International Immunology, Vol. 26, No. 2, pp. 61–69
doi:10.1093/intimm/dxt060
Advance Access publication 9 December 2013

Foxp3$^+$ T_{reg} cells in humoral immunity

James B. Wing and **Shimon Sakaguchi**

Department of Experimental Immunology, WPI Immunology Frontier Research Center, Osaka University, Suita 565–0871, Osaka, Japan

Correspondence to: S. Sakaguchi; E-mail: shimon@ifrec.osaka-u.ac.jp

Received 27 September 2013, *accepted* 23 October 2013

REVIEW

T_{reg} cells are essential for the maintenance of immune homeostasis and prevention of autoimmunity. In humoral immune responses, loss of T_{reg} cell function causes increased levels of serum autoantibodies, hyper-IgE, spontaneous generation of germinal centres, and enhanced numbers of specialised T follicular helper cells (T_{fh} cells) controlled by the lineage-defining transcription factor BCL-6 (B-cell lymphoma 6). Recent studies have demonstrated that a subset of T_{reg} cells [T follicular regulatory (T_{freg}) cells] are able to co-opt the follicular T-cell program by gaining expression of BCL-6 and travelling to the follicle where they have an important role in the control of expansion of T_{fh} cells and the germinal centre reaction. However, the mechanisms by which they exert this control are still under investigation. In this review, we discuss the effects of T_{reg} cells on humoral immunity and the mechanisms by which they exert their regulatory function.

Keywords: antibody, CTLA-4, T-follicular helper cells, T-follicular regulatory cells

Introduction

The generation of high-affinity antibody is critical to protection from infectious disease and other threats. During the T-dependent antibody response, antigen-presenting B cells at the border of the T-cell zone form cognate interactions with T_h cells and, following co-stimulatory interactions, may either form short-lived extrafollicular plasmablast cells—responsible for rapid but low-affinity antibody production (1)—or traverse to the B-cell follicle. Once activated *in situ* there, B cells begin to proliferate and form a germinal centre where longer-lasting, high-affinity responses are induced (2,3).

Although germinal centres are critical for the generation of protective antibody responses, their dysregulation may lead to autoimmunity (4). A large proportion of B cells are autoreactive at an early stage of development (5) and, despite central tolerance mechanisms such as receptor editing, clonal deletion and anergy (6–8), autoreactive B cells are still present in the periphery. Additionally, somatic hypermutation within the germinal centre allows the generation of autoreactive B cells during the response to foreign antigens (9) and also allows chromosomal translocations that have a causative role in the generation of lymphomas (10–12).

As such, it is evident that self-reactive germinal centres must be controlled to avoid autoimmunity but also that some level of regulation of even non-self-reactive germinal centres must be in place. This is achieved by a number of mechanisms such as antibody feedback (13) and follicle-resident CD8$^+$ T cells (14,15). However, in this article we will focus on the contribution of Foxp3-expressing T_{reg} cells to the control of the humoral response. Various phenotypes of T cells have immunosuppressive properties, but the best understood are CD4$^+$CD25$^+$Foxp3$^+$ T_{reg} cells. These cells are critical to the regulation of humoral immunity as both mice and humans with loss of Foxp3 function have raised levels of serum antibodies (16,17). More recently, it has also become clear that T_{reg} cells are able to travel into the B-cell follicle and directly regulate the germinal centre response (18–20).

T_{reg} cells

Foxp3$^+$ T_{reg} cells make up around 10% of peripheral CD4$^+$ T cells and have a critical role in the maintenance of immune self-tolerance and homeostasis (21,22). The function of T_{reg} cells is controlled by two key features: (i) the expression of the transcription factor Foxp3 (23–25), responsible for the maintenance of several key phenotypic factors in T_{reg}-cell function such as CTLA-4 expression and repression of IL-2 production and (ii) the maintenance of T_{reg}-type epigenetics, a specific DNA hypomethylation pattern that is required for the stability and full functional capabilities of T_{reg} cells (26). In cases where expression of Foxp3 is lost, such as in the scurfy mouse strain and immune dysregulation polyendocrinopathy enteropathy X-linked syndrome (IPEX) patients, the resulting loss of T_{reg}-cell function causes a range of severe immune disorders such as widespread autoimmunity, immunopathology and lymphoproliferation (17,24,27–29).

In terms of humoral immunity, Foxp3-deficient mice have uncontrolled germinal centre reactions and large numbers of plasma cells and T follicular helper cells (T_{fh} cells) (20,30). In the K/BxN mouse model of autoantibody-driven arthritis, loss of T_{reg}-cell function, owing to the introduction of the Foxp3 scurfy mutation, leads to accelerated production of

pathogenic autoantibodies and accumulation of spleenic plasma cells (30,31). In humans, B cells from IPEX patients produce large amounts of autoreactive antibodies (32). Another characteristic feature of the loss of Foxp3 function is hyper-IgE in both mice (33) and IPEX patients (29). This may be partly because of the disproportionate effect of the loss of T_{reg}-cell function on IL-4, critical for IgE production, in comparison with other cytokines (34).

Although valuable information can be gained from the study of systems where T_{reg} cells are absent from birth, it can be difficult to determine whether particular phenotypes are specifically the result of T_{reg}-cell depletion or secondary to the high levels of inflammation seen in such circumstances. This issue has been addressed by models in which mammalian diphtheria toxin receptor is co-expressed with Foxp3, making T_{reg} cells sensitive to diphtheria-toxin-induced cell death and allowing specific depletion of T_{reg} cells in adult mice, which in turn leads to the induction of widespread autoimmunity similar to scurfy mice (35,36). The use of anti-CD25 antibodies to deplete T_{reg} cells is also effective.

A number of studies have used these systems to address the consequences of peripheral T_{reg}-cell depletion on the humoral immune response and the generation of autoantibodies. CD25-based depletion of T_{reg} cells leads to increased autoantibody production in a murine model of arthritis (37) or in lupus-prone NZB/NZW F_1 mice (38). Accordingly, transfer of *ex-vivo*-expanded T_{reg} cells into NZB/NZW F_1 mice ameliorates disease (39). The maintenance of peripheral anergy in autoreactive B cells is a critical tolerance mechanism, preventing activation of autoreactive B cells (8,40,41). In the absence of T_{reg} cells, normally anergic, dsDNA-specific B cells lose their anergic phenotype and, following provision of T-cell help, produce anti-ds-DNA antibodies. Transfer of CD25⁺ T_{reg} cells prevents this process (42). Correspondingly, specific T_{reg}-cell depletion via diphtheria toxin also leads to a loss of B-cell anergy (43). Glucocorticoid-induced TNFR-related protein (GITR) is expressed at high levels on T_{reg} cells and is important in their suppressor functions; depletion of GITR-expressing T_{reg} cells also leads to the development of anti-myosin autoantibodies and myocarditis (44).

Since T_{reg} cells are generated in the thymus through selection of high-affinity, self-reactive TCRs (45,46), their TCR repertoire is skewed towards recognition of self-antigens. This raises the question of whether T_{reg} cells will be capable of efficiently recognizing complexes of MHC class II (MHCII) plus foreign peptide or are restricted to control of self-reactive cells. Indeed several studies examining the effects of T_{reg}-cell depletion on autoantibodies have shown no similar effect on foreign antigens (37,47).

