etemire et al. demonstrated that addition of anti-CD28 antibody to the B-T cell co-culture decreased the suppressive activity of Treg-of-B cells. Lower activity of the PI3K/AKT pathway was associated with Foxp3^+ regulatory T cell generation [66]. IL-10-deficient Treg-of-B cells and Treg-of-B cells induced in the presence of anti-IL-10 neutralizing antibody remained their suppressive function suggesting that IL-10 was not critical for their induction [64, 67, 68]. These results suggest that the interaction between B-T cells is indispensable for the differentiation of Treg-of-B cells.

**Treg-of-B cells differ from well-known Treg cells**

To date, several molecules have been identified for their strong association with Treg-of-B cells that are conserved in single peptide-induced and anti-CD3/CD28 antibodies-induced methods. Treg-of-B cells expressed higher levels of LAG3, ICOS, PD1, GITR, OX40 (CD134), and CTLA4 compared to those on naïve CD4
"CD25" T cells (Fig. 1). Another group demonstrated that antigen-presenting B cells facilitated naïve T cells to convert into CD4⁺CD25⁺CD62L⁺Foxp3⁺ IL-10-producing regulatory T cells [65]. Our published and unpublished data showed that Treg-of-B cells did not express Foxp3, Helios, or neuropilin-1 [67, 69], and these also confirmed by using Foxp3-GFP reporter mice [64]. These evidence differentiates Treg-of-B cells from Foxp3-expressing Treg cells (Table 1).

Th3 cells are well-known that they exert TGF-β-dependent inhibition and express LAP on surface [26]. Although Treg-of-B cells produced TGF-β compared with naïve CD4⁺CD25⁻ T cells [68, 69], TGF-β did not play a role in their suppressive mechanism [64]. In our unpublished data, Treg-of-B cells did not express LAP. These indicate that Treg-of-B cells are different from Th3 cells.

Tr1 cells are characterized by IL-10-mediated suppression and the higher production of IL-10 [27]. In recent years, CD49b, LAG3, and CD226 were identified as the surface markers for human and mouse Tr1 cells [35]. In our results, Treg-of-B cells produced a higher amount of IL-10 compared with naïve CD4⁺CD25⁻ T cells [50, 64]. Repeated stimulation of B cells induced long-term Treg-of-B cells with higher expression of ICOS, CTLA4, CD49b, and c-Maf, but not CD226. In addition to the difference in surface marker, IL-10 seems to be dispensable in the inhibitory mechanism of Treg-of-B cells and these would be described in the later section. These observations suggest that this Treg-of-B cell is a new type of regulatory T cells and different from Tr1 cells.

In addition to regulatory T cells, Treg-of-B cells did not share characteristics with follicular T helper (T_{FH}) cells. T_{FH} cells expressed BCL-6, CXCR5, ICOS, PD1, and c-Maf and CXCR5 conferred T_{FH} cells migration to B follicles [67, 70]. Although Treg-of-B cells expressed ICOS, PD1 and c-Maf, they did not express the critical molecule BCL-6 and CXCR5 (data not shown). These indicate that Treg-of-B cells could not migrate into follicle to facilitate B cell as T_{FH} cells did.

Furthermore, Treg-of-B cells were hypoproliferative to stimulation and did not express T-bet, GATA3, or ROR-γt ([64] and our unpublished data). Treg-of-B cells produced higher level of IL-10, TGF-β, and IL-4 and lower or no IL-2, IFN-γ, IL-17, or tumor necrosis factor (TNF)-α [68, 69, 71]. These data confirm that Treg-of-B cells have anergic characteristics and are not proinflammatory T helper cells.

