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Regulatory T cells: how do they suppress immune responses?

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Abstract

Regulatory T cells (Tregs), either natural or induced, suppress a variety of physiological and pathological immune responses. One of the key issues for understanding Treg function is to determine how they suppress other lymphocytes at the molecular level *in vivo* and *in vitro*. Here we propose that there may be a key suppressive mechanism that is shared by every forkhead box p3 (Foxp3)$^+$ Treg *in vivo* and *in vitro* in mice and humans. When this central mechanism is abrogated, it causes a breach in self-tolerance and immune homeostasis. Other suppressive mechanisms may synergistically operate with this common mechanism depending on the environment and the type of an immune response. Further, Treg-mediated suppression is a multi-step process and impairment or augmentation of each step can alter the ultimate effectiveness of Treg-mediated suppression. These findings will help to design effective ways for controlling immune responses by targeting Treg suppressive functions.

Introduction

Regulatory T cells (Tregs), especially naturally arising $CD25^+CD4^+$ Tregs, in which expression of the transcription factor forkhead box p3 (Foxp3) occurs in the thymus (as opposed to 'induced' Tregs, in which Foxp3 is induced in the periphery), actively engage in the maintenance of immunological self-tolerance and immune homeostasis (1). Their contribution is best illustrated by the spontaneous development of autoimmune disease in normal rodents when $CD25^+CD4^+$ T cells are depleted and also by the occurrence of severe autoimmune disease, allergy and immunopathology in humans and rodents with mutated Foxp3 genes (1, 2). This means that deficiency or dysfunction of natural $CD25^+CD4^+Foxp3^+$ Tregs alone is sufficient to break self-tolerance in otherwise normal animals.

During the past decade, evidence has accumulated regarding the essential roles of natural Tregs in the control of a variety of physiological and pathological immune responses, including anti-microbial and anti-tumour responses, and transplantation immunity (3–5). Yet, it is still obscure as to how they control other lymphocytes at the molecular level (6–9). This issue is of cardinal importance since dysfunction of the Treg suppressive mechanism is presumed to be causative of autoimmune and immunopathological diseases as seen in Treg deficiency. Furthermore, the molecular events specific for Treg-mediated suppression can be key targets for immune intervention or potentiation, and the molecules, if any, that are specific for the immunosuppressive mechanisms may be ideal Treg-specific markers with functional relevance.

The advances in our understanding of the cellular and molecular basis of Treg-mediated suppression have been mainly built on the following key findings. First, Foxp3-expressing $CD25^+CD4^+$ natural Tregs can inhibit the development of autoimmune disease or inflammatory bowel disease (IBD) elicited by Treg depletion (10, 11). Second, in *in vitro* culture, Tregs are able to suppress the proliferation of antigen-stimulated naive T cells (12, 13). Third, induction or forced expression of the Foxp3 gene in normal naive T cells is able to convert them to Treg-like cells with *in vivo* and *in vitro* suppressive function, thereby indicating that Foxp3 is likely to control the expression of key molecules mediating suppression (14–16).

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Efforts to analyse these cellular and molecular events, *in vivo* and *in vitro*, in rodents and humans have revealed multiple mechanisms of suppression mediated by Foxp3$^+$ Tregs (6–9). However, it remains obscure as to (i) how and to what extent each mechanism contributes to the maintenance of self-tolerance and immune homeostasis; (ii) how the findings made *in vitro* correlate with *in vivo* suppression; (iii) which suppressive mechanism is controlled by Foxp3 and, more practically, (iv) which mechanism is a suitable target for effective control of immune responses via Tregs.

In this article, we discuss these issues after briefly reviewing the mechanisms of Treg-mediated suppression that have been proposed. Although the issues are highly contentious, we hope that our view would help to understand Treg function and to design their clinical use.

Many possible mechanisms have been suggested for Treg-mediated suppression

Soon after the discovery that CD25$^+$CD4$^+$ T cells that were physiologically present in normal rodents were engaged in suppressing the development of autoimmune disease, a short-term *in vitro* assay was established that has been widely used to assess the suppressive activity of CD25$^+$CD4$^+$ Tregs in rodents and humans (12, 13). In this simple 3-day *in vitro* assay, CD25$^+$CD4$^+$ Tregs potently suppress proliferation of other CD4$^+$ and CD8$^+$ T cells when Treg and responder populations are co-cultured and stimulated with specific antigen or a polyclonal TCR stimulator (such as anti-CD3 mAb) in the presence of antigen-presenting cells (APCs). CD25$^+$CD4$^+$ Tregs also suppress cytokine production (especially IL-2 production) by CD4$^+$ and CD8$^+$ responder T cells and their effector activities such as CD8$^+$ T cell cytotoxicity. As summarized below, this *in vitro* assay, together with *in vivo* suppression of autoimmune disease and IBD, revealed the contributions of both cell contact-dependent and cell contact-independent (i.e. humoral factor mediated) mechanisms of suppression as well as the molecules involved in each mechanism.