Recent work by Lee *et al*. (48) demonstrates that the thymic selection process that results from T_{reg} cell possession of high-affinity TCRs may be stochastic, rather than a certain TCR signal strength guaranteeing selection of either an effector T cell or a T_{reg} cell. This has been addressed experimentally by the Jenkins group using MHCII tetramers (i.e. specific antigen bound to four MHCII monomers to increase avidity) to demonstrate that T_{reg} cells with foreign-antigen-specific TCRs are present in both the thymus and periphery of naive animals (49).When the same antigen was transgenically expressed, effectively converting it to a 'neo' self-antigen, numbers of both Foxp3⁻ and Foxp3⁺ tetramer-binding cells were reduced due to clonal deletion. However, Foxp3⁺ cells were more resistant to deletion, ensuring that the ratio of antigen-specific Foxp3⁺ cells to Foxp3⁻ cells is higher for self-antigens (49).

As a molecular mechanism of this self-skewing of the T_{reg}-cell TCR repertoire, CTLA-4 expression by developing T_{reg} cells may play a key role. Both CTLA-4 and CD28 on T cells can bind either CD80 (B7-1) or CD86 (B7-2). Binding of CD28 provides co-stimulatory signals to the T cell, but as detailed later, CTLA-4 interferes with this by depletion of CD80 and CD86 from the surface of APCs (50–52). CTLA-4 may thus attenuate the TCR signal to developing T_{reg} cells, allowing self-reactive T_{reg} cells that would otherwise receive a signal sufficiently strong to induce their apoptosis to escape negative selection (53).

Taken together, these findings suggest that T_{reg} cells are able to control both foreign-antigen-reactive and autoantigen-reactive cells but that the relative ratios between antigen-specific Foxp3⁻ and Foxp3⁺ T cells are altered, dependent on recognition of foreign or self-antigens. For foreign antigens, there are more Foxp3⁻ than Foxp3⁺ T_{reg} cells, whereas for self-antigens, there are more Foxp3⁺ than Foxp3⁻ T_{reg} cells. These different ratios mean that T_{reg} cells may tightly control autoreactive responses, while the level of control of foreign reactive cells is less stringent but not non-existent, allowing proper immune responses to pathogens while still maintaining a level of control to prevent excessive responses.

Since the effect on the response to foreign antigens is likely to be more subtle, this may explain why it has not always been observed. Accordingly, it has been demonstrated that loss of T_{reg} cells or T follicular regulatory cells (T_{freg} cells; see below) may enhance humoral immune responses to foreign antigens (18,20,54). T_{reg} cells also have an important protective role in a wide range of infection models (55). For instance, T_{reg}-cell depletion enhances the CD8⁺ T-cell response to pathogens such as herpes simplex virus (56,57) and *Listeria monocytogenes* (58).

In some cases, T_{reg}-cell effects on pathogen–host interactions may be because of a broader role in damping inflammation; however, the specificity of T_{reg} cells for pathogen epitopes has been demonstrated in several studies. T_{reg} cells that bind MHCII tetramers specific for peptides from influenza nucleoprotein are detectable following influenza infection (59) and their depletion during secondary infection enhances numbers of antigen-specific CD8⁺ cells, whereas T_{reg} cells recognizing epitopes from the rJ2.2 stain of mouse coronavirus block immunopathological damage and demyelination following infection (60).

T_{freg} cells

A critical factor in the regulation of germinal centres is the availability of T-cell help (61,62). Recently it has become clear that a subset of CD4⁺ T_h cells, T_{fh} cells, have a specialised function in providing T-cell help to follicular B cells (63,64).

T_{fh} cells form via a multistage process following initial contacts with dendritic cells (DCs), leading to induction of the lineage-defining transcription factor BCL-6 (B-cell lymphoma 6) (65). This is followed by contact with cognate B cells delivering co-stimulation—via CD80/CD86 and inducible

co-stimulator ligand (ICOSL) binding T-cell CD28 and ICOS that allows the full commitment and maintenance of the T_{fh} lineage (66–70). Expression of the chemokine receptor CXCR5, in combination with a loss of CCR7 expression, allows T_{fh} cells to travel into the B-cell follicle (71). T_{fh}-cell CD40 ligand (CD40L) expression provides help for B cells and production of various cytokines, notably IL-21, by T_{fh} cells drives the survival, differentiation and class-switching of B cells (72,73). These actions expand and maintain the germinal centre reaction, allowing the generation of high-affinity antibody and production of long-lasting plasma cells and memory B cells (63,64).

The vital role of T_{fh} cells in the class-switching of antibody isotypes was underlined by elegant experiments demonstrating that cytokines produced by T_{fh} cells directly control B-cell isotype class-switching. T-cell–B-cell doublets were isolated from reporter mice producing the relevant cytokines, and it was found that B cells in contact with IL-4-producing T_{fh} cells had switched to IgG1 production, whereas B cells in contact with IFN-γ-producing T_{fh} cells had switched to IgG2a production (73). T_{fh} cells also appear to have a critical role in the generation of IgE because inhibition of T_{fh}-cell IL-4 production has profound effects on IgE production, whereas loss of T_h2-cell IL-4 production had little effect (74).

Although it is clear that T_{fh} cells have a vital role in the development of protective responses to pathogens, their excessive expansion in ICOS-overexpressing mice leads to lupus-like pathology and autoantibody production (75,76). Correspondingly, in humans, large-scale expansion of T_{fh} cells is associated with severe systemic lupus erythematosus (SLE) (77), demonstrating the need for tight peripheral control of these cells.

Given the role of T_{reg} cells in the control of humoral immunity, an important question to answer was whether, similarly to T_{fh} cells, T_{reg} cells are able to travel into the follicle where they might directly regulate the germinal centre reaction. Pioneering work from Lim and colleagues demonstrated that, upon activation, $CD4^+CD25^+CD69^-$ T_{reg} cells are able to downregulate CCR7 while simultaneously upregulating CXCR5, allowing them to migrate into the B-cell zone (78). As a result, these T_{freg} cells could be visualized in the germinal centres of human tonsillar sections (78,79). The T_{freg} cells are able to suppress T-cell-dependent antibody production as well as directly inhibit antibody production by CD40-stimulated B cells in the absence of T_h cells (78,79).

More recently, several groups were able to significantly expand on these earlier observations with the discovery that a subset of T_{reg} cells are able to co-opt the T_{fh}-cell pathway by gaining expression of the transcription factor BCL-6 (18–20). These T_{freg} cells are able to travel to the germinal centre and control the expansion of T_{fh} cells and germinal centre B cells (18–20). Similar to T_{fh} cells, T_{freg} cells express high levels of CXCR5, PD-1 (programmed cell death 1) and ICOS, but differ from T_{fh} cells by a lack of IL-4, IL-21 and CD40L expression, while also expressing high levels of T_{reg}-cell associated suppressive molecules such as CTLA-4 and IL-10 (19). T_{freg} cells appear to differentiate from natural T_{reg} cells (which already have their regulatory phenotype in the thymus), rather than being a form of induced T_{reg} cells (which develop their regulatory phenotype only in the periphery), and require many of the same signals as T_{fh} cells as their formation is dependent on CD28, and SAP (signalling lymphocytic activation molecule-associated protein) signalling and interactions with B cells (18–20).

The *in vivo* role of T_{freg} cells has been demonstrated by both adoptive transfer and bone marrow chimera of cells from CXCR5, BCL-6 or SAP-knockout (KO) mice. Interestingly, while it is clear that T_{freg} cells control expansion of T_{fh} cells and germinal centre B cells, conflicting results were obtained regarding their control of the specific response to a foreign vaccine antigen. It was reported that a loss of T_{freg} cells leads to an increase in antigen-specific B cells and the resulting antibody response (18,20). This contrasts with the observation that although total numbers of germinal centre B cells increased, there was an overall drop in the number of antigen-specific B cells and the resulting serum antibody levels (19). It was suggested that a large increase in autoreactive cells effectively outcompeted antigen-specific B cells to the extent that antigen-specific antibody was reduced (19).