**Application of Treg-of-B cells**

The therapeutic effects of CD4⁺Foxp3⁺ Treg-of-B cells has been described in several murine disease models (Fig. 2). Adoptive transfer of Treg-of-B cells prevented mice from graft-versus-host disease in a murine model of heart transplantation [65]. Peyer’s patch B-cell-induced ovalbumin (OVA)-specific Treg-of-B cells

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**Fig. 1** Treg-of-B cells differ from well-known regulatory T cells and T helper cells. With regard to transcription factors, Treg-of-B cells do not express Foxp3, ROR-γt, T-bet, or BCL-6. Repeated stimulation increased the expression of c-Maf in long-term Treg-of-B cells. Treg-of-B cells produce a higher amount of IL-10 and TGF-β and lower amounts of IL-17 and IFN-γ. Several Treg-associated molecules have been described in Treg-of-B cells, including LAG3, PD1, ICOS, CTLA4, and GITR. Long-term cultured Treg-of-B cells express CD49b but do not express CD226 as Tr1 cells. Treg-of-B cells do not express ROR-γt as Th17 cells do, do not express T-bet as Th1 cells do, do not express CXCR5 or BCL-6 as T_{FH} cells do, and do not express LAP as Th3 cells do. These indicate Treg-of-B cell is a new type of CD4⁺ regulatory T cells.
protected mice from Th2-cell-mediated airway hyperresponsiveness (AHR), airway inflammation, and IgE hyperproduction in allergic asthma in an antigen-specific fashion [50]. In addition, splenic B-cell-induced OVA-specific Treg-of-B cells shared several characteristics with oral antigen administration activated CD4⁺CD25⁺ T cells, including elevated expression levels of ICOS, PD1, and CTLA4 and enhanced non-antigen-specific suppressive functions [69]. Monoclonal antibody-induced Treg-of-B cells prevented mice from osteolysis and joint inflammation in collagen-induced arthritis [71]. Prophylactic transfer of Treg-of-B cells also protected mice from T-cell-induced Th1- and Th17-dominant inflammatory bowel disease [68]. Taken together, naïve B cell without cytokines or chemical supplements is able to induce functional CD4⁺Foxp3⁻ regulatory T cells and that B-cell-induced regulatory T cells is an economical strategy for cellular therapy for different T-helper-cell-dominant inflammatory diseases.

**Table 1** The differences between Treg-of-B cells and the well-known Treg cells, including Foxp3⁺ Treg, Th3, and Tr1 cells

| Treg cells | Biomarkers | Effector molecules | Transcription factors | Assisted cell types |
|------------|------------|--------------------|-----------------------|-------------------|
| Treg-of-B  | CD4⁺CD25⁺Foxp3⁻LAG3⁻ICOS⁻PD1⁺GITR⁺OX40⁺ | Majorly contact-dependent IL-10, LAG3, and CTLA4 has reported in reference | Undefined | B cells |
| Foxp3⁺ Treg | CD4⁺Foxp3⁺Helios has reported in reference | IL-10, TGF-β, IL-35, LAP, CD39/CD73, cAMP, CTLA4, LAG3, IL-2/IL-7 consumption, granzyme B, galectin-1, DR5…etc | Foxp3 | DCs, macrophages, B cells |
| Th3        | CD4⁺Foxp3⁺LAP⁺ | Majorly TGF-β IL-10 has reported in reference | Undefined | DCs |
| Tr1        | CD4⁺Foxp3⁺CD49b⁺LAG3⁺CD226⁺ | Majorly IL-10 TGF-β, CTLA4, and CD226 has reported in reference | Undefined | DCs, macrophages, B cells, mast cells…etc |

Treg-of-B cells possess both IL-10-dependent and IL-10-independent suppressive functions

IL-10 as an anti-inflammatory cytokine is an issue in Treg-of-B cells suppressive function. As described above, IL-10 does not play a crucial role in Treg-of-B cells differentiation. Chen and Chu et al. reported that LAG3⁺Treg-of-B cells produced higher amount of IL-10 and both IL-10 and LAG3 play the roles in their inhibitory mechanisms [71, 72]. Long-term Treg-of-B cells increased expression levels of CTLA4 and IL-10, both of which were involved in their suppressive functions [67]. IL-10-deficient mice were used to confirm the role of IL-10 in the regulation; however, IL-10-deficient Treg-of-B cells remained suppressive activities [64, 68]. IL-10 seems to be dispensable in the inhibitory mechanism of Treg-of-B cells. Although IL-10 plays a more important role in long-term Treg-of-B cells than in short-term Treg-of-B cells, three-day short-term culture is sufficient
for the generation of Treg-of-B cells. These suggest that there might be unknown inhibitory factors in Treg-of-B cells suppressive functions.

