Chapter 2

Physiology and Pathology of Immune Dysregulation: Regulatory T Cells and Anergy

Maria Isabel Torres, Miguel Angel López-Casado, Candelaria Ponce de León, Pedro Lorite and Teresa Palomeque

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.71770

Abstract

The immune system is responsible for the defense of the organism. It controls what is introduced into it and identifies it as self from non-self. The defensive mechanisms activated by the immune system are directed against pathological microbes and toxic or allergenic proteins, and it must avoid responses that produce excessive damage of self-tissues, inducing tolerance to avoid autoimmunity and other immunopathologies. Regulatory T cells play an essential role in these active processes, using several distinct suppressive mechanisms. The immune dysregulatory diseases result from defects affecting regulatory T cell development and/or function, including the impact of essential genes mutations for T regulatory cell functions and the associated autoimmune syndromes.

Keywords: anergy, T cell exhaustion, regulatory T cells, IPEX syndrome, tolerance, autoimmunity

1. Introduction

The immune system requires strict control and self-regulation in order for its functioning to be the most efficient possible and adjusted to the defensive needs of each moment, thus inducing an appropriate immune response against pathogens and tumors. Immune tolerance is based on the fact that the immune system has to distinguish between itself and any non-self in order not to destroy its own components, which must be previously recognized as such in the thymus and bone marrow. When tolerance for some reason fails, multiple pathologies appear, as autoimmune diseases. In this chapter, we analyze general aspects of dysfunctional T cell responses such as anergy and T cell exhaustion, some of the phenotypic markers associated

with them, and the importance of these processes in the establishment of tolerance and autoimmunity. Also, we consider the main pathogenic event of regulatory T cell dysfunction leading to multi-organ autoimmunity in the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Clonal anergy, a well-known regulatory mechanism, can be deemed a hyporeactive state arising when the T cell antigen receptor activates T cells despite the lack of suitable co-stimulatory signals. T cells have proved to be important in stimulating and/or maintaining anergy, so the anergic T cells may change their transcriptional and epigenetic programs and turn into regulatory T cells. Anergic T cells appear to represent the intermediate reprogramming stage before becoming regulatory T cells, which maintain self-tolerance. T cell exhaustion is a state phenotypically similar to anergy. When exhausted, T cells neither secrete cytokines nor lyse target cells, and furthermore fail to proliferate. Such chronic stimulation prompts the sustained high expression of co-inhibiting molecules, including TIM-3, 2B4, PD-1, and LAG-3, which act blocking the activation of T cells. Whereas anergy is a programmed transcriptional process induced by minimal signaling, exhaustion occurs at the pathological level by the presence of abundant inflammatory signals maintained over time. Certain conserved mechanisms promote both anergy and depletion of T cells in the immune system. The dysfunction of Treg cells is the main pathogenic event leading to the multi-organ autoimmunity that characterizes the IPEX syndrome, a paradigm of genetically determined primary immunodeficiency due to mutations of *FOXP3*, a key transcription factor for naturally occurring Treg cells, with autoimmunity.

2. Dysfunctional T cell responses

Mechanisms have been developed by the immune system to direct effective responses to a broad gamut of pathogens. Responses of the immune system protect against many lymphocyte antigen receptors that are generated by realignments of somatic genes. Although this process enables hosts to combat pathogens effectively, these organisms quickly evolve to present many challenges, prompting detrimental immune responses to, for example, self-tissue antigens and non-harmful components including food antigens or non-pathogenic agents of the intestinal tract [1]. Various states of T cell dysfunction have been described as a consequence of altered activation and differentiation processes. Terms such as exhaustion, tolerance, anergy, senescence, and even ignorance have been used to describe the dysfunctional state of T cells, depending on the clinical settings and the phenotypic and functional features of the T cells.

