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The Foxp3⁺ regulatory T cell: a jack of all trades, master of regulation

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Abstract

The function of regulatory T cells (T_{reg} cells) has been attributed to a growing number of diverse pathways, molecules and processes. Seemingly contradictory conclusions regarding the mechanisms underlying T_{reg} cell suppressive activity have revitalized skeptics in the field who challenge the core validity of the idea of T_{reg} cells as central immune regulators. However, we note that a consensus may be emerging from the data: that multiple T_{reg} cell functions act either directly or indirectly at the site of antigen presentation to create a regulatory milieu that promotes bystander suppression and infectious tolerance. Thus, the versatility and adaptability of the Foxp3⁺ T_{reg} cells may in fact be the best argument that these cells are ‘multitalented masters of immune regulation’.

Armed with the potential to destroy invading microorganisms and stop aberrant outgrowth of tumor cells, the immune system has extensive built-in mechanisms for preventing attack of healthy self tissues. The first line of such ‘self-tolerance’ is the elimination of self-reactive T lymphocytes and B lymphocytes during negative selection in the thymus and bone marrow, respectively. However, there has long been a belief that the immune system must have peripheral mechanisms in place to deal with immune cells that ‘escape’ central tolerance. For almost 40 years, immunologists have postulated the existence of suppressor T cells that police the immune system to avert unwanted immune responses^{1–3}. However, that phenomenology was cast into doubt as various labs presented unique, hard-to-reproduce systems, each with complexities and idiosyncrasies that raised credibility issues. This situation was not unlike the early years of cytokine biology, during which dozens of activities were found in sera and cell supernatants without consistent molecular or biochemical ‘signatures’. Fortunately, as biochemistry and the molecular biology revolution rescued the field of cytokine biology by identifying genes and biochemical structures tied to the varied biologic activities, the identification of a constellation of cell surface, transcriptional and biochemical markers that uniquely mark ‘regulatory’ T cells (T_{reg} cells) has made possible a rebirth of the suppressor T cell field over the past decade.

The realization that T_{reg} cells have a unique surface expression profile incorporating CD25, CD62L and specific CD45 isoforms^{4–6}, together with the identification of the T_{reg} cell-specific transcription factor Foxp3, catapulted T_{reg} cells from a rare CD4⁺ T cell subset to

what many regard as ‘master regulators’ of immune homeostasis⁷⁻⁹. However, the waters are becoming murky once again. First, there are myriad subpopulations of T_{reg} cells, including the CD4⁺CD25⁺ Foxp3⁺ cells, interleukin 10 (IL-10)–producing ‘Tr1’ cells¹⁰, transforming growth factor-β (TGF-β)–producing T helper type 3 cells¹¹, CD8⁺ T suppressor cells¹², natural killer T cells¹³, CD4⁺CD8⁺ T cells¹⁴ and γδ T cells¹⁵. Some of these T_{reg} cells, such as the CD4⁺Foxp3⁺ cells, originate in the thymus during ontogeny and are referred to as ‘natural’ T_{reg} cells. T_{reg} cells can also be induced from naive T cells in the periphery¹⁶. Some but not all of these peripherally induced ‘adaptive’ T_{reg} cells also express Foxp3. Now it is rare to find a high-profile journal that does not include a new functional activity ascribed to T_{reg} cells. This explosion of molecules, processes, mechanisms of action and phenomenology has led to an undercurrent of cynicism in the field. In this review, we will attempt to make some sense of many (although not all) of the reported functions of T_{reg} cells. We will focus our discussion on Foxp3⁺ CD4⁺ natural T_{reg} cells (which develop in the thymus) because these cells are central for immune homeostasis, as illustrated by the fatal consequence of their absence from mice deficient in IL-2, CD25 or Foxp3 or of their depletion from normal adult mice^{7-9,17,18}. Notably, like mice, humans with mutations in *FOXP3* develop multiorgan autoimmune diseases with fatal consequences¹⁹.