Studies have demonstrated that ICOS controls IL-10 production and functional CTLA4 expression in Treg cells [73–75]. PD1 recruits SHP-1 and SHP-2 to intrinsically downregulate T cell receptor signaling, which maintains an anergic phenotype in Treg cells [76, 77]. Mouse Treg cells constitutively expressed GITR and OX40 and involved the tTreg cells development as well as their functions [78–80]. All regulatory-T-related molecules on Treg-of-B cells, including IL-10, TGF-β, LAG3, CTLA4, ICOS, PD1, GITR, and OX40, might confer partial suppressive activities to compensate for single blockage or neutralization. The critical molecules controlling Treg-of-B cell phenotype and regulatory mechanisms remain priorities for investigation. The inhibitory functions of Treg-of-B cell depend on the suppressive molecules on the surface or soluble mediators that require short distance.

**B-cell-induced CD4*Treg-of-B**

Reports have revealed the role of B cells in the development of Treg cells. Naïve primary B cells preferentially induced the expansion of allogenic CD4*Treg-of-B cells rather than CD4*Treg-of-B T cells [81, 82]. Splenic B cells converted allogenic naïve T cells into Foxp3* regulatory T cells in the presence of TGF-β and IL-2, and peritoneal B cells induce Th17 cells [83]. Human CD40-activated B cells induced the differentiation of CD25+Foxp3*CD62L regulatory T cells more efficiently than immature DCs [84, 85]. In contrast, reports demonstrated that murine CD40-activated B cells promoted CD4*T cell proliferation and effector functions [86, 87]. Furthermore, the frequency of intrathymic B cells correlated with that of tTreg cells, and B cells colocalized with tTreg cells in the thymus [88, 89]. Intrathymic B cells expressed autoimmune regulator (Aire), increased the levels of MHC class II and CD80, and contributed T cell negative selection for central T cell tolerance [90, 91]. Taken together, there are unknown criteria, such as MHC class II-TCR signaling, the B cell activation status, and different types of tissue resident B cells, that may fine-tune the expression of Foxp3 in B-cell-induced regulatory T cells.

**Conclusions**

To date, we know that naïve antigen-presenting B cell is sufficient to induce CD4*Treg-of-B regulatory T cells without additional cytokines or chemicals in an IL-10- and IL-27-dispensable and cell-cell contact-dependent manner. The expression levels of characteristic molecules differentiate Treg-of-B cells from well-known T helper and regulatory T cells as a brand-new type of CD4*Foxp3 regulatory T cells (Fig. 1). Treg-of-B cells possess IL-10-dependent, IL-10-independent, and cell-cell contact-dependent suppressive abilities in antigen-specific and non-antigen specific fashions. Compared to long-term Treg-of-B cells, short-term Treg-of-B cells act through multiple suppressive pathways, and thus a blockade strategy would be more easily overcome through compensation by other pathways. Treg-of-B cells exhibit immunomodulatory effects in Th2-, Th1-, and Th17-mediated diseases and even allogeneic transplantation. Nevertheless, the physiological conditions or cues necessary for Treg-of-B cell generation remain unknown. What is the fine-tuning mechanism for B cells to induce CD4*Treg-of-B or expand CD4*Treg-of-B T cells? What factors determine the kinetics, memory, and maintenance? And, most importantly, how could we use Treg-of-B cells in immunotherapy?

**Abbreviations**

Breg: Regulatory B; Foxp3: Forkhead box P3; ICOS: Inducible T-cell co-stimulator; IL-10: Interleukin 10; iTreg: in vitro-induced Treg; LAG3: Lymphocyte-activation gene 3; PD1: Programmed cell death protein 1; pTreg: Peripherally derived regulatory T; TGF-β: Transforming growth factor-β; Th3: Type 3 helper; Treg: Type 1 regulatory T; Treg: Regulatory T; Treg-of-B: B-cell-induced regulatory T; tTreg: Thyimus-derived regulatory T

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**Authors’ contributions**

C-H C performed the literature reviewed and drafted the manuscript. B-L C supervised and critically reviewed the manuscript. Both authors read and approved the final manuscript.

**Authors’ information**

None.

**Ethics approval and consent to participate**

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**Consent for publication**

None.

**Competing interests**

The authors declare that they have no competing interests.

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**Reference**

1. Sakaguchi S, et al. Immuno logic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a
single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155(3):1151–64.