Earlier studies examining depletion of total T_{reg} cells have also reported that T_{reg}-cell depletion may (54) or may not (37,47,80) enhance the responses to foreign antigens. In our hands, we are able to generate both results using different T_{reg}-cell depletion strategies, suggesting it is essentially a dose-dependent phenomenon.

Thus, we suggest a model in which temporary depletion of T_{reg} cells leads to activation of antigen-presenting cells (APCs) and increased formation of T_{fh} cells without disrupting immune homeostasis, allowing antigen-specific cells to expand effectively. However, beyond a certain threshold, homeostasis is disrupted and the overwhelming numbers of antigen-non-specific or autoantigen-specific cells expanding in the absence of T_{reg} cells may prove deleterious to an antigen-specific response (Fig. 1). Indeed we have found that while short-term depletion of T_{reg} cells around the time of vaccination leads to enhanced numbers of tetramer-binding T_{fh} cells and antigen-specific germinal centre B cells, prolonged T_{reg}-cell depletion leads to high levels of T_{fh} cells and germinal centre expansion but a reduction in the total number of tetramer-binding T_{fh} cells, with the percentage of

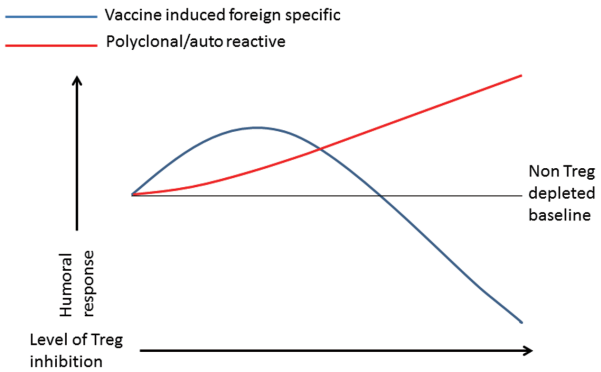

Fig. 1. Model of vaccine-specific and polyclonal responses and correlation with level of T_{reg}-cell depletion. As T_{reg}-cell depletion increases, the vaccine-induced response to foreign antigens (*blue line*) is first enhanced by a loss of antigen-specific T_{reg} cells and increased CD28 signalling but later, the response is reduced by large-scale expansion of autoreactive cells (*red line*) competing for resources.

antigen-recognizing B cells dropping in correlation with germinal centre expansion (J. B. Wing *et al*., in preparation).

Mechanisms of T_{reg}-cell modulation of humoral immunity

T_{reg} cells have a wide range of suppressive mechanisms that may be available for use in different contexts and dependent on the level of stimuli that individual T_{reg} cells have been exposed to (81). A number of mechanisms by which T_{reg} cells regulate humoral immunity either by direct contact with B cells or suppression of T-cell help have been proposed, which are summarized below.

CTLA-4

The inhibitory molecule CTLA-4 is highly expressed on the surface of T_{reg} cells and plays a critical role in their inhibitory function both *in vitro* and *in vivo* by limiting availability of CD80 and CD86 (52,82–85). CD80 and CD86 expressed by APCs provide essential co-stimulatory signals to T cells via ligation of CD28 in addition to TCR signalling (86). T cells from CD28-deficient mice lack expression of CXCR5 and have a reduced ability to differentiate to T_{fh} cells (87,88), despite the near-total absence of T_{reg} cells (89). As such, CD28-deficient mice also fail to form germinal centres (87,90).

T_{reg}-cell-specific conditional KO (cKO) of CTLA-4 leads to the generation of fatal autoimmune and lymphoproliferative disorders similar to those seen in Foxp3-deficient scurfy mice or full CTLA-4 KO mice (52,83,84). Total loss of CTLA-4 expression has profound effects on humoral immunity as effector T cells produce large amounts of IL-4 leading to hyper-IgE production (82). In T_{reg}-cell-specific CTLA-4 cKO mice, we have also previously reported hyper-IgE and enhanced IgG in the serum (52). More recently we have observed high levels of spontaneous development of T_{fh} cells and germinal centres in CTLA-4 cKO mice, similar to those seen in scurfy mice, suggesting that, in the absence of CTLA-4, other T_{reg}-cell effector mechanisms are insufficient to regain control of humoral immunity despite a large number of T_{reg}/T_{freg} cells (20) (J. B. Wing *et al*., in preparation).

It is well established that CD80 and CD86 are critical for humoral immune responses and that it is possible to efficiently block their function with a solubilized form of CTLA-4 (CTLA-4–Ig), resulting in drastically inhibited vaccine responses and reduced germinal centre formation (91,92). However, *in vivo*, the blocking effect of large doses of CTLA-4–Ig may not be an accurate correlate for the *in vivo* function of membrane-bound CTLA-4 found on T_{reg} cells, which appears to act primarily via downregulation of surface expression of CD80/CD86 on DCs and B cells (50,52).

The exact mechanism by which CTLA-4 is able to deplete CD80/CD86 had been sought for some time. However, recent elegant work by Qureshi *et al.* has shed light on this process by demonstrating that CTLA-4 is able to trans-endocytose CD80/CD86 from the surface of the APC and take it into the T_{reg} cell, where it is then degraded (51). In addition to its role in the control of CD80 and CD86 expression, CTLA-4 may also directly inhibit the function of CD80/CD86-expressing DCs by inducing nuclear translocation of the transcription factor Foxo3, leading to a loss of IL-6 expression (93) or modulating tryptophan catabolism by induction of the immunoregulatory enzyme indoleamine 2,3-dioxygenase (IDO) (94).

CTLA-4 binding to B-cell CD80/CD86 may also have a direct effect on antibody production within established germinal centres. Consistent with this notion, putative $CD25^{+}CD69^{-}$ T_{freg} cells are able to directly reduce B-cell antibody production *in vitro,* but this suppressive effect was partially abrogated by the addition of CTLA-4-blocking antibodies (79). However, definitive proof, such as a true understanding of the molecular pathways involved in this reverse signalling into B cells, has remained elusive.

As previously noted, CD28-, CD80- or CD86-deficient mice have impaired formation of T_{fh} cells (95,96). As such, it seems likely that the key role of CTLA-4 in humoral immunity may be via control of CD80 and CD86 expression by APCs and the resultant effects on T_{fh}-cell formation. Interestingly, it seems that B-cell expression of CD80 and CD86 is of particular importance, as addition of CD86-expressing B cells to CD86-KO mice is sufficient to restore formation of T_{fh} cells (95); and B-cell-specific expression of CD80 also affects T_{fh}-cell generation and subsequent production of plasma cells (96).

Interestingly, given the particular role of both T_{reg} cells and CTLA-4 in the control of IgE production, Tian *et al.* demonstrated that T_{reg}-cell depletion has a disproportionate effect on IL-4 production (34). Although they interpreted this as evidence of control of $T_{h}2$ cells, more recent evidence suggests that IL-4 produced by T_{fh} cells may be critical for control of the IgE response (74). Given that both loss of T_{reg}-cell function and T_{reg}-cell-specific expression of CTLA-4 have a profound effect on the formation of T_{fh} cells, it seems likely that this characteristic hyper-IgE seen in T_{reg}-cell-deficient mice and IPEX patients (29,33) may be due to the loss of T_{reg}-cell CTLA-4-dependent control of T_{fh}-cell expansion and IL-4 production.

PD-1

The inhibitory receptor PD-1 is part of the same superfamily as CD28 and CTLA-4 and is believed to be important in the regulation of a number of immune pathways (97). PD-1 is upregulated following activation of B cells, T cells and myeloid cells and notably, is highly upregulated on both T_{fh} cells and T_{freg} cells, suggesting that it may have a role in the function of these cells (19,20,70).