Autoimmunity, one of the most serious problems of the immune system, causes many diseases that are difficult to cure. One cause of autoimmunity is self-reactive T cells that start to attack the body of the host in the periphery [2, 3], although most self-reactive immature T cells are eliminated by negative selection in the thymus [4]. Multiple mechanisms are at work to prevent autoimmunity, including regulatory T cells [5], T cell ignorance [6], and T cell anergy [7]. The development the other pathologies such as chronic infections and cancer is facilitated by a variety of immune-subversion mechanisms, with the production of anti-inflammatory cytokines, induction of regulatory T (Treg) cells, and expression of immune checkpoint molecules.

http://dx.doi.org/10.5772/intechopen.71770

3. Regulatory T cells

Regulatory cells (Tregs) play a critical role in the establishment and maintenance of immune homeostasis as well as in the limitation of chronic inflammatory responses directed against pathogens and environmental factors [8–10]. This cell-mediated suppression is considered a vital mechanism of negative regulation of immunomediated inflammation, and plays a prominent role in autoimmunity and auto-inflammatory disorders, allergies, acute and chronic infections, cancer, and metabolic inflammation; these are important candidates for the therapeutic treatment in these inflammatory and autoimmune diseases.

Treg cells represent 5–10% of peripheral CD4+ T cell compartment in humans. In this section, we present the characteristics that define regulatory T cells, the phenotypic and functional heterogeneity that they present, with particular reference to the consequences of T cell dysfunction in contributing to the development of autoimmunity and deregulation of the immune system [11].

3.1. Treg phenotypes

Treg cells represent highly differentiated populations in that they are distinguished phenotypes based on the expression of specific markers and mechanism of action. Different Treg subsets have been identified, but two major types expressing Foxp3+ transcription factor can be distinguished based on their origin: (i) natural or Treg cell thymus-derived (nTreg or tTreg) and (ii) induced Tregs that develop in the periphery from naïve conventional CD4+ T cells (iTregs or pTregs) [12, 13]. The nTregs are the major mediators of central immune tolerance, whereas iTregs are involved in the regulation of peripheral immune tolerance in sites of inflammation [14].

The phenotype as well as function of nTregs, as opposed to iTregs, have been difficult to study in humans, given the shortage of markers used for discriminating these cell types. It has recently been argued that the expression of Helios, which is a transcription factor of the Ikaros family, can discriminate nTregs from iTregs on the basis of most thymically derived FOXP3+ cells expressed by Helios [15]. Nevertheless, the Helios used as a marker for nTregs has been disputed because, depending on the cell-activation conditions, Helios is also expressed in conventional T cells (T conv) of humans [16]. Helios cannot be used as an nTreg/iTreg discrimination marker but may serve as a useful activation/differentiation marker for Tregs. In this sense, the subset of nTreg cells could be subdivided on the basis of Helios expression, representing a stable and suppressive Treg population that differs only in cytokine/chemokine production [17].

Other Treg cells are found in the periphery, such as Tr1 cells, which lack the expression of the transcription factor FOXP3 [18] with immunosuppressive functions as IL-10 and TGF-β secretion [19], and Th3 cells with a variable level of FOXP3 expression [20]. CD8+CD25+ Treg cells are also developed in the thymus, expressing several molecules characteristic of nTregs, namely CD25, FOXP3, CTLA-4, and TNF-receptor. CD8+CD28+ Tregs inhibit priming of CD8 + and CD4+ T cells, and antibody-mediated against oral antigens. CD8+CD28-Tregs can be induced from naïve CD8+ T cells upon activation by allogenic antigen presentation cells (APCs) in the presence of IL-2 and granulocyte-macrophage colony-stimulating factor (GM-CSF). The γδ T cells are commonly of the CD8 + FOXP3-phenotype and are found mainly in the

intestinal epithelium associated with mucosal tolerance. These cells can also regulate autoimmunity and tumor immunity by producing IL-10 and TGF-β, similarly to Tr1 cells [21].

3.2. Treg functions

Treg cells have been considered key players in dominant immune tolerance [22]. Treg cells have performed functions such as to suppress inflammatory responses in mucosal interfaces that are constantly exposed to allergens [23], commensal gut microbiota [24, 25], transplanted organs [26], pathogenic infections [24], and tumors [27]. Recent studies have suggested a role for Tregs in other situations, such as adipose tissue resident Tregs controlling metabolic disorders [28, 29] and Tregs limiting organ rejection [30]. In certain cases, the suppressive function of Tregs limits beneficial effector responses of the host against tumors and chronic infections [31, 32]. Hence, the activities of this suppressive population need to be controlled by allowing the balance between restricting deleterious inflammatory and autoimmune insults, while facilitating protective responses against infections and tumors.