2. Wildin RS, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet. 2001;27(1):18–20.

3. Bennett CL, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet. 2001;27(1):20–1.

4. Tommasini A, et al. X-chromosome inactivation analysis in a female carrier of FOXP3 mutation. Clin Exp Immunol. 2002;130(1):127–30.

5. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2002;299(5609):1057–61.

6. Sugimoto N, et al. Foxp3-dependent and -independent molecules specific for CD25+CD4+ natural regulatory T cells revealed by DNA microarray analysis. Int Immunol. 2006;18(8):1197–209.

7. Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol. 2000;164(1):183–90.

8. Collison LW, et al. The inhibitory cytokine IL-35 contributes to regulatory T-cell function. Nature. 2007;450(7169):566–9.

9. Nakamura K, Kitani A, Strober W. Cell contact-dependent immunosuppression by CD4+CD25+ regulatory T cells is mediated by cell surface-bound transforming growth factor beta. J Exp Med. 2001;194(5):629–44.

10. Deaglio S, et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression. J Exp Med. 2007;204(6):1257–65.

11. Bopp T, et al. Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression. J Exp Med. 2007;204(6):1303–10.

12. Pandiyan P, et al. CD4+CD25+Foxp3+ regulatory T cells induce cytokine deprivation-mediated apoptosis of effector CD4+ T cells. Nat Immunol. 2007;8(12):1353–62.

13. Gondek DC, et al. Cutting edge: contact-mediated suppression by CD4+CD25+ regulatory cells involves a granzyme B-dependent, perforin-independent mechanism. J Immunol. 2005;174(4):1783–6.

14. Garin MI, et al. Galectin-1: a key effector of regulation mediated by CD4+CD25+ T cells. Blood. 2007;109(5):2058–65.

15. Ren X, et al. Involvement of cellular death in TRAIL/DR5-dependent suppression induced by CD4+CD25+ regulatory T cells. Cell Death Differ. 2007;14(12):2076–84.

16. Wing K, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science. 2008;322(5909):271–5.

17. Liang B, et al. Regulatory T cells inhibit dendritic cells by lymphocyte activation gene-3 engagement of MHC class II. J Immunol. 2008;180(9):5916–26.

18. Abbas AK, et al. Regulatory T cells: recommendations to simplify the nomenclature. Nat Immunol. 2013;14(4):307–8.

19. Chen W, et al. Conversion of peripheral CD4+CD25-naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med. 2003;198(12):1875–86.

20. Huter EN, et al. TGF-beta-induced Foxp3+ regulatory T cells rescue scurfy mice. Eur J Immunol. 2008;38(7):1814–21.

21. Muraida D, et al. Oral tolerance in the absence of naturally occurring Tregs. J Clin Invest. 2005;115(7):2015–23.

22. Coombes JL, et al. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. J Exp Med. 2007;204(8):1757–64.

23. Soroosh P, et al. Lung-resident tissue macrophages generate Foxp3+ regulatory T cells and promote airway tolerance. J Exp Med. 2013;210(4):755–88.

24. Cao X, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity. 2007;27(2):463–46.

25. Yadav M, et al. Neuruplin-1 distinguishes natural and inducible regulatory T cells among regulatory T cell subsets in vivo. J Exp Med. 2012;209(10):1713–22.

26. Weiner HL, et al. Oral tolerance. Immunol Rev. 2011;241(1):241–59.

27. Zeng H, et al. Type I regulatory T cells: a new mechanism of peripheral immune tolerance. Cell Mol Immunol. 2015;12(5):566–71.

28. Chen Y, et al. Regulatory T cell clones induced by oral tolerance: suppression of autoimmune encephalomyelitis. Science. 1994;265(5176):1237–40.
54. Mizoguchi A, et al. Chronic intestinal inflammatory condition generates IL-10-producing regulatory B cell subset characterized by CD1d upregulation. Immunity. 2002;16(2):219–30.

55. Mizoguchi A, Bhan AK. A case for regulatory B cells. J Immunol. 2006;176(2):705–10.

56. Miyagaki T, Fujimoto M, Sato S. Regulatory B cells in human inflammatory and autoimmune diseases: from mouse models to clinical research. Int Immunol. 2015;27(10):495–504.