Many investigations have firmly established the involvement of natural T_{reg} cells in controlling autoimmunity, inflammatory disorders such as asthma and colitis, and immune responses to tissue transplants, tumors and various infectious agents²⁰. T_{reg} cells are purported to use many cellular processes to control immune responses. However, to fully consider the varied mechanisms of action of T_{reg} cells, it is critical to consider the two core T_{reg} cell-mediated phenomena: bystander suppression and infectious tolerance.

Although the suppressive activity of T_{reg} cells requires their prior activation through their T cell receptor, once activated, T_{reg} cells suppress in an antigen-nonspecific way called ‘bystander suppression’. Thus, T_{reg} cells with one antigen specificity can suppress effector T cells (T_{eff} cells) with many other distinct antigen specificities.

The phenomenon of infectious tolerance is proposed on the basis of *in vivo* transfer studies in which one population of suppressor T cells creates a regulatory milieu that promotes the outgrowth of a new population of T_{reg} cells with antigen specificities distinct from those of the original T_{reg} population. For example, in the setting of transplantation and type 1 diabetes, the tolerant state induced by T_{reg} cells is maintained even after loss or removal of the original T_{reg} cell population^{21,22}. Furthermore, new antigen specificities can be acquired by adaptive T_{reg} cell populations as long as the new antigen is present on the same tissue that the antigen recognized by the original T_{reg} cells was²¹. Thus, through the processes of bystander suppression and infectious tolerance, T_{reg} cells effectively establish a state of dominant and stable tolerance.

Investigations of the mechanisms of T_{reg} cell function have identified not one or two but an ever-growing list of molecules and processes that contribute to T_{reg} cell-suppressive activities (Table 1). Most of these studies have relied on the widely used *in vitro* suppression assay and a limited number of *in vivo* disease models. Three complementary approaches have been used to define T_{reg} cell functional biology by examining the influence of T_{reg} cells on other T cells or on antigen-presenting cells (APCs) or by molecular characterization of T_{reg} cell-derived suppressive molecules.

T_{reg} cells influence other T cells

Both *in vitro* and *in vivo* analyses suggest that T_{reg} cells can suppress the proliferation and/or cytokine production of T_{eff} cells. Additionally, T_{reg} cells prevent CD8⁺ cells from differentiating into cytolytic effector cells *in vivo* without affecting their proliferation or

interferon- γ (IFN- γ) production²³. Because the suppression of T_{eff} cell proliferation by T_{reg} cells was observed *in vitro* in an APC-free system, it was suggested that T_{reg} cells suppress through direct contact with T_{eff} cells²⁴. A mechanism to explain the contact dependence of *in vitro* suppression has been proposed in a publication showing that T_{reg} cells have large amounts of cytoplasmic cAMP and could deliver this potent immunosuppressive compound to T_{eff} cells by contact through gap junctions²⁵. Other studies have shown that T_{reg} cells can kill T_{eff} cells directly in culture through the release of granzyme B and perforin²⁶⁻²⁹. However, imaging studies have shown that T_{eff} cells and T_{reg} cells do not interact stably during suppression *in vivo* and *in vitro*^{30,31}.

Because natural T_{reg} cells constitutively express CD25, the high-affinity receptor for IL-2, it has long been suspected that T_{reg} cells suppress by 'sopping up' IL-2 produced by T_{eff} cells thereby preventing their proliferation and differentiation. This hypothesis has been revisited in studies examining the physiological changes in T_{eff} cells after their encounter with T_{reg} cells³². T_{eff} cells undergo apoptosis after being exposed to T_{reg} cells. This form of T_{eff} cell death is distinct from those mediated by granzyme B or perforin mentioned earlier and is dependent on the proapoptotic factor Bim, expressed by T_{eff} cells. Preventing Bim-mediated cell death by either Bim deficiency or forced expression of Bcl-2, a protein shown to counteract the proapoptotic activity of Bim, renders the T_{eff} cells resistant to T_{reg} cell-mediated suppression *in vitro* and *in vivo*. In this context, it is notable that the galectins also mediate cell death by a perforin- and granzyme-independent mechanism, which suggests a potential link between these two pathways^{33,34}. Unfortunately, it is difficult to explain bystander suppression or infectious tolerance *in vivo* on the basis of these T_{eff} cell-killing activities.