PD-1 has been implicated in the control of humoral immunity. Total KO of PD-1 leads to dysregulation of humoral immunity in several settings. In BALB/c mice, anti-parietal autoantibodies and anti-cardiac troponin I autoantibodies are produced (98,99), and in C57BL/6 mice, development of lupus-like disease and arthritis occurs, while anti-nuclear antibody levels are enhanced in autoimmunity-prone FcγRII-deficient mice (100). This increase in autoimmunity and autoantibody production may be because of the observed expansion of T_{fh} cells in either PD-1-deficient mice or following PD-1 blocking (101,102). However, it seems that this dysregulation also results in a loss of quality as the generated T_{fh} cells may have reduced IL-4 and IL-21 expression leading to reductions in the numbers of long-lasting plasma cells (103) and the production of large amounts of low-affinity IgA in the Peyer's patches that fail to properly control the gut microbiota (104).

More recently, PD-1 expression has also been demonstrated to be critical to maintaining normal T_{freg}-cell function. In the absence of PD-1, T_{freg} cells have increased suppressive potential and have an enhanced ability to inhibit antibody production both *in vitro* and *in vivo*, presumably because the lack of PD-1 cell-intrinsic inhibitory signals allows the cells to become more highly activated (105). It seems likely that since T_{freg} cells and T_{fh} cells are present in the B-cell follicle where stimulatory signals may be in excess, it is necessary for them to express inhibitory receptors such as PD-1 to prevent their overactivation, leading to either autoimmunity or excessive suppression of the immune response for T_{fh} cells and T_{freg} cells.

Expression of PD-1 ligands on T_{reg} cells may also play a role in their suppressive function. Recently Gotot and colleagues demonstrated that T_{reg}-cell-specific expression of PD-1 ligand 1 and PD-1 ligand 2 directly inhibits the function of autoreactive, PD-1-expressing B cells without the need for intermediate interactions with T_h cells (106).

IL-10

The pleotropic cytokine IL-10 has a complex role in humoral immunity, having functions that may either inhibit or enhance the antibody response. *In vitro*, addition of exogenous IL-10 to IL-4-stimulated human PBMCs suppresses IgE production and enhances IgG4 production (107,108) while also acting as a switching factor for human IgG1 and IgG3 (109). In addition, IL-10 stimulation of B-cells isolated from SLE patients, but not healthy donors, enhances their production of IgM, IgA and IgG, demonstrating that in certain pathological circumstances, IL-10 may be capable of directly enhancing auto-antibody production (110). Furthermore, following stimulation with IL-10, germinal centre B cells upregulate Bcl-2 expression, preventing their apoptosis (111), although this does not appear to be the mechanism of enhanced antibody production from B-cells isolated from lupus patients (110). On the other hand, IL-10 downregulates the inflammatory function of macrophages and DCs (112), a notion that is implicated in MRL-Fas^{lpr} IL-10-deficient mice which suffer an increased severity of lupus (113).

Total KO of IL-10 leads to inflammation in the gut mucosa (114). However, it is difficult to be certain to what extent results from total IL-10 KO mice are due to a loss of T_{reg}-cell IL-10 production and not IL-10 production by other cells, notably other described immunoregulatory subsets such as $Foxp3^{-}CD4^{+}$ IL-10-producing Tr1 cells and IL-10-producing B cells (115,116). Interestingly, IL-10R KO mice also have quantitative increases in $CXCR5^{+}$ T-cell formation following vaccination in addition to qualitative increases in IL-21 and IL-17 expression by said cells (117). However, since T_{reg} cells themselves appear to require IL-10 signalling to maintain their full function and control of IL-17-producing cells, the possibility of reduced IL-10 signalling inhibiting T_{reg}-cell function must be also be considered (118). Also, purified DCs from IL-10R KO mice had enhanced IL-6 and IL-23 expression following *in vitro* stimulation, again demonstrating a cell-intrinsic role in suppressing DC function (117).

IL-10 is not critical to the *in vitro* suppressive function of T_{reg} cells (119,120). However, similar to full IL-10 KO mice, cKO of IL-10 in T_{reg} cells leads to the development of colitis but not the systemic autoimmunity seen in Foxp3- or CTLA-4-deficient mice (120). The colitis does not seem to be primarily driven by autoantibodies but instead by IL-17-producing T_h17 cells (121). As such, it appears that T_{reg}-cell-expressed IL-10 is most critical at maintaining immune homeostasis at the mucosal surfaces.

The role of IL-10 in humoral immunity is complex, since B cells, DCs, effector T cells and T_{reg} cells all express IL-10R and can also produce IL-10 itself. Although there is currently little direct evidence that IL-10 produced specifically by Foxp3-expressing T_{reg} cells is critical to the control of humoral immunity, it seems likely that given the ability of IL-10 to regulate DC function and T_{fh}-cell differentiation while also directly inhibiting production of at least some subclasses of antibody, particularly IgE T_{reg}-cell production of IL-10 may have some role in the control of antibody production. Importantly, T_{freg} cells have been reported to have increased levels of IL-10 mRNA expression (19), suggesting that this may play some role in their suppressive function within the B-cell follicle.

TGFβ

The suppressive cytokine TGFβ has also been implicated in T_{reg}-cell suppression of antibody responses. Membrane-bound, but not soluble, TGFβ is able to mediate contact-dependent suppression of B-cell antibody production (122). Additionally, *in vitro* treatment of T_{reg} cells with anti-TGFβ antibody has been reported to suppress B-cell antibody production, either by direct action on the B cells themselves (79) or via inhibition of T-cell help (54).

However, the role of TGFβ may be more complex than simple suppression of antibody responses, since it is also able to induce isotype-switching to IgA (123). As such, T_{reg}-cell TGFβ production has been demonstrated to enhance the induction of IgA production in the mucosa (124).

Induction of B-cell apoptosis

A particularly direct mechanism by which T_{reg} cells may control B-cell responses is by induction of their apoptosis. *In vitro*, activated T_{reg} cells preferentially kill antigen-presenting B cells rather than bystander cells. Similarly, T_{reg} cells from both human sufferers of SLE and lupus-prone NZB/NZW F_1 mice also induce B-cell apoptosis via granzyme and perforin (47,125,126). In addition to granzyme-based induction of apoptosis, Fas–FasL-induced lysis of B cells by T_{reg} cells may occur (127). Interestingly Fas–FasL interactions have also been recently demonstrated to be critical to the maintenance of germinal centre homeostasis (128). Currently no information on the role of apoptosis induction by T_{freg} cells is available, but T_{freg} cells have been reported to have decreased levels of granzyme B mRNA expression, suggesting that this particular pathway may not be critical to their function (19).