While FOXP3 is an indispensable transcription factor to define the majority of the Treg transcriptional and functional subsets, FOXP3+ Treg cells express on the cell surface high levels of interleukin-2 receptor α (CD25) and a low level of IL-7 receptor α (CD127) [33]. Thus, the majority of Treg cells constitutively express high levels of the inhibitory molecule cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and the glucocorticoid-induced TNFR family related (GITR), as well as the regulatory cytokines IL-10 and TGF-β [34–36].

According to a number of studies, not all FOXP3+ T cells are functional Tregs, and it is possible to induce a portion of the Treg signature without the presence of FOXP3 [37, 38] since activated human T cells express Foxp3 transiently without acquiring suppressor capacity [39, 40] The essential aspect of the Treg cell (FOXP3 expression and suppressive capability) can be maintained in differing Treg sub-populations identified in various anatomical locations as well as under pathological conditions [41–43]. Their characteristics allow phenotypic/functional adaptation to block full immune responses. Within the FOXP3+ Treg subsets, the diversity can be characterized by: (i) differential transcription-factor expression [44–47]; (ii) different expression of chemokine receptors [41, 42, 47], and (iii) differing expression of suppressor markers that control various types of target cell in diverse environmental and pathological conditions [48–50].

Treg cells, on losing FOXP3 expression as well as their suppressive capability, form an unstable population, taking on characteristics similar to those of the effector T cell reacting to environmental cues [51–53]. Though convincing evidence is available for Treg cell stability under healthy immune conditions [54, 55], numerous studies propose that inflammatory conditions may be related to downregulation/loss of FOXP3, secretions of effector cytokines, and also the proliferation of the so-called “ex-Treg” cells [13, 56]. This implies that Treg cells may be reprogrammable as inflammatory cells in reaction to microenvironmental signals. Treg cells show no terminal differentiation, though they do retain plasticity and can differentiate into specialized hybrids to control immune responses [57]. Thus, for Treg function, two models have been proposed: one in which Treg-specific expression of FOXP3 would encode the expression of Treg suppressor characteristics (greater CD25 and CTLA-4), whereas their ability to adapt to the shifting environmental cues would induce further suppressive modules (e.g. miRNAs, suppressive pathways, transcription factors, and chemokine receptors) for suitable immune regulation [58].

Another key question is the role of Treg cells in preventing autoimmunity and their therapeutic potential based on Treg cell transfer or activation leading to the definition of the signals responsible for generating and maintaining of Treg cells [59]. Several studies have focused on two sets of signals—interleukin-2 (IL-2) and antigen itself [60, 61]. Thus, IL-2 is required for the survival of Treg and for maintaining their functional activity by promoting expression of FOXP3 and mediators of suppression, particularly CTLA-4 [62]. Answers to environmental antigens may provide enough IL-2 to maintain a Treg cell repertoire in healthy individuals. The dependence of Treg cells based on IL-2 received from conventional T cells provides a negative feedback through which the ratio of Treg cells and conventional T cells is controlled [63].

3.3. Regulatory T cells and tolerance

Oral tolerance to foods is an active immunological process that involves allergen-specific Treg cells [64–66]. Genetic and immunological evidence supports an important role for Treg cells in enforcing oral tolerance to foods [67–69]. This tolerance depends on iTreg-cell development from naïve conventional CD4+ T cells (CD4+ Tconv), which are activated in presence of TGF-β1 and CD103+ dendritic cells (DCs) [70–72] regulating T helper 2 cell responses at the mucosal surfaces [73, 74]. In food allergy, a deficient formation and impaired function of allergen-specific Treg cells is present.

Treg cells in the intestine are important in bringing about a tolerogenic environment for maintaining immune homeostasis in commensal bacteria [75, 76]. The question of commensal bacteria-inducing Treg and effector cells is basic in explaining the way in which the immune system receives instructions from particular species of bacteria and in determining the dynamics of Treg versus effector-cell selection of bacterial antigens [77–79]. T cell differentiation may be guided by innate stimulators of commensal bacteria as TLRS selectively activate cytokine production from APC subsets, TLRS being major sensors capable of recognizing conserved molecular motifs in bacteria [80, 81]. However, the adaptive immune system may react to pathogenic rather than commensal bacteria, so that the pre-existing effector and Treg cell reactions to commensal bacteria may alter the course of the infection. In addition, infection may upset the balance between effector versus Treg cell reactions to commensal bacteria, disturbing immune homeostasis as well as potential immunopathology [75, 80].