In contrast, some studies suggest that T_{reg} cells can alter the differentiation of other T cells. For example, CD4⁺ T_{eff} cells differentiate into IL-10- or TGF- β -producing adaptive T_{reg} cells in the presence of T_{reg} cells *in vitro* and *in vivo*, an observation more consistent with the idea of bystander suppression and infectious tolerance³⁵⁻³⁷.

T_{reg} cells alter APCs

A second approach to defining T_{reg} cell function has been to evaluate their effect on APCs. It has been shown that T_{reg} cells directly interact with antigen-presenting dendritic cells (DCs) *in vivo* within hours of transfer. The interaction of T_{reg} cells with DCs profoundly affects the ability of T_{eff} cells to subsequently engage and become activated by the same DCs. T_{reg} cells either abrogate the antigen-presenting activity of the DC or promote the secretion of suppressive factors by the target DC population. It has been shown that T_{reg} cells can stimulate APCs to upregulate the activity of indoleamine 2,3-dioxygenase (IDO), a potent immunosuppressive enzyme associated with pregnancy and tumor immune evasion^{38,39}. In those studies, IDO induction was found to depend on high expression of the inhibitory receptor CTLA-4 on T_{reg} cells⁴⁰. However, there is no clear evidence of the involvement of IDO in T_{reg} cell function *in vivo* or *in vitro*. The effect of CTLA-4 on T_{reg} cell function is also complex. Blocking CTLA-4 has no effect on *in vitro* suppression by T_{reg} cells, and in most circumstances, T_{reg} cells from CTLA-4-deficient mice show suppressive activity similar to that of cells from their wild-type littermates^{41,42}. However, *in vivo* CTLA-4 blockade abrogates T_{reg} cell-mediated suppression in mouse models of inflammatory bowel disease (IBD), autoimmune gastritis and transplantation (Table 1). In the setting of IBD, CTLA-4-deficient T_{reg} cells are able to prevent disease. However, unlike the protection afforded by wild-type T_{reg} cells, disease protection mediated by CTLA-4-deficient T_{reg} cells is dependent on IL-10 (ref. 43). That *in vivo* finding is consistent with *in vitro* analyses showing that CTLA-4-deficient T_{reg} cells are distinct from wild-type T_{reg} cells in the dependency of their suppressive function on TGF- β ⁴². Finally, mice deficient in

the recombination-activating gene product, when reconstituted with a mixture of CTLA-4-deficient and Foxp3-deficient bone marrow cells, live longer than mice reconstituted with either CTLA-4-deficient or Foxp3-deficient bone marrow cells alone, which shows that CTLA-4⁺ and Foxp3⁺ cells complement each other's function⁴⁴. These findings suggest that T_{reg} cells rely not on one mechanism for their function but instead use many alternative mechanisms to control unwanted autoimmune and uncontrolled adaptive immune responses.

Another relevant observation is the potential of T_{reg} cells to activate TGF- β on the surfaces of DCs. Latent TGF- β binds to DCs through interaction with $\alpha_V\beta_8$ integrins on the basis of an Arg-Gly-Asp motif. This binding is essential for the provision of a substrate for TGF- β activation and prevention of colitis⁴⁵. Evidence has been presented that furin, an enzyme purported to cleave latent TGF- β , is made by T cells after stimulation with IL-12 and thus might influence the creation of inflammatory and regulatory milieus⁴⁶. Additionally, TGF- β , together with a subpopulation of DCs, promotes the differentiation of T_{eff} cells into Foxp3⁺ T_{reg} cells⁴⁷. Thus, elaborate cross-talk between T_{reg} cells and DCs lead to DC silencing, local TGF- β activation and expansion of the T_{reg} cell repertoire, all of which help to actively establish and reinforce immune quiescence and self-tolerance.