Conclusions

T_{reg} cells have a vital role in the control of T-dependent antibody responses. Given their self-antigen-skewed TCR repertoire, it seems likely that T_{reg} cells should preferentially control expansion of autoreactive T- and B-cell

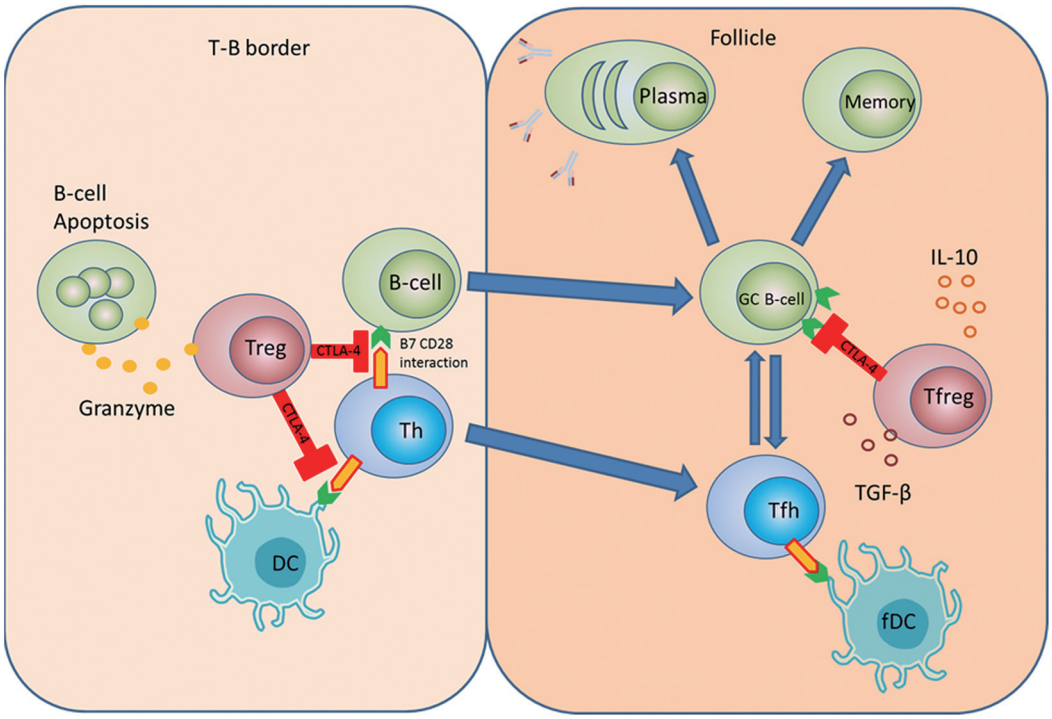

Fig. 2. Mechanisms of T_{reg}-cell suppression. During the events of germinal centre (GC) formation, T_{reg} cells acting outside the follicle, possibly at the T–B border, act to regulate CD28 co-stimulation via CTLA-4, while also inducing apoptosis of specific B cells via granzyme. Within the GC, it is likely that T_{freg} cells use a number of mechanisms such as IL-10 and TGFβ in addition to CTLA-4.

clones. However, sufficient evidence exists to suggest that T_{reg} cells may also be important for the control of non-self-responses.

It is clear from the many proposed mechanisms that T_{reg}-cell modulation of humoral immunity is a complex process, likely to involve several different mechanisms acting either synergistically or redundantly. Given its clear effects on antibody production, we suggest that CTLA-4 is the core mechanism of T_{reg}-cell function controlling the formation of germinal centres. However, T_{freg} cells, similar to T_{reg} cells from other sites where stimulatory signals are in excess (81), may utilize a range of other suppressive mechanisms such as IL-10 and TGFβ, thereby allowing them to play an important role in the fine-tuning of established germinal centre responses (Fig. 2).

Polyclonal depletion of T_{reg} cells is a valuable tool that has allowed us to assess the function of T_{reg} cells in humoral immunity. However, because of the role of T_{reg} cells in the maintenance of immune homeostasis, it is perhaps too blunt a tool to allow fine modulation of antigen-specific responses, due to the possibility of inhibiting the antigen-specific response following excessive expansion of polyclonal cells and the inherent risk of autoimmune side effects. In the future, it will be imperative to develop new methods that allow the preferential depletion or expansion of antigen-specific T_{reg} cells following vaccination, allowing the enhancement or negation of antigen-specific responses while maintaining immune homeostasis by preserving the polyclonal T_{reg}-cell repertoire.

Funding

Japan Society for Promotion of Science (JSPS) Grant-in-Aid for Young Scientists B (25860356) to J.B.W.; Grant-in-Aid for Specially Promoted Research (No. 20002007) to S.S. and Core Research for Evolutional Science and Technology (CREST) from Japan Science and Technology Agency.

Acknowledgements

The authors thank Drs A. Tanaka and D. O. Adeegbe for critical reading of the manuscript.

References

1 McHeyzer-Williams, L. J., Malherbe, L. P. and McHeyzer-Williams, M. G. 2006. Checkpoints in memory B-cell evolution. *Immunol. Rev.* 211:255.
2 Klein, U. and Dalla-Favera, R. 2008. Germinal centres: role in B-cell physiology and malignancy. *Nat. Rev. Immunol.* 8:22.
3 MacLennan, I. C. 1994. Germinal centers. *Annu. Rev. Immunol.* 12:117.
4 Vinuesa, C. G., Sanz, I. and Cook, M. C. 2009. Dysregulation of germinal centres in autoimmune disease. *Nat. Rev. Immunol.* 9:845.
5 Wardemann, H., Yurasov, S., Schaefer, A., Young, J. W., Meffre, E. and Nussenzweig, M. C. 2003. Predominant autoantibody production by early human B cell precursors. *Science* 301:1374.
6 Nemazee, D. and Buerki, K. 1989. Clonal deletion of autoreactive B lymphocytes in bone marrow chimeras. *Proc. Natl Acad. Sci. USA* 86:8039.
7 Nemazee, D. 2006. Receptor editing in lymphocyte development and central tolerance. *Nat. Rev. Immunol.* 6:728.

8 Goodnow, C. C., Crosbie, J., Adelstein, S. *et al.* 1988. Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. *Nature* 334:676.

9 Ray, S. K., Putterman, C. and Diamond, B. 1996. Pathogenic autoantibodies are routinely generated during the response to foreign antigen: a paradigm for autoimmune disease. *Proc. Natl Acad. Sci USA* 93:2019.

10 Robbiani, D. F., Bunting, S., Feldhahn, N. *et al.* 2009. AID produces DNA double-strand breaks in non-Ig genes and mature B cell lymphomas with reciprocal chromosome translocations. *Mol. Cell.* 36:631.

11 Robbiani, D. F., Bothmer, A., Callen, E. *et al.* 2008. AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. *Cell* 135:1028.

12 Ramiro, A. R., Jankovic, M., Eisenreich, T. *et al.* 2004. AID is required for c-myc/IgH chromosome translocations in vivo. *Cell* 118:431.

13 Zhang, Y., Meyer-Hermann, M., George, L. A. *et al.* 2013. Germinal center B cells govern their own fate via antibody feedback. *J. Exp. Med.* 210:457.

14 Kim, H. J., Verbinnen, B., Tang, X., Lu, L. and Cantor, H. 2010. Inhibition of follicular T-helper cells by CD8(+) regulatory T cells is essential for self tolerance. *Nature* 467:328.

15 Noble, A., Zhao, Z. S. and Cantor, H. 1998. Suppression of immune responses by CD8 cells. II. Qa-1 on activated B cells stimulates CD8 cell suppression of T helper 2 responses. *J. Immunol.* 160:566.

16 Godfrey, V. L., Wilkinson, J. E. and Russell, L. B. 1991. X-linked lymphoreticular disease in the scurfy (sf) mutant mouse. *Am. J. Pathol.* 138:1379.

17 Wildin, R. S., Ramsdell, F., Peake, J. *et al.* 2001. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. *Nat. Genet.* 27:18.

18 Wollenberg, I., Agua-Doce, A., Hernandez, A. *et al.* 2011. Regulation of the germinal center reaction by Foxp3+ follicular regulatory T cells. *J. Immunol.* 187:4553.

19 Linterman, M. A., Pierson, W., Lee, S. K. *et al.* 2011. Foxp3+ follicular regulatory T cells control the germinal center response. *Nat. Med.* 17:975.

20 Chung, Y., Tanaka, S., Chu, F., *et al.* 2011. Follicular regulatory T cells expressing Foxp3 and Bcl-6 suppress germinal center reactions. *Nat. Med.* 17:983.