A dynamically regulated Treg cell population would be in tune with the commensal microbiota and thus would be more responsive when confronted with a strong influx of commensal antigens after mucosal injury, limiting the activation of effector T cell, and controlling excessive inflammation [75–77]. By contrast, bacteria new to the digestive system would not trigger Treg or effector T cells already present, but rather would need a new selection of effector versus regulatory T cell reactions. This situation would enable quicker effector responses to microbes, limiting the generation of effector T cells meeting commensal bacteria, and this could prompt inflammatory bowel disease (IBD) development [82].

Commensal bacteria are major initiators of effector T cell reactions that lead to inflammation. The immune system responds to commensal antigens as non-self, not only because bacterial antigens are unlikely to be present during the selection of thymic T cells, but also because bacteria bear a number of ligands used in recognizing immune receptors [83]. It is widely accepted that commensal bacteria also induce T cells that decrease inflammation in order to

sustain intestinal tolerance. Thus, Treg cells play a vital part in maintaining homeostasis of the gut immune system and in deterring effector cells from triggering immunopathology as a response against commensal bacteria.

Several studies have identified microbial products from a specific bacterial species that affects Treg cell function. Polysaccharide A (PSA) from *Bacteroides fragilis* was found to activate TLR2 expressed on Treg cells, inducing the production of IL-10 [84], facilitating the persistence of *B. fragilis*. Many studies have reported a possible "universal" mechanism driving Treg cell expansion that is mediated by bacterially derived short-chain fatty acids (SCFAs) produced through the metabolism of dietary fiber [85–87]. The microbial products are perceived by the intestinal immune system to facilitate homeostasis and tolerance instead of inflammation, consistent with the notion of an evolutionary mutualistic relationship between commensal bacteria and the host [84, 88]. In this sense, colonic Treg cells utilize a unique set of T cell receptors (TCRs), suggesting that they recognize antigens found only in this tissue including colon-specific self-antigens and antigens derived from commensal bacteria [89].

Alterations in the composition of commensal bacterial populations are linked to multiple metabolic and inflammatory diseases including, but not limited to, inflammatory bowel disease (IBD), obesity, type 2 diabetes, atherosclerosis, allergy, and colon cancer [90–92]. Recent studies have identified a critical role for commensal bacteria and their products in regulating the development, homeostasis, and function of innate and adaptive immune cells [93–95]. However, an emerging and interesting area that has received relatively little attention is how metabolites and nutrients derived from commensal bacteria regulate the host immune system.

4. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome

As discussed above, Treg cells play a key role in immune homeostasis by maintaining a balanced adaptive immune response. The spectrum of manifestations due to Treg cell defect might range from mild allergy or autoimmunity to lethal immune dysregulation disorders (IPEX) [96]. Several human genetic disorders have recently been described and noted to have an extraordinary impact on Treg cell development and functional activity [97]. A loss of function mutation in FOXP3, the key transcriptional factor for Treg cell differentiation, leads to an IPEX phenotype. Subsequently, a number of other gene defects have been reported to cause IPEX-related phenotypes, including the loss of function mutations in theCD25, STAT5B, LRBA, and CTLA4 gene [98].

IPEX, a rare genetic disorder, results from a dearth of functional Treg cells caused by losses of function mutations in FOXP3. It affects only males because of its X-linked recessive inheritance. Also, it is frequently fatal in the early years of life if the patient receives no bone marrow transplant [99]. In clinical terms, IPEX presents three maladies: autoimmune enteropathy, autoimmune endocrinopathy, and eczematous dermatitis. The most frequent manifestation, enteropathy, gives way to endocrinopathy particularly insulin-dependent type 1 diabetes mellitus [99]. Other manifestations include lung disease, immune-mediated cytopenia,

autoimmune nephropathy, anemia and/or thrombocytopenia, and hepatitis. Furthermore, food allergies with high serum IgE and peripheral eosinophilia prove very common, indicating a clear failure of oral tolerance in this disorder. Usually, IPEX patients show a broad range of autoantibodies because of adaptive immune dysregulation. With over 60 FOXP3 mutations reported up to now, observations from the clinical phenotype reported for these mutations have led to postulations of genotype/phenotype relationships [100].