The contribution of cell contact-dependent mechanisms was suggested by the *in vitro* inability of Tregs to suppress the proliferation of responder T cells when the two populations were separated by a semi-permeable membrane (12, 13). Culture supernatant of antigen-stimulated Tregs also fails to exhibit suppressive activity. Following cell contact, Tregs may kill responder T cells by a granzyme-dependent or perforin-dependent mechanism (17, 18) or deliver a negative signal to responder T cells via (i) up-regulating intracellular cyclic AMP, which leads to inhibition of T cell proliferation and IL-2 formation (19); (ii) generating pericellular adenosine catalyzed by CD39 (ectonucleoside triphosphate diphosphohydrolase 1) and CD73 (ecto-5′-nucleotidase) expressed by Tregs (20) and (iii) interacting with B7 (CD80 and CD86) expressed by responder T cells (21).

Concerning modification of APC function, activated Tregs may hamper the up-regulation or down-modulate the expression of CD80 and CD86 on APCs, as well as stimulate dendritic cells (DCs) to express the enzyme indoleamine 2,3-dioxygenase (IDO) (22, 23). IDO catabolizes conversion of the essential amino acid tryptophan to kynurenine, which is toxic to T cells neighbouring the DCs. Both of these APC-modifying pathways appear to be dependent on the Treg expression of CTL-associated protein 4 (CTLA-4 or CD152; this binds CD80 and CD86). Activated Tregs can also kill APCs including B cells (24). Lymphocyte activation gene 3 (LAG-3; CD223), a CD4-related, activation-induced cell surface molecule highly expressed on Foxp3$^+$ Tregs, also plays a role in Treg–APC interaction (25).

As short-range suppressive humoral factors involved in Treg-mediated suppression, IL-10 and transforming growth factor β (TGF-β) were first suspected to mediate Treg suppression. However, neutralization of either IL-10 or TGF-β does not abrogate *in vitro* suppression (12, 13). In contrast, IL-10 and TGF-β contribute, at least in part, to the *in vivo* suppression of IBD induced in mice by Treg depletion. For example, IL-10-deficient Tregs are unable to suppress IBD in a mouse model (26). Blockade of IL-10R and neutralization of TGF-β can also abolish Treg-mediated inhibition of the disease (27). In contrast with IBD, IL-10-deficient Tregs are fully able to suppress autoimmune gastritis that is produced by Treg depletion (28).

TGF-β may act as a mediator of suppression as a membrane-bound form (29), although this is a controversial. It may condition responder T cells to be sensitive to suppression, maintain Foxp3 expression and suppressive activity and might contribute to the differentiation of other T cells into Treg-like cells (infectious tolerance) (30–34). A more recent study demonstrates that Foxp3$^+$ natural Tregs predominantly produce immunosuppressive IL-35, a novel member of the IL-12 family; IL-35-deficient Tregs are less suppressive in controlling IBD *in vivo* and in the suppression assay *in vitro* (35). In addition, cytokine absorption by Tregs induces cytokine deprivation-mediated apoptosis in responder T cells (36). Other molecules including carbon monoxide and galectins produced by Tregs are also reported to play roles in suppression (37, 38).

Taken together, these *in vivo* and *in vitro* findings suggest that multiple mechanisms may operate in Treg-mediated suppression and that various molecules may be secreted or expressed on the cell surface of Tregs and directly contributing to their suppressive functions. This prompts one to ask how such multiple mechanisms or modes of suppression interact in the maintenance of self-tolerance and immune homeostasis. It is conceivable that there is a single core suppressive mechanism shared by every Treg and several complementary mechanisms. Alternatively, a particular mechanism may play a dominant role under a particular condition, with different mechanisms operating in various situations. Furthermore, another possibility is that multiple suppressive mechanisms operate simultaneously and synergistically and that dysfunction of any of them is not sufficient to seriously impair suppression.