21 Sakaguchi, S., Yamaguchi, T., Nomura, T. and Ono, M. 2008. Regulatory T cells and immune tolerance. *Cell* 133:775.

22 Wing, K. and Sakaguchi, S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. *Nat. Immunol.* 11:7.

23 Hori, S., Nomura, T. and Sakaguchi, S. 2003. Control of regulatory T cell development by the transcription factor Foxp3. *Science* 299:1057.

24 Fontenot, J. D., Gavin, M. A. and Rudensky, A. Y. 2003. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. *Nat. Immunol.* 4:330.

25 Khattri, R., Cox, T., Yasayko, S. A. and Ramsdell, F. 2003. An essential role for Scurfin in CD4+CD25+ T regulatory cells. *Nat. Immunol.* 4:337.

26 Ohkura, N., Hamaguchi, M., Morikawa, H. *et al.* 2012. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. *Immunity* 37:785.

27 Brunkow, M. E., Jeffery, E. W., Hjerrild, K. A. *et al.* 2001. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. *Nat. Genet.* 27:68.

28 Bennett, C. L., Christie, J., Ramsdell, F. *et al.* 2001. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. *Nat. Genet.* 27:20.

29 Wildin, R. S., Smyk-Pearson, S. and Filipovich, A. H. 2002. Clinical and molecular features of the immunodysregulation, polyendocrinopathy, enteropathy, X linked (IPEX) syndrome. *J. Med. Genet.* 39:537.

30 Jang, E., Cho, W. S., Cho, M. L. *et al.* 2011. Foxp3+ regulatory T cells control humoral autoimmunity by suppressing the development of long-lived plasma cells. *J. Immunol.* 186:1546.

31 Nguyen, L. T., Jacobs, J., Mathis, D. and Benoist, C. 2007. Where FoxP3-dependent regulatory T cells impinge on the development of inflammatory arthritis. *Arthritis Rheum.* 56:509.

32 Kinnunen, T., Chamberlain, N., Morbach, H. *et al.* 2013. Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. *Blood* 121:1595.

33 Lin, W., Truong, N., Grossman, W. J. *et al.* 2005. Allergic dysregulation and hyperimmunoglobulinemia E in Foxp3 mutant mice. *J. Allergy Clin. Immunol.* 116:1106.

34 Tian, L., Altin, J. A., Makaroff, L. E. *et al.* 2011. Foxp3(+) regulatory T cells exert asymmetric control over murine helper responses by inducing Th2 cell apoptosis. *Blood* 118:1845.

35 Lahl, K., Loddenkemper, C., Drouin, C. *et al.* 2007. Selective depletion of Foxp3+ regulatory T cells induces a scurfy-like disease. *J. Exp. Med.* 204:57.

36 Kim, J. M., Rasmussen, J. P. and Rudensky, A. Y. 2007. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. *Nat. Immunol.* 8:191.

37 Morgan, M. E., Sutmuller, R. P., Witteveen, H. J. *et al.* 2003. CD25+ cell depletion hastens the onset of severe disease in collagen-induced arthritis. *Arthritis Rheum.* 48:1452.

38 Hsu, W. T., Suen, J. L. and Chiang, B. L. 2006. The role of CD4CD25 T cells in autoantibody production in murine lupus. *Clin. Exp. Immunol* 145:513.

39 Scalapino, K. J., Tang, Q., Bluestone, J. A., Bonyhadi, M. L. and Daikh, D. I. 2006. Suppression of disease in New Zealand Black/New Zealand White lupus-prone mice by adoptive transfer of ex vivo expanded regulatory T cells. *J. Immunol.* 177:1451.

40 Merrell, K. T., Benschop, R. J., Gauld, S. B. *et al.* 2006. Identification of anergic B cells within a wild-type repertoire. *Immunity* 25:953.

41 Gauld, S. B., Merrell, K. T. and Cambier, J. C. 2006. Silencing of autoreactive B cells by anergy: a fresh perspective. *Curr. Opin. Immunol.* 18:292.

42 Seo, S. J., Fields, M. L., Buckler, J. L. *et al.* 2002. The impact of T helper and T regulatory cells on the regulation of anti-double-stranded DNA B cells. *Immunity* 16:535.

43 Leonardo, S. M., De Santis, J. L., Gehrand, A., Malherbe, L. P. and Gauld, S. B. 2012. Expansion of follicular helper T cells in the absence of Treg cells: implications for loss of B-cell anergy. *Eur. J. Immunol.* 42:2597.

44 Ono, M., Shimizu, J., Miyachi, Y. and Sakaguchi, S. 2006. Control of autoimmune myocarditis and multiorgan inflammation by glucocorticoid-induced TNF receptor family-related protein(high), Foxp3-expressing CD25+ and CD25- regulatory T cells. *J. Immunol.* 176:4748.

45 Jordan, M. S., Boesteanu, A., Reed, A. J. *et al.* 2001. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. *Nat. Immunol.* 2:301.

46 Hsieh, C. S., Liang, Y., Tyznik, A. J., Self, S. G., Liggitt, D. and Rudensky, A. Y. 2004. Recognition of the peripheral self by naturally arising CD25+ CD4+ T cell receptors. *Immunity* 21:267.

47 Ludwig-Portugall, I., Hamilton-Williams, E. E., Gottschalk, C. and Kurts, C. 2008. Cutting edge: CD25+ regulatory T cells prevent expansion and induce apoptosis of B cells specific for tissue autoantigens. *J. Immunol.* 181:4447.

48 Lee, H. M., Bautista, J. L., Scott-Browne, J., Mohan, J. F. and Hsieh, C. S. 2012. A broad range of self-reactivity drives thymic regulatory T cell selection to limit responses to self. *Immunity* 37:475.

49 Moon, J. J., Dash, P., Oguin, T. H., 3rd *et al.* 2011. Quantitative impact of thymic selection on Foxp3+ and Foxp3- subsets of self-peptide/MHC class II-specific CD4+ T cells. *Proc. Natl Acad. Sci. USA* 108:14602.

50 Onishi, Y., Fehervari, Z., Yamaguchi, T. and Sakaguchi, S. 2008. Foxp3(+) natural regulatory T cells preferentially form aggregates on dendritic cells in vitro and actively inhibit their maturation. *Proc. Natl Acad. Sci. USA* 105:10113.

51 Qureshi, O. S., Zheng, Y., Nakamura, K. *et al.* 2011. Trans-endocytosis of CD80 and CD86: a molecular basis for the cell-extrinsic function of CTLA-4. *Science* 332:600.

52 Wing, K., Onishi, Y., Prieto-Martin, P. *et al.* 2008. CTLA-4 control over Foxp3(+) regulatory T cell function. *Science* 322:271.