CD25 deficiency properties shared with IPEX include chronic eczema, enteropathy, lymphoproliferation, and autoimmunity disorders such as alopecia, diabetes mellitus, thyroiditis, and autoimmune hemolytic anemia [101–103]. CD25 deficiency is permissive to Treg cell differentiation, with normal count of FOXP3+ Treg cells found in circulation [104]. The loss of CD25 expression impairs Treg cell suppressive function by defective production of suppressive cytokine IL10. Their failure deprives Tconv cells of IL-2 production, leading to their apoptosis [62, 101]. Finally, the decreased sensitivity of CD25-deficient Treg cells to IL-2 impairs their metabolic competence in the context of an immune response [105, 106].

Evidence from studies on human and murine models show that Type-1 regulatory T (Tr1) cells can contribute to suppressing the development of autoimmunity in addition to nTreg cells [106, 107]. Tr1 cells can develop in IPEX patients regardless of FOXP3 expression [108]. This observation suggests that FOXP3-independent immune regulation can potentially help control the disease, although Tr1 cells alone do not seem adequate to suppress the initial acute phase of the disease.

5. T cell exhaustion

T cell exhaustion is distinguishable from other dysfunctions such senescence or anergy, based on molecular mechanisms [109, 110]. That is, exhausted T cells come from cells that initially developed an effector function but then gradually lose it because of continuous stimulation of the T cell receptor (TCR) from the persistent antigen helping to build peripheral tolerance as well as to modulate immune responses [111, 112]. As such, exhausted T cells present in patients having autoimmune disorders correlate with positive prognoses [113]. However, in cancers, exhausted T cells may block tumor clearance, thereby contributing to immune escape [114, 115]. This also leads to chronic infections, and viral immune evasion results from the persistence of activated T cells that have no effector function [116].

Regarding the origin of exhausted T cells, recent work has shown that exhausted CD4+ and CD8+ T cells bear a notably different transcriptional profile from that of effector and memory CD4+ or CD8+ T cells. These differences include shifts in the expression of co-stimulatory and inhibitory receptors (IRs), as well as signaling molecules, transcription factors, chemokines receptors, cytokines, and genes that are involved in metabolism. Also, genomic research supports the contention that exhausted T cells constitute a unique stage of T cell differentiation [110, 117].

With respect to the causes behind T cell exhaustion, CD8+ T cell exhaustion likely involves altered inflammatory and tissue microenvironments as well as other populations of

lymphocytes such as CD4+ T cells, regulatory T cells, B cells, and inhibitory cues from cytokines and inhibitory as well as co-stimulatory cell-surface receptors [110]. The major feature appears to be a chronic and presumably continual antigen exposure instead of acutely terminated or intermittent exposure. Also, the severity of the exhaustion and the deletion of antigen-specific T cells have been found to correlate with (i) the expression of stimulatory and inhibitory receptors; (ii) the levels of stimulatory and suppressive cytokines; and (iii) the degree of antigen stimulation [118, 119].

The gradual dysfunction of exhausted T cells is accompanied by the expression of multiple inhibitory receptors, by progressive loss of IL-2 production and TNF-α and IFN-γ depletion [112], as well as by altered cell metabolism with a markedly different transcriptional profile [120, 121]. T cells do not exhaust uniformly during chronic diseases or cancer, but instead specific subsets with different memory and proliferative potentials emerge after exposure to persistent antigen [122, 123].

While exhaustion was first viewed as a dysfunctional T cell state, this phenotype is now considered an appropriate response to chronic infection, because a persistent effector function could cause excessive damage to healthy cells. T cell exhaustion prevents optimal control of infection and tumors, modulating pathways overexpressed in exhaustion that can reverse this functional state and reinvigorate immune responses [124] by targeting programmed cell-death protein 1 (PD1) and cytotoxic T lymphocyte antigen 4 (CTLA4) [125, 126]. Exhausted T cells are not inert, given that they retain crucial functions at the suboptimal level that limits ongoing pathogen replication or tumor progression. These cells are not effective at eradicating pathogens or tumors, and have been considered of interest in avoiding or reversing exhaustion.