T_{reg} cell-derived suppressor molecules

A third and perhaps the most straightforward approach to understanding T_{reg} cell biology has been to identify T_{reg} cell-specific molecules that are directly responsible for their potent tolerogenic effect. Like the other two approaches, this approach has led to complex and contradictory results. Many studies seem to definitively describe a mechanism of action that is essential for T_{reg} cell function, whether IL-10, TGF- β , CTLA-4, granzyme B, perforin, IFN- γ , IL-9, heme oxygenase-1 (HO-1), cAMP, CD39, galectins or IL-35 (Table 1). Yet with the possible exception of CTLA-4 and TGF- β (the latter of which is also essential for T_{reg} cell development), disruption of the genes encoding these molecules does not result in the catastrophic effects found in T_{reg} cell-deficient mice. In an effort to categorize the various pathways and perhaps identify common characteristics, several are summarized below (Fig. 1).

Many studies have shown that T_{reg} cells can make suppressive cytokines such as IL-10 and TGF- β . Early *in vitro* studies demonstrated that neutralizing antibodies to IL-10 or TGF- β do not block T_{reg} cell activity and that T_{reg} cells from mice lacking IL-10 or TGF- β show similar suppressive activity^{24,41,48}. It should be pointed out, however, that although often dismissed, there were some early suggestions that TGF- β is involved in T_{reg} cell suppressive function *in vitro*^{49,50}. In contrast to those *in vitro* studies, it has been found that neutralizing IL-10 or TGF- β abolishes T_{reg} cell suppression *in vivo* in mouse models of IBD, type 1 diabetes, leishmania skin infection and transplantation (Table 1). In a few *in vivo* models, expression of IL-10 or TGF- β specifically in T_{reg} cells is required for T_{reg} cell function⁵¹⁻⁵³. However, in some other situations, the source of these suppressive cytokines is not limited to T_{reg} cells^{37,54,55}.

HO-1, which catalyzes the formation of carbon monoxide through heme degradation, has been linked to T_{reg} cell function^{56,57}. Similarly, CD39 and CD73, which are 'preferentially' expressed on the surfaces of T_{reg} cells, catalyze the generation of adenosine from the extracellular nucleotide ATP or ADP⁵⁸. Adenosine can bind to its receptor A2A expressed on T_{eff} cells to suppress their responses. Additionally, it has been suggested that several members of the galectin family of carbohydrate-binding proteins are involved in T_{reg} cell function⁵⁹⁻⁶¹. It has also been proposed that another cytokine, IL-35, is responsible for close-range suppression by T_{reg} cells. IL-35, a heterodimeric protein composed of the IL-12 p35 chain and Ebi3, is regulated in T_{reg} cells by Foxp3 (ref. 62). T_{reg} cells lacking either p35

or Ebi3 are less suppressive *in vitro* and are unable to control IBD induced by T_{eff} cells. Thus, it would seem that many molecules can mediate T_{reg} cell functions both *in vitro* and in certain *in vivo* settings.

The 'big picture'

Although the results presented above seem overwhelming and confusing at first glance, the following theme emerges from the vast array of data obtained in various disease settings: T_{reg} cells probably use many mechanisms to control unwanted immune responses *in vivo*. Depending on the nature of the immune response, the eliciting agent, the immunological makeup of the host and the site of suppression, certain mechanism(s) may seem to dominate.