CTLA-4-dependent or IL-2-dependent suppression as possible core mechanisms of Treg-mediated suppression

Identifying core mechanisms contributing to Treg-mediated suppression

One may argue that if disruption of any suppressive mechanism discussed above breaches self-tolerance and immune

homeostasis as seen in Treg or Foxp3 deficiency, that mechanism should be considered as central (core) or, at least, essential. Among many mouse strains deficient in specific genes involved in the putative suppressive mechanisms described above, it is worth noting that systemic deficiency of TGF-β, CTLA-4, IL-2 or its receptor, which consists of CD25 (IL-2R α-chain) and CD122 (IL-2R β-chain), causes fatal autoimmune or inflammatory disease.

Although the role of TGF-β is controversial (see above), TGF-β1-deficient or -intact $CD25^+CD4^+$ Tregs are equally able to suppress the development of IBD when each population is co-transferred with normal $CD4^+CD45RB^{high}$ cells into SCID mice (30). This means that TGF-β is not a direct mediator of suppression. IL-10 deficiency produces IBD but not autoimmune disease, suggesting that IL-10-dependent mechanism is important for mucosal immune homeostasis but may not be indispensable for systemic self-tolerance, as discussed above. Deficiency of IDO, IL-35, LAG-3, granzyme or perforin reportedly fails to produce autoimmune disease in mice. However, if a molecule is essential for the functions of both Tregs and non-Treg cells, deficiency of that molecule may fail to reveal a key suppressive mechanism. Nonetheless, if there exists a central suppressive mechanism indispensable for the maintenance of self-tolerance, then a CTLA-4-dependent and possibly an IL-2-dependent mechanism are two plausible candidates.

Possible core contribution of CTLA-4 in Treg-mediated suppression

The role of CTLA-4 in Treg function has been controversial for over a decade. It was first found that $CD25^+CD4^+$ T cells in normal mice constitutively express high levels of CTLA-4 (27, 39, 40). In humans, terminally differentiated $Foxp3^{high}CD25^{high}CD4^+$ T cells are highly suppressive *in vitro* and are the only constitutive expressers of CTLA-4 (41).

The following findings support the notion that CTLA-4 is essential for Treg function. First, blockade of CTLA-4 by administration of a mAb produces organ-specific autoimmune disease and colitis in otherwise normal mice (27, 40) and exacerbates diabetes in diabetes-prone non-obese diabetic mice (42). In addition, blockade of CTLA-4 expressed by natural Tregs and not by responder T cells abrogates Treg suppression as observed when Tregs from CTLA-4-intact mice are co-cultured with CTLA-4-deficient responder T cells *in vitro* or co-transferred into SCID mice (40, 43, 44). Second, Foxp3, together with other transcription factors, up-regulates the expression of CTLA-4 by binding to the promoter region of the CTLA-4 gene, thereby indicating that Foxp3 may sustain the high expression of CTLA-4 in $Foxp3^+$ Tregs (45–48). Furthermore and most importantly, a recent study using mice in which CTLA-4 deficiency was Treg specific clearly demonstrates that these mice succumb to lymphoproliferation with splenomegaly, a variety of autoimmune diseases, and develop hyper-production of IgE, as seen in Foxp3 deficiency (49).

The Treg-specific CTLA-4 deficiency affects Treg suppressive activity but not their thymic production, their *in vivo* survival or their activation status (49). When naive Tregs are prepared from autoimmunity-free, female conditional knockout (CKO) mice (in which half of Tregs are CTLA-4 deficient due to the localization of *Foxp3* on the X chromosome and its random inactivation in female Tregs), their suppressive activity is severely impaired *in vivo* and *in vitro*. There are also accumulating findings that mixed bone marrow (BM) chimeras of CTLA-4-deficient and CTLA-4-intact BM cells fail to develop autoimmunity or systemic inflammation (50). Further, CTLA-4-deficient non-Treg cells from these chimeric mice are functionally normal (51). All these findings collectively support the notion that CTLA-4 expressed by $Foxp3^+$ Tregs is essential for Treg function to sustain self-tolerance and immune homeostasis. However, these results do not exclude the possible function of CTLA-4 expressed by activated effector T cells as a brake to their activation.