Inhibitory receptors (IRs) are negative regulatory pathways that control autoreactivity and immunopathology and are transiently expressed in functional effector T cells during activation. A higher and sustained expression of inhibitory receptors is a hallmark of exhausted T cells. The molecular mechanisms by which inhibitory receptors control T cell exhaustion are not entirely known. Although PD1 is the best characterized inhibitory receptor, exhausted T cells express a range of other cell-surface inhibitory molecules to impair T cell responses during chronic infections, such as CTLA4, LAG3, 2B4, TIM3, CD160, and many others [127]. The co-expression of multiple inhibitory receptors is a chief feature because the simultaneous blockage of IRs results in synergistic reversal of T cell exhaustion. Results of several clinical trials using immune checkpoint inhibitors are very encouraging. Blocking antibodies for CTLA-4, PD1, and PDL1 appear to have a strong therapeutic potential given alone or in combination with standard treatment in many tumors.

In addition, the soluble molecules regulate T cell exhaustion. These include immunosuppressive cytokines such as IL-10, TGF-β, and inflammatory cytokines such as IFNs type I and IL-6 [110]. Blockage of IL-10 restores T cell function and improves viral control during chronic viral infections, demonstrating that IL-10 promotes T cell exhaustion [128, 129]. Many cell types can be the source of IL-10 during chronic infection, including dendritic cells (DCs), monocytes, and CD4+ T cells [130, 131]. The blocking of IL-10 and the PD1 pathway in a simultaneous manner, synergistically reverses CD8+ T cell exhaustion and enhances viral control, indicating a role for IL-10 in controlling CD8+ T cell exhaustion [132]. Depletion of CD4+ T cells help during pathogen persistence and can contribute to defective CD8+ T cell responses. Therefore, in HIV

infection, the loss of the CD4+ T cell response can result in exhausted CD8+ T cells and disease progression [133].

6. T cell anergy

Immunological tolerance is the essential mechanism for maintaining immune homeostasis. T cell anergy, one of the major mechanisms involved in immunological tolerance [134–136], is a hyporesponsive state of T cells under antigen stimulation. The expression of several anergy-specific genes are known to change in anergic T cells, such as DGK-a, an intracellular signaling molecule (also known as an anergy-related gene) and EGR2, a transcription factor, and this reportedly increases in anergic T cells [137, 138]. However, the degree of contribution and relevance of each anergic gene and the mechanism of this gene regulation are not understood. It is known that the increased expression of anergic genes is maintained over the long term. However, it seems unlikely that every gene associated with anergy induction would be epigenetically regulated, because there are too many genes with an altered expression level in anergic T cells to be independently regulated [139, 140].

Effective mechanisms of peripheral tolerance are required to eliminate circulating autoreactive T cells and thereby prevent undesired immune responses against self-antigens. The key players in this process are DCs, which induce tolerance by different control mechanisms such as T cell deletion, the generation of Tregs, and/or the induction of anergy [141, 142]. Interaction between DCs and T cells occurs through three independent signals: (i) recognition of peptide-MHC complexes presented on DCs via specific TCR on T lymphocytes, (ii) binding of co-stimulatory molecules expressed on DCs to their respective receptors on T cells, and (iii) polarizing cytokines secreted by DCs [143]. When antigen peptides are presented by DCs in the absence of co-stimulation, T cells become anergic [144].

The induction of T cell anergy occurs when negative signals acquire more weight than the activatory signals from APCs. Anergy was originally defined as an unresponsive state provoked in T cells recognizing an antigen without co-stimulatory signals [145], normally when CD28 on T cells binds to its ligands, that is, B7 molecules, and expresses on DCs [146]. As a result, T cell proliferation and cytokine production are impaired when the same antigen is encountered again. Anergy also results from coinhibitory signals by PD-1 or CTLA-4 receptors [147, 148]. The latter interacts with B7 molecules, with preference toward CD80, whereas PD-1 binds to PD-L2 and/or PD-L1 ligands on DCs. Furthermore, adenosine from tissue, acting by the adenosine A2A receptor (A2AR), acts as another key negative regulator for the activation of T cells, having the ability to drive long-term anergy, even with co-stimulation [149]. Therapies known as the "checkpoint blockade" treat cancer patients using blocking antibodies against those receptors. This approach is clinically quite promising given that blocking antibodies can alleviate hyporesponsiveness and encourage the rejection of tumors. Unraveling this process is the focus in designing therapies to counteract autoreactive T cells involved in autoimmune diseases [150].