This phenomenon is most vividly exemplified by the IBD model⁶³. Reliance on IL-10 for T_{reg} cell-mediated control of IBD varies depending on how the disease is elicited and the timing of T_{reg} cell treatment. Control of more aggressive forms of disease induced by memory T cells or pathogenic bacteria and reversion of established disease requires IL-10 in addition to CTLA-4 and TGF- β . In contrast, in the absence of one of the suppressive mechanisms (such as CTLA-4), T_{reg} cells rely more on alternative suppressive functions such as IL-10 and TGF- β *in vitro* and *in vivo*^{42,43}. Thus, it is possible that T_{reg} cell-mediated control of IBD, a disease at the highly inflammation-prone mucosal surface, may require a wider array of suppressive mechanisms operating in synergy and thus that the presence of IL-10, TGF- β , CTLA-4, and IL-35 as well as IL-2 deprivations are necessary for complete protection. This scenario is mirrored in the transplant setting. Immune responses to organ transplants are one of the most vigorous forms of immune responses because of the exceptionally high frequency of alloreactive T cells. Thus, T_{reg} cells may depend on many suppressive mechanisms working in concert to keep alloimmune responses under control. In contrast, one or two mechanisms may suffice to control slowly progressing organ-specific autoimmune diseases such as type 1 diabetes and autoimmune gastritis. In these disease settings, when one pathway is blocked, alternative mechanisms may fully compensate; therefore, T_{reg} cell function might not be dependent on any one particular mechanism.

The site of T_{reg} cell action *in vivo* is certainly not limited to lymphoid organs. T_{reg} cells have been detected at sites of inflammation, and in many situations, their ability to migrate to and remain in inflamed tissues is important for their function *in vivo*⁵⁰. Moreover, the molecular mechanisms of suppression used by T_{reg} cells seem to differ depending on their localization in nonlymphoid versus lymphoid tissues. T_{reg} cell-mediated prevention of IBD induced by the transfer of naive T cells is confined to lymphoid organs and is dependent on TGF- β and CTLA-4. In contrast, T_{reg} cell-mediated control of colitogenic bacteria-induced IBD requires the presence of T_{reg} cells in lymph nodes and in the inflamed colon, as well as IL-10 (ref. 64). In addition, control of an acute immune response to leishmania is located mainly in lymphoid organs and is independent of IL-10, whereas control of a chronic immune response in the infected skin requires local retention of T_{reg} cells and their production of IL-10 (ref. 64). Finally, tumor-infiltrating T_{reg} cells suppress tumor immunity by a mechanism dependent on TGF- β and IL-10, unlike T_{reg} cells isolated from the peripheral blood of the same person⁶⁵. These data from various disease models collectively demonstrate differential requirements for distinct suppressive functions of T_{reg} cells at different tissue sites. In general, T_{reg} cells rely on more suppressive mechanisms to control responses at sites of inflammation than in lymphoid organs.

On the basis of the experimental evidence collected so far, we propose a three-tiered 'sequential' model of T_{reg} cell function *in vivo* (Fig. 1). In the steady state, T_{reg} cells exert 'homeostatic control' over the immune system in lymphoid organs to prevent the potential outgrowth of auto-reactive T cells. By virtue of their constitutive expression of CD25, T_{reg}