Possible core contribution of IL-2 and IL-2R in Treg-mediated suppression

In addition to CTLA-4, IL-2-related molecules including IL-2 itself, CD25 and CD122 may contribute to Treg suppression as key suppressive mechanisms for the following reasons. Deficiency in each of the IL-2-related molecules produces fatal autoimmune or inflammatory disease (52, 53). Foxp3 binds to the promoter of the *Il2* and *Cd25* genes, repressing the former and activating the latter (45–48). Further, addition of IL-2 to the *in vitro* Treg suppression assay abrogates suppression and allows the proliferation of responder T cells (12, 13). Recent studies have shown that IL-2 is required for the survival of natural Tregs (54–56). However, although the reduction of Tregs in number and frequency in IL-2-deficient mice or in IL-2-neutralized mice is only to 50% of the levels present in control, IL-2-intact mice, it is sufficient to cause autoimmune disease (54, 57).

These findings, taken together, suggest the possibility that IL-2 may be required not only for the maintenance of natural Tregs and *de novo* induction of $Foxp3^+$ Tregs from naive T cells in the presence of TGF-β but also for Treg-mediated suppression. For example, although IL-2-deficient non-Treg cells are able to proliferate upon antigenic stimulation (58), Tregs may absorb IL-2 and thereby hamper the activation of other T cells (36). IL-2 may also be required for Treg activation because IL-2 up-regulates Foxp3 expression via signal transducer and activator of transcription 5 (STAT5) (59, 60). It is thus interesting to observe that sub-optimally activated Tregs need IL-2 for their full *in vitro* suppression, whereas optimally stimulated Tregs do not (61).

In vitro cell contact-dependent suppression as a multi-step process

Assuming that CTLA-4, and possibly IL-2, plays a key role during *in vivo* and *in vitro* suppression, how does CTLA-4 contribute to Treg-mediated suppression? To address this question, we have revisited the *in vitro* Treg-mediated suppression assay systems that were established over 10 years ago (see above) and further analysed the cellular and molecular basis of the process.

When the *in vitro* behaviour of Tregs and responder T cells is visualized by dye labelling of Tregs and naive T cells, which are co-cultured in the presence of DCs and antigen, Tregs out-compete responder T cells in forming aggregates around DCs, thereby apparently physically deterring the

access of responder T cells to DCs (62). This aggregation process is antigen dependent as there is no aggregate formation without antigen. It is lymphocyte function-associated antigen 1 (LFA-1; CD11a–CD18) dependent but CTLA-4 independent since CTLA-4-deficient Tregs efficiently outcompete, whereas LFA-1-deficient Tregs do not.

By forming aggregates, Tregs inhibit the up-regulation of CD80 and CD86 on immature DCs and also down-regulate the expression of CD80 and CD86 by mature DCs without affecting the expression of CD40 and class II MHC (49, 62). This modification of CD80 and CD86 expression is CTLA-4 dependent as CTLA-4-deficient Tregs from complete CTLA-4-KO mice or Treg-specific CKO mice fail to modify expression of CD80 or CD86. The aggregate formation and down-modulation of CD80 and CD86 are robust processes and occur even in the presence of strong DC-activating stimuli such as LPS, Zymosan and type I IFN (62).

Thus, *in vitro*, Treg-mediated, contact-dependent suppression can be dissected into two steps based upon CTLA-4 dependency: (i) the LFA-1-dependent, CTLA-4-independent initial formation of Treg aggregates with DCs and (ii) LFA-1-dependent and CTLA-4-dependent, active down-modulation of CD80 and CD86 expression on DCs. Both steps are required to prevent stable interaction between DCs and responder T cells and thereby inhibit activation of the latter.

LFA-1 is thus critical for the suppressive function of Tregs. Yet, LFA-1 deficiency does not produce autoimmune disease, as LFA-1 is required for functions of both Tregs and responder T cells; we highlighted this kind of concern above when discussing the use of KO mice for analysing Treg function. A recent study utilizing Tregs from genetically LFA-1-deficient individuals supports the findings obtained in mice (61).

How CTLA-4 contributes to Treg-mediated suppression is currently under active investigation. CTLA-4 ligation of CD80 or CD86 may not only down-regulate expression of CD80 and CD86 but also induce IDO in DCs, leading to the production of immunosuppressive kynurenin (23). In addition, activated Tregs, which express the high-affinity IL-2R at high levels, may absorb IL-2 from the surroundings, thereby synergistically hindering the activation of other T cells recruited to the DCs (36). These possible suppressive mechanisms are in accord with *in vivo* finding utilizing intra-vital two-photon microscopy, which demonstrate that Tregs apparently inhibit stable contacts between antigen-activated T cells and DCs (63, 64).

Key features of multi-step *in vitro* suppression

The two-step (LFA-1 dependent and CTLA-4 independent; then LFA-1 dependent and CTLA-4 dependent) model of *in vitro* suppression has the following features and provides solutions to some controversial issues regarding *in vitro* Treg-mediated suppression.