53 Yamaguchi, T. K. A., Osaki, M., Morikawa, H. *et al.* 2013. Construction of self-recognizing regulatory T cells from conventional T cells by controlling CTLA-4 and IL-2 expression. *Proc. Natl Acad. Sci. USA* 110:E2116.
54 Eddahri, F., Oldenhove, G., Denanglaire, S., Urbain, J., Leo, O. and Andris, F. 2006. CD4+ CD25+ regulatory T cells control the magnitude of T-dependent humoral immune responses to exogenous antigens. *Eur. J. Immunol.* 36:855.
55 Rowe, J. H., Ertelt, J. M. and Way, S. S. 2012. Foxp3(+) regulatory T cells, immune stimulation and host defence against infection. *Immunology* 136:1.
56 Suvas, S., Kumaraguru, U., Pack, C. D., Lee, S. and Rouse, B. T. 2003. CD4+CD25+ T cells regulate virus-specific primary and memory CD8+ T cell responses. *J. Exp. Med.* 198:889.
57 Toka, F. N., Suvas, S. and Rouse, B. T. 2004. CD4+ CD25+ T cells regulate vaccine-generated primary and memory CD8+ T-cell responses against herpes simplex virus type 1. *J. Virol.* 78:13082.
58 Ertelt, J. M., Rowe, J. H., Mysz, M. A. *et al.* 2011. Foxp3+ regulatory T cells impede the priming of protective CD8+ T cells. *J. Immunol.* 187:2569.
59 Brincks, E. L., Roberts, A. D., Cookenham, T. *et al.* 2013. Antigen-specific memory regulatory CD4+Foxp3+ T cells control memory responses to influenza virus infection. *J. Immunol.* 190:3438.
60 Zhao, J., Fett, C., Trandem, K., Fleming, E. and Perlman, S. 2011. IFN-gamma- and IL-10-expressing virus epitope-specific Foxp3(+) T reg cells in the central nervous system during encephalomyelitis. *J. Exp. Med.* 208:1571.
61 Victora, G. D., Schwickert, T. A., Fooksman, D. R. *et al.* 2010. Germinal center dynamics revealed by multiphoton microscopy with a photoactivatable fluorescent reporter. *Cell* 143:592.
62 Allen, C. D., Okada, T., Tang, H. L. and Cyster, J. G. 2007. Imaging of germinal center selection events during affinity maturation. *Science* 315:528.
63 Breitfeld, D., Ohl, L., Kremmer, E. *et al.* 2000. Follicular B helper T cells express CXC chemokine receptor 5, localize to B cell follicles, and support immunoglobulin production. *J. Exp. Med.* 192:1545.
64 Schaerli, P., Willimann, K., Lang, A. B., Lipp, M., Loetscher, P. and Moser, B. 2000. CXC chemokine receptor 5 expression defines follicular homing T cells with B cell helper function. *J. Exp. Med.* 192:1553.
65 Deenick, E. K., Chan, A., Ma, C. S. *et al.* 2010. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* 33:241.
66 Baumjohann, D., Okada, T. and Ansel, K. M. 2011. Cutting Edge: distinct waves of BCL6 expression during T follicular helper cell development. *J. Immunol.* 187:2089.
67 Choi, Y. S., Kageyama, R., Eto, D. *et al.* 2011. ICOS receptor instructs T follicular helper cell versus effector cell differentiation via induction of the transcriptional repressor Bcl6. *Immunity* 34:932.
68 Kerfoot, S. M., Yaari, G., Patel, J. R. *et al.* 2011. Germinal center B cell and T follicular helper cell development initiates in the interfollicular zone. *Immunity* 34:947.
69 Qi, H., Cannons, J. L., Klauschen, F., Schwartzberg, P. L. and Germain, R. N. 2008. SAP-controlled T-B cell interactions underlie germinal centre formation. *Nature* 455:764.
70 Crotty, S. 2011. Follicular helper CD4 T cells (TFH). *Annu. Rev. Immunol.* 29:621.
71 Haynes, N. M., Allen, C. D., Lesley, R., Ansel, K. M., Killeen, N. and Cyster, J. G. 2007. Role of CXCR5 and CCR7 in follicular Th cell positioning and appearance of a programmed cell death gene-1high germinal center-associated subpopulation. *J. Immunol.* 179:5099.
72 Chtanova, T. T. S., Newton, R., Frank, N., Hodge, M. R., Rolph, M. S. and Mackay, C. R. 2004. T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *J. Immunol.* 173:68.
73 Reinhardt, R. L., Liang, H. E. and Locksley, R. M. 2009. Cytokine-secreting follicular T cells shape the antibody repertoire. *Nat. Immunol.* 10:385.
74 Harada, Y., Tanaka, S., Motomura, Y. *et al.* 2012. The 3' enhancer CNS2 is a critical regulator of interleukin-4-mediated humoral immunity in follicular helper T cells. *Immunity* 36:188.
75 Linterman, M. A., Rigby, R. J., Wong, R. K. *et al.* 2009. Follicular helper T cells are required for systemic autoimmunity. *J. Exp. Med.* 206:561.
76 Vinuesa, C. G., Cook, M. C., Angelucci, C. *et al.* 2005. A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. *Nature* 435:452.
77 Simpson, N., Gatenby, P. A., Wilson, A. *et al.* 2010. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. *Arthritis Rheum.* 62:234.
78 Lim, H. W., Hillsamer, P. and Kim, C. H. 2004. Regulatory T cells can migrate to follicles upon T cell activation and suppress GC-Th cells and GC-Th cell-driven B cell responses. *J. Clin. Invest.* 114:1640.
79 Lim, H. W., Hillsamer, P., Banham, A. H. and Kim, C. H. 2005. Cutting edge: direct suppression of B cells by CD4+ CD25+ regulatory T cells. *J. Immunol.* 175:4180.
80 Ludwig-Portugall, I., Hamilton-Williams, E. E., Gotot, J. and Kurts, C. 2009. CD25+ T(reg) specifically suppress auto-Ab generation against pancreatic tissue autoantigens. *Eur. J. Immunol.* 39:225.
81 Yamaguchi, T., Wing, J. B. and Sakaguchi, S. 2011. Two modes of immune suppression by Foxp3(+) regulatory T cells under inflammatory or non-inflammatory conditions. *Semin. Immunol.* 23:424.
82 Bour-Jordan, H., Grogan, J. L., Tang, Q., Auger, J. A., Locksley, R. M. and Bluestone, J. A. 2003. CTLA-4 regulates the requirement for cytokine-induced signals in T(H)2 lineage commitment. *Nat. Immunol.* 4:182.
83 Waterhouse, P., Penninger, J. M., Timms, E. *et al.* 1995. Lymphoproliferative disorders with early lethality in mice deficient in Ctla-4. *Science* 270:985.
84 Tivol, E. A., Borriello, F., Schweitzer, A. N., Lynch, W. P., Bluestone, J. A. and Sharpe, A. H. 1995. Loss of CTLA-4 leads to massive lymphoproliferation and fatal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. *Immunity* 3:541.
85 Walker, L. S. 2013. Treg and CTLA-4: Two intertwining pathways to immune tolerance. *J. Autoimmun.* 45:49.
86 Lenschow, D. J., Walunas, T. L. and Bluestone, J. A. 1996. CD28/B7 system of T cell costimulation. *Annu. Rev. Immunol.* 14:233.
87 Linterman, M. A., Rigby, R. J., Wong, R. *et al.* 2009. Roquin differentiates the specialized functions of duplicated T cell costimulatory receptor genes CD28 and ICOS. *Immunity* 30:228.
88 Walker, L. S., Gulbranson-Judge, A., Flynn, S. *et al.* 1999. Compromised OX40 function in CD28-deficient mice is linked with failure to develop CXC chemokine receptor 5-positive CD4 cells and germinal centers. *J. Exp. Med.* 190:1115.
89 Salomon, B., Lenschow, D. J., Rhee, L. *et al.* 2000. B7/CD28 costimulation is essential for the homeostasis of the CD4+CD25+ immunoregulatory T cells that control autoimmune diabetes. *Immunity* 12:431.
90 Ferguson, S. E., Han, S., Kelsoe, G. and Thompson, C. B. 1996. CD28 is required for germinal center formation. *J. Immunol.* 156:4576.
91 Linsley, P. S., Wallace, P. M., Johnson, J. *et al.* 1992. Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. *Science* 257:792.
92 Walker, L. S., Wiggett, H. E., Gaspal, F. M. *et al.* 2003. Established T cell-driven germinal center B cell proliferation is independent of CD28 signaling but is tightly regulated through CTLA-4. *J. Immunol.* 170:91.
93 Dejean, A. S., Beisner, D. R., Ch'en, I. L. *et al.* 2009. Transcription factor Foxo3 controls the magnitude of T cell immune responses by modulating the function of dendritic cells. *Nat. Immunol.* 10:504.
94 Grohmann, U., Orabona, C., Fallarino, F. *et al.* 2002. CTLA-4-Ig regulates tryptophan catabolism in vivo. *Nat. Immunol.* 3:1097.
95 Salek-Ardakani, S., Choi, Y. S., Rafii-El-Idrissi Benhnia, M. *et al.* 2011. B cell-specific expression of B7-2 is required for follicular Th cell function in response to vaccinia virus. *J. Immunol.* 186:5294.