57. Mauri C, Bosma A. Immune regulatory function of B cells. Annu Rev Immunol. 2012;30:221–41.

58. Gray M, et al. Apoptotic cells protect mice from autoimmune inflammation by the induction of regulatory B cells. Proc Natl Acad Sci U S A. 2007;104(35):14080–5.

59. Eynon EE, Parker DC. Small B cells as antigen-presenting cells in the induction of tolerance to soluble protein antigens. J Exp Med. 1992;175(1):131–8.

60. Raimondi G, et al. Induction of peripheral T cell tolerance by antigen-presenting B cells. I. Chronic antigen presentation overrules antigen-presenting B cell activation. J Immunol. 2006;176(7):4021–8.

61. Raimondi G, et al. Induction of peripheral T cell tolerance by antigen-presenting B cells. II. Relevance of antigen presentation persistence. J Immunol. 2006;176(7):4012–20.

62. Murray SE, Toren KG, Parker DC. Peripheral CD4+ T-cell tolerance is induced in vivo by rare antigen-bearing B cells in follicular, marginal zone, and B-1 subsets. Eur J Immunol. 2013;43(7):1818–27.

63. Wang L, et al. T regulatory cells and B cells cooperate to form a regulatory loop that maintains gut homeostasis and suppresses dextran sulfate sodium-induced colitis. Mucosal Immunol. 2015;8(6):297–312.

64. Hsu LH, et al. A B-1a cell subset induces Foxp3+ T regulatory cells in vivo. EMBO J. 2015;34(11):1536–46.

65. Reichardt P, et al. Naive B cells generate regulatory T cells in the presence of a mature immunologic synapse. Blood. 2007;110(5):1519–29.

66. Etemire E, et al. Transiently reduced PI3K/Akt activity drives the development of regulatory function in antigen-stimulated naive T-cells. PLoS One. 2013;8(7):e68378.

67. Chien CH, et al. Characterization of c-Maf+Foxp3- regulatory T cells induced by repeated stimulation of antigen-presenting B cells. Sci Rep. 2017;7:46348.

68. Shao TY, et al. Novel Foxp3(-) IL-10(-) regulatory T-cells induced by B-cells alleviate intestinal inflammation in vivo. Sci Rep. 2016;632415.

69. Chien CH, Yu HH, Chiang BL. Single allergen-induced oral tolerance inhibits airway inflammation in conjunctival allergen immunized mice. J Allergy Clin Immunol. 2015;136(4):1110–3, e4.

70. Qi H. T follicular helper cells in space-time. Nat Rev Immunol. 2016;16(10):612–25.

71. Chen SY, et al. Lymphocyte-activation gene 3(+) (LAG3(+)) forkhead box protein 3(-) (FOXP3(-)) regulatory T cells induced by B cells alleviates joint inflammation in collagen-induced arthritis. J Autoimmun. 2016;68:75–85.

72. Chu KH, Chiang BL. Characterization and functional studies of forkhead box protein 3(-) lymphocyte activation gene 3(+) CD4(+) regulatory T cells induced by mucosal B cells. Clin Exp Immunol. 2015;180(2):316–28.

73. Kôhyama M, et al. Inducible costimulator-dependent IL-10 production by regulatory T cells specific for self-antigen. Proc Natl Acad Sci U S A. 2004;101(12):4192–7.

74. Tuettenberg A, et al. The role of ICOS in directing T cell responses: ICOS-dependent induction of T cell anergy by tolerogenic dendritic cells. J Immunol. 2009;182(6):3349–56.

75. Zheng J, et al. ICOS regulates the generation and function of human CD4+ Treg in a CTLA-4-dependent manner. PLoS One. 2013;8(12):e82280.

76. Chemitz JM, et al. SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation. J Immunol. 2004;173(2):945–54.

77. Fife BT, et al. Interactions between PD-1 and PD-L1 promote tolerance by blocking the TCR-induced stop signal. Nat Immunol. 2009;10(11):185–92.

78. Ronchetti S, et al. Glucocorticoid-induced tumour necrosis factor receptor-related protein: a key marker of functional regulatory T cells. J Immunol Res. 2015;2015:171520.

79. Grisetti T, et al. OX40 is required for regulatory T cell-mediated control of colitis. J Exp Med. 2010;207(4):699–709.