The requirement for cell–cell contact

The model is consistent with cell contact-dependent suppression, which is a key feature of *in vitro* Treg-mediated suppression. Since the advent of the *in vitro* Treg suppression assay, the requirement for cell contact in order to achieve suppression is only demonstrated by the inability of Treg to suppress across a semi-permeable membrane (12, 13). The finding that the interaction between LFA-1 on Tregs and intercellular adhesion molecule 1 on APCs is essential for Treg aggregation, the consequent physical out-competition of responder T cells on the surface of APCs, and the subsequent down-regulation of CD80 and CD86 provides unequivocal evidence for cell contact as an indispensable prerequisite for *in vitro* suppression. It remains to be determined whether other adhesion molecules are also involved in these steps or, as in the case for LAG-3 and galectins, may contribute to intensifying the adhesion of Treg and APCs.

The phenomenon of bystander suppression

The two-step process is the basis of cell contact-dependent bystander suppression, another important feature of the *in vitro* Treg-mediated suppression (12, 65). Both steps hamper the activation of responder T cells with different antigen specificities. Further, via this mechanism, Tregs are able to suppress not only CD4$^+$ T cells but also CD8$^+$ T cells recruited to the same APC. It remains to be determined whether the mechanism is also responsible for suppressing the activation and proliferation of other types of lymphocytes, such as NK cells and NKT cells.

The capabilitiy of immature DCs to activate Treg

Immature DCs can preferentially activate natural Tregs. It is well documented that Treg must be first activated by antigen in order to exert their suppressive functions (12, 13). Notably, Treg aggregation around DCs does not occur without antigen (62). Further, as demonstrated with TCR transgenic mice, Tregs can be activated and exert suppression at a 1/10 to 1/100 lower concentration of antigen than required for the activation of naive T cells specific for the same antigen (12). These findings taken together indicate that Treg can be activated by a small amount of antigen presented by immature DCs irrespective of their low expression of CD80 and CD86. Such activated Tregs may further up-regulate LFA-1 expression, strongly adhere to DCs, aggregate around them and sustain expression of CD80 and CD86 below the level required for activation and expansion of responder T cells. Further, Foxp3$^+$ Tregs exert *in vitro* suppression on plasmacytoid DCs, which expresses low to undetectable levels of CD80 and CD86 (62).

The discrepancies between CTLA-4-KO mouse strains in Treg-mediated suppression

There is a discrepancy in the suppressive activity between Tregs from complete CTLA-4-KO mice and Tregs from mice in which CTLA-4 CKO are Treg specific (40, 43, 66). The former retain *in vitro* suppressive activity almost equivalent to normal CTLA-4-intact Tregs. This finding has made it difficult for a decade to accept CTLA-4 as a key molecule for Treg suppressive function. This *in vitro* suppressive activity of CTLA-4-KO Tregs has been attributed to their abundant secretion of IL-10 or TGF-β. However, neutralization of IL-10 and TGF-β fails to abolish the suppression (43).

The two-step model of suppression indicates that the first step, in which Tregs strongly aggregate to DC in a CTLA-4-independent manner, is sufficient to exert *in vitro* suppression. Indeed, Tregs from CTLA-4-deficient mice with severe systemic inflammation are strongly activated, express high levels of adhesion molecules (including LFA-1) and therefore efficiently out-compete responder T cells by forming aggregates with DCs in a CTLA-4-independent fashion (62). Further, such activated CTLA-4-deficient Tregs from CTLA-4-KO or CTLA-4-CKO mice are suppressive *in vitro* in the presence of X-irradiated splenic non-T cells, but much less so with live DCs. Overall, these findings indicate that the activation status of Tregs and the type of APCs (and their maturation states) significantly contribute to the effectiveness of *in vitro* Treg-mediated suppression.