cells effectively 'sop up' IL-2 produced by T_{eff} cells, thereby terminating local incidental T_{eff} cell activation while boosting T_{reg} cell fitness and function. This phenomenon helps to explain some of the antigen-non-specific functions of T_{reg} cells noted in several *in vivo* systems. T_{reg} cells also function in an antigen-specific way. The T_{reg} cell antigen receptor repertoire is skewed toward recognition of self antigens⁶⁶. In addition, T_{reg} cells show greater sensitivity to antigens than do their T_{eff} cell counterparts^{47,67}. These properties allow T_{reg} cells to effectively patrol the lymphoid organs to prevent potential priming of autoreactive T cells. Most likely the type of T_{reg} cell target in this setting is self antigen-presenting DCs. This contention is supported by the observation that T_{reg} cells are stably conjugated with tissue antigen-bearing DCs during *in vivo* suppression in lymph nodes. In addition, complete T_{reg} cell ablation in adult mice leads to the expansion of populations of activated DCs and to subsequent fatal multiorgan autoimmune disease¹⁸. The molecular mechanisms used by T_{reg} cells during this stage probably involve TGF- β and CTLA-4, as suggested by the IBD studies mentioned above⁶³. In addition, the massive lymphoproliferation noted in CTLA-4-deficient and TGF- β -deficient mice also supports the idea of an essential function for T_{reg} cells in normal immune homeostasis^{68,69}. Other mechanisms involving IL-10, IDO, HO-1 and CD39-CD73 may also contribute to T_{reg} cell function at this stage, but they are probably not essential, as indicated by the lack of overt changes in immune homeostasis in their absence. It is notable that one attribute of the galectins is their ability to bind to multiple ligands and soluble molecules because of the 'sticky' nature of the lectin moieties. Thus, it is possible that the galectins, by forming functional scaffolds that bind several of the small molecules purported to be mediators of T_{reg} cell suppression, help to establish a local immunosuppressive milieu that influences many cell types, such as T_{eff} cells, DCs, macrophages and B cells, to actively maintain immunological quiescence.

When steady-state self-tolerance is breached, as after Toll-like receptor engagement during infection⁷⁰ and in the presence of heightened frequencies of self-reactive T_{eff} cells in autoimmune and transplantation settings, T_{reg} cells become further activated to engage the second tier of 'damage control'. After being activated, T_{reg} cells shed CD62L and upregulate the chemokine receptors and adhesion molecules needed to leave lymphoid organs and gain entry into inflamed tissues. Once in inflamed tissue, T_{reg} cells reactivated by tissue DCs upregulate CD25 and therefore become more effective at competing with T_{eff} cells for IL-2. Activated T_{reg} cells express more IL-10, CTLA-4, IL-35, TGF- β , HO-1 and CD39-CD73 and so are more potent suppressors. In addition, chronically activated T_{reg} cells have high expression of cytotoxic granules such as granzyme B, which enable T_{reg} cells to actively kill APCs and T_{eff} cells in extreme conditions that lead to resolution of the immune response.

Infectious tolerance is established during the final stage to stabilize the tolerant state. Tissue destruction associated with the immune response leads to the presentation of newly exposed tissue antigens on the T_{reg} cell-silenced DCs. Together with TGF- β , also a byproduct of immune activation, these tolerogenic DCs induce the differentiation of T_{eff} cells into adaptive T_{reg} cells, thus expanding the T_{reg} cell antigen receptor repertoire. In the intestine, it has been shown that CD103⁺ DCs have the unique ability to induce adaptive T_{reg} cells because of their ability to produce retinoic acid, which is needed to induce naive T cells to differentiate into Foxp3-expressing T_{reg} cells⁴⁷. TGF- β induces CD103 expression⁴⁵; thus, TGF- β may contribute to the induction and/or maintenance of these specialized DCs. This model is consistent with the 'hygiene hypothesis', proposed to explain the reciprocal rates of infection and atopic and autoimmune disease. Improved hygienic standards and a decrease in both infections and the resulting active immune responses may also deprive people of the opportunity to expand T_{reg} cell antigen receptor repertoires through infectious tolerance and thereby lead to higher rates of allergy and autoimmune disease.