96 Good-Jacobson, K. L., Song, E., Anderson, S., Sharpe, A. H. and Shlomchik, M. J. 2012. CD80 expression on B cells regulates murine T follicular helper development, germinal center B cell survival, and plasma cell generation. *J. Immunol.* 188:4217.

97 Okazaki, T. and Honjo, T. 2006. The PD-1-PD-L pathway in immunological tolerance. *Trends Immunol.* 27:195.

98 Okazaki, T., Tanaka, Y., Nishio, R. *et al.* 2003. Autoantibodies against cardiac troponin I are responsible for dilated cardiomyopathy in PD-1-deficient mice. *Nat. Med.* 9:1477.

99 Okazaki, T., Otaka, Y., Wang, J. *et al.* 2005. Hydronephrosis associated with antiurothelial and antinuclear autoantibodies in BALB/c-Fcgr2b-/-Pdcd1-/- mice. *J. Exp. Med.* 202:1643.

100 Nishimura, H., Nose, M., Hiai, H., Minato, N. and Honjo, T. 1999. Development of lupus-like autoimmune diseases by disruption of the PD-1 gene encoding an ITIM motif-carrying immunoreceptor. *Immunity* 11:141.

101 Hams, E., McCarron, M. J., Amu, S. *et al.* 2011. Blockade of B7-H1 (programmed death ligand 1) enhances humoral immunity by positively regulating the generation of T follicular helper cells. *J. Immunol.* 186:5648.

102 Good-Jacobson, K. L., Szumilas, C. G., Chen, L., Sharpe, A. H., Tomayko, M. M. and Shlomchik, M. J. 2010. PD-1 regulates germinal center B cell survival and the formation and affinity of long-lived plasma cells. *Nat. Immunol.* 11:535.

103 Good-Jacobson, K. L. and Shlomchik, M. J. 2010. Plasticity and heterogeneity in the generation of memory B cells and long-lived plasma cells: the influence of germinal center interactions and dynamics. *J. Immunol.* 185:3117.

104 Kawamoto, S., Tran, T. H., Maruya, M. *et al.* 2012. The inhibitory receptor PD-1 regulates IgA selection and bacterial composition in the gut. *Science* 336:485.

105 Sage, P. T., Francisco, L. M., Carman, C. V. and Sharpe, A. H. 2013. The receptor PD-1 controls follicular regulatory T cells in the lymph nodes and blood. *Nat. Immunol.* 14:152.

106 Gotot, J., Gottschalk, C., Leopold, S. *et al.* 2012. Regulatory T cells use programmed death 1 ligands to directly suppress autoreactive B cells in vivo. *Proc. Natl Acad. Sci. USA* 109:10468.

107 Meiler, F., Klunker, S., Zimmermann, M., Akdis, C. A. and Akdis, M. 2008. Distinct regulation of IgE, IgG4 and IgA by T regulatory cells and toll-like receptors. *Allergy* 63:1455.

108 Jeannin, P., Lecoanet, S., Delneste, Y., Gauchat, J. F. and Bonnefoy, J. Y. 1998. IgE versus IgG4 production can be differentially regulated by IL-10. *J. Immunol.* 160:3555.

109 Malisan, F., Briere, F., Bridon, J. M. *et al.* 1996. Interleukin-10 induces immunoglobulin G isotype switch recombination in human CD40-activated naive B lymphocytes. *J. Exp. Med.* 183:937.

110 Llorente, L., Zou, W., Levy, Y., Richaud-Patin, Y. *et al.* 1995. Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. *J. Exp. Med.* 181:839.

111 Levy, Y. and Brouet, J. C. 1994. Interleukin-10 prevents spontaneous death of germinal center B cells by induction of the bcl-2 protein. *J. Clin. Invest.* 93:424.

112 Banchereau, J., Pascual, V. and O'Garra, A. 2012. From IL-2 to IL-37: the expanding spectrum of anti-inflammatory cytokines. *Nat. Immunol.* 13:925.

113 Yin, Z., Bahtiyar, G., Zhang, N. *et al.* 2002. IL-10 regulates murine lupus. *J. Immunol.* 169:2148.

114 Kuhn, R., Lohler, J., Rennick, D., Rajewsky, K. and Muller, W. 1993. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 75:263.

115 Pot, C., Apetoh, L. and Kuchroo, V. K. 2011. Type 1 regulatory T cells (Tr1) in autoimmunity. *Semin. Immunol.* 23:202.

116 Mauri, C. and Bosma, A. 2012. Immune regulatory function of B cells. *Annu. Rev. Immunol.* 30:221.

117 Cai, G., Nie, X., Zhang, W. *et al.* 2012. A regulatory role for IL-10 receptor signaling in development and B cell help of T follicular helper cells in mice. *J. Immunol.* 189:1294.

118 Chaudhry, A., Samstein, R. M., Treuting, P. *et al.* 2011. Interleukin-10 signaling in regulatory T cells is required for suppression of Th17 cell-mediated inflammation. *Immunity* 34:566.

119 Takahashi, T., Kuniyasu, Y., Toda, M. *et al.* 1998. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. *Int. Immunol.* 10:1969.

120 Rubtsov, Y. P., Rasmussen, J. P., Chi, E. Y. *et al.* 2008. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. *Immunity* 28:546.

121 Huber, S., Gagliani, N., Esplugues, E. *et al.* 2011. Th17 cells express interleukin-10 receptor and are controlled by Foxp3 and Foxp3+ regulatory CD4+ T cells in an interleukin-10-dependent manner. *Immunity* 34:554.

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

123 Coffman, R. L., Lebman, D. A. and Shrader, B. 1989. Transforming growth factor beta specifically enhances IgA production by lipopolysaccharide-stimulated murine B lymphocytes. *J. Exp. Med.* 170:1039.

124 Cong, Y., Feng, T., Fujihashi, K., Schoeb, T. R. and Elson, C. O. 2009. A dominant, coordinated T regulatory cell-IgA response to the intestinal microbiota. *Proc. Natl Acad Sci. USA* 106:19256.

125 Zhao, D. M., Thornton, A. M., DiPaolo, R. J. and Shevach, E. M. 2006. Activated CD4(+)CD25(+) T cells selectively kill B lymphocytes. *Blood* 107:3925.

126 Iikuni, N., Lourenco, E. V., Hahn, B. H. and La Cava, A. 2009. Cutting edge: Regulatory T cells directly suppress B cells in systemic lupus erythematosus. *J. Immunol.* 183:1518.

127 Janssens, W., Carlier, V., Wu, B., VanderElst, L., Jacquemin, M. G. and Saint-Remy, J. M. 2003. CD4+CD25+ T cells lyse antigen-presenting B cells by Fas-Fas ligand interaction in an epitope-specific manner. *J. Immunol.* 171:4604.

128 Hao, Z., Duncan, G. S., Seagal, J. *et al.* 2008. Fas receptor expression in germinal-center B cells is essential for T and B lymphocyte homeostasis. *Immunity* 29:615.