The suppressive capabilities of Foxp3-non-expressing Tregs and non-Tregs

Foxp3$^+$ Tregs may share a suppressive mechanism with Tregs that do not express Foxp3. Assuming the key contribution of the LFA-1-dependent and CTLA-4-dependent mechanism to Treg-mediated suppression, one can ask whether activated non-Treg cells that highly express LFA-1 and CTLA-4 have a similar suppressive activity. Notably, anergic T cells and IL-10-secreting Tr1 cells, both of which fail to produce IL-2 but express CTLA-4 upon activation, exert *in vitro* cell contact-dependent suppression precisely in the manner of Foxp3$^+$ Tregs (67, 68). Further, activated conventional T cells expressing CTLA-4 can down-modulate CD80 and CD86 expression on DCs via CTLA-4, thereby suggesting that activated effector T cells may concurrently down-modulate APC function in a negative feedback manner via CTLA-4 (49). It is thus tempting to speculate that non-Treg cells, whether being effector, anergic or exhausted, might exhibit some suppressive activity, albeit transiently and weakly, and contribute to peripheral immune homeostasis together with Foxp3$^+$ natural Tregs (69, 70). This might partly explain why systemic autoimmunity and inflammation in mice with complete CTLA-4 KO are more severe than in mice with CTLA-4 CKO specifically in Tregs.

Differentiation of Tregs to conduct context-dependent suppression *in vivo*

Assuming that every Foxp3$^+$ Treg exercises LFA-1-dependent and CTLA-4-dependent suppression at any place as a core suppressive mechanism, Tregs may additionally utilize other suppressive mechanisms, which may synergistically operate with the LFA-1-dependent and CTLA-4-dependent one. Such auxiliary mechanisms, whose dysfunction *per se* does not seriously affect self-tolerance or immune homeostasis, may operate concurrently with the core one or subsequently to the differentiation of Tregs depending on the environment, the context and the type of an immune response.

For example, IL-10-producing Foxp3$^+$ Tregs are rare in the spleen but abundant in the lamina propria of the intestine, which supports the observation that Treg-specific IL-10 deficiency causes immunopathology in the mucosal surface of the intestine and the lung (71–73). Perforin-expressing or granzyme-expressing Foxp3$^+$ Tregs are also rare in the spleen but abundant in a tumour environment (18).

Similarly, Foxp3$^+$ Tregs can functionally differentiate to acquire the ability to specifically control T_h1- or T_h2-type immune responses by modifying their expression of T_h1-related or T_h2-related transcription factors. Thus, a fraction of Foxp3$^+$ natural Tregs express the T_h1-specifying transcription factor T-bet, which increases in Tregs stimulated in a T_h1 cytokine milieu, causing the subsequent up-regulation of C–X–C chemokine receptor 3, a T-bet-controlled chemokine receptor, leading to Treg recruitment to type 1 inflammation sites (74). Foxp3 also directly controls the expression of the transcription factor IFN regulatory factor 4 (IRF4), which is required for T_h2 differentiation (75). Interestingly, Treg-specific deletion of IRF4 impairs the control of T_h2, but not T_h1 immune responses, resulting in spontaneous development of T_h2-type inflammation.

As such, Tregs and effector T cells recruited to the same inflammation sites generally express similar sets of chemokine receptors, which is exemplified by C–C chemokine receptor 6 (CCR6)$^+$ Tregs preferentially recruited to T_h17 inflammation site where T_h17 cells express CCR6 under the control of retinoic acid-related orphan receptor γt (76). It remains to be investigated whether such inflammation-type-specific Tregs also exercise an environment-specific suppressive activity, for example, by acting as 'cytokine sink' for distinct effector cytokines, such as IFN-γ, in a type 1 inflammatory environment.

Conclusion

We have discussed how Foxp3$^+$ natural Tregs suppress other lymphocytes *in vivo* and *in vitro*. Our main argument is that the LFA-1-dependent and CTLA-4-dependent two-step mechanism, as revealed *in vitro*, may be the core mechanism of Treg-mediated suppression, thereby supporting the indispensable role of CTLA-4 for Treg function *in vivo*. Indeed, blockade of CTLA-4 by specific mAb in humans provokes effective tumour immunity and, at the same time, affects self-tolerance, which can elicit autoimmunity (77). In addition to this core mechanism, auxiliary suppressive mechanisms may also operate depending on the environment and the type of the immune response. These core and auxiliary suppressive mechanisms may be exploited for effective control of immune responses via Tregs.

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Abbreviations

APC	antigen-presenting cell
BM	bone marrow
CCR6	C–C chemokine receptor 6
CKO	conditional knockout

CTLA-4	CTL-associated protein 4
DC	dendritic cell
Foxp3	forkhead box p3
IBD	inflammatory bowel disease
IDO	indoleamine 2,3-dioxygenase
IRF4	IFN regulatory factor 4
LAG-3	lymphocyte activation gene 3
LFA-1	lymphocyte function-associated antigen 1
STAT5	signal transducer and activator of transcription 5
TGF-β	transforming growth factor β
Treg	regulatory T cell

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