Overall, T_{reg} cells probably use many mechanisms *in vivo* to prevent and terminate immune responses and to establish a regulatory milieu that will promote a long-lasting, durable state of tolerance. Short-range mechanisms such as cytokine deprivation and the secretion of molecules such as IL-10, adenosine, IL-35, galectins and carbon monoxide ‘shut down’ local T_{eff} cells during adaptive immune responses. These pathways are designed to act quickly and locally and in many cases are sufficient to dampen immunity. However, in the long term, T_{reg} cells create a regulatory environment by producing TGF- β and/or by substantially changing DC functions to promote new T_{reg} cell production. These processes promote bystander suppression and are critical to the creation of infectious tolerance that can spread beyond the local tissue and exert long-lasting influence over the immune system. It is notable that some of the mechanisms that T_{reg} cells use to suppress immunity also enhance T_{reg} cell function. For example, by ‘sopping up’ IL-2, T_{reg} cells simultaneously starve T_{eff} cells and promote their own population expansion and survival. Similarly, the direct or indirect production of TGF- β by T_{reg} cells ‘feeds back’ to stabilize and enhance Foxp3 expression in T_{reg} cells, thereby boosting their suppressive function.

Concluding remarks

In conclusion, *in vitro* and *in vivo* T_{reg} cell function cannot be attributed to a single dominant pathway or molecule. Instead, T_{reg} cells use many suppressive mechanisms that can target various cell types depending on the circumstances and their surroundings. The versatility and adaptability of T_{reg} cells makes them true ‘masters of immune regulation’. The chief function of T_{reg} cells in a normal person is to maintain immune homeostasis in the lymphoid organs. After the onset of an immune response, T_{reg} cells use additional suppressive strategies to resolve inflammation and limit tissue damage. The dynamic interaction between T_{reg} cells and DCs is vital for T_{reg} cell function. This model provides a conceptual framework for the ongoing quest to discover more suppressive mechanisms used by T_{reg} cells, which are intricately involved in the pathogenesis of autoimmune disease, infectious disease, cancer and the rejection of transplanted organs. Better understanding of their biology will create opportunities for the development of new therapeutic intervention in these disease settings.

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Figure 1.

A three-tiered model of the functions of T_{reg} cells in maintaining normal immune homeostasis. This model shows mechanisms potentially used by T_{reg} cells for homeostatic control in the steady state, during ‘damage control’ at the site of inflammation and for infectious tolerance after the resolution of an immune response. a T_{reg} , adaptive T_{reg} cell; TGF- β R, TGF- β receptor; n T_{reg} , natural T_{reg} cell; CO, carbon monoxide; IFN- γ R, IFN- γ receptor.

Table 1

Molecular mechanisms underlying T_{reg} cell-mediated suppression

Mechanism	<i>In vivo</i>						
	IBD	Type 1 diabetes	Autoimmune gastritis	Asthma	Infection	Tumor	Transplantation
IL-10	No (refs. 41,48)	Yes (ref. 64)	No (ref. 72)	No (refs. 37,54)	Yes (ref. 64)	Yes (ref. 65) ^{a,b}	Yes (ref. 73) ^c
TGF-β	No (refs. 24,41,48)	Yes (ref. 53); no (ref. 55)	No (ref. 76)			Yes (ref. 65) ^{a,b}	No
CTLA-4	Yes (refs. 77); no (refs. 41,42)	Yes (ref. 43)	Yes (ref. 77) ^c				Yes (ref. 79) ^c
Granzyme B	Yes (refs. 27-29)					Yes (ref. 80)	
Perforin	No (ref. 27); yes (refs. 26,29) ^b					Yes (ref. 80)	
IFN-γ	No (ref. 41)						Yes (ref. 81) ^d
IL-9							Yes (ref. 82)
HO-1	Yes (refs. 56,57) ^c ; no (ref. 83)			Yes (ref. 57) ^c			Yes (ref. 84)
cAMP	Yes (ref. 25)						
Galectins	Yes (refs. 59,60)						
CD39-CD73; adenosine	Yes (ref. 58)						Yes (ref. 58)
'IL-2 sink'	Yes (ref. 32)	Yes (ref. 32)					
IL-35	Yes (ref. 62)	Yes (ref. 62)					

^aDependence on IL-10 and TGF-β determined *in vitro*.

^bData derived from human studies.

^cSource of suppressive molecule may or may not be natural T_{reg} cells.

^dT_{reg} cells in these settings may be natural or adaptive T_{reg} cells.