Treg cells, important for inducing and/or maintaining anergy and anergic T cells, can in turn alter their epigenetic and transcriptional programs to become Treg cells [151]. Anergic T cells may represent the intermediate reprogramming stage before they themselves become surveying Treg

cells that maintain self-tolerance. T cell anergy and Treg induction are crucial mechanisms for re-establishing tolerance [152], and although presenting different phenotypic and functional characteristics, both mechanisms have in common the expression regulation of some genes, such as *PD-1*, *ICOS*, *LAG3*, *CTLA-4*, *EGR2* [151], *GRAIL* [152, 153], *CBL-B*, and *ITCH* [154, 155].

The suppression of antigen-specific T cell responses either through the expansion of Tregs or the induction of anergy represents an attractive immunotherapeutic approach to target autoreactive T cells in autoimmune diseases [156]. The generation of Tregs has been of interest, but Tregs can exert unspecific regulation and may be prone to conversion into proinflammatory Th17 cells [157]. By contrast, the induction of a stable hyporesponsive state appears to be a promising strategy to specifically silence self-reactive T cells in autoimmune diseases without undesired adverse effects. *In vivo* anergy induction in autoreactive $CD4^+$ T cells has been demonstrated to control disease onset and progression in murine models of autoimmune diseases [158]. The possibility that anergic T cells can also acquire suppressive capacities supports their fundamental role in the control of immune responses. Thus, T cell anergy is an effective mechanism to eradicate aberrant T cell responses to "self" and for the reestablishment of self-tolerance in patients with autoimmune diseases [159, 160].

Acknowledgements

We are very grateful to David Nesbitt for his assistance with the language review.

Funding statement

Supported by Junta de Andalucía throughout the program "Ayudas a grupos de investigación", group BIO-220.

Author details

Maria Isabel Torres[1]*, Miguel Angel López-Casado[2], Candelaria Ponce de León[1], Pedro Lorite[1] and Teresa Palomeque[1]

*Address all correspondence to: mitorres@ujaen.es

1 Department of Experimental Biology, University of Jaén, Jaén, Spain

2 Department Pediatric Gastroenterology, Virgen de las Nieves Hospital, Granada, Spain

References

[1] Sawant DV, Vignali DAA. Once a Treg, always a Treg? Immunological Reviews. 2014; **259**(1):173-191

[2] Sakaguchi S, Sakaguchi N. Thymus and autoimmunity: Capacity of the normal thymus to produce pathogenic self-reactiveTcells and conditions required for their induction of autoinmune disease. The Journal of Experimental Medicine. 1990;**172**:537-545

[3] Seddon B, Mason D. Peripheral autoantigen induces regulatory T cells that prevent autoimmunity. The Journal of Experimental Medicine. 1999;**189**:877-881

[4] Kappler JW, Roehm N, Marrack P. T cell tolerance by clonal elimination in the thymus. Cell. 1987;**49**:273-280

[5] Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self- tolerance maintained by activated T cells expressing IL-2 receptor a-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. Journal of Immunology. 1995;**155**:1151-1164

[6] Ohashi PS, Oehen S, Buerki K, Pircher H, Ohashi CT, Odermatt B, Malissen B, Zinkernagel RM, Henartner H. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell. 1991;**65**:305-317

[7] Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T-cell unresponsiveness in vitro and in vivo. The Journal of Experimental Medicine. 1987;**165**:302-319

[8] Sakaguchi S, Powrie F, Ransohoff RM. Re-establishing immunological self-tolerance in autoimmune disease. Nature Medicine. 2012;**18**:54-58

[9] Shevach EM. Biological functions of regulatory T cells. Advances in Immunology. 2011; **112**:137-176

[10] Vignali DA, Collison LW, Workman CJ. How regulatory T cells work. Nature Reviews. Immunology. 2008;**8**:523-532

[11] Alroqi FJ, Chatila TAT. Regulatory cell biology in health and disease. Current Allergy and Asthma Reports. 2016;**16**(4):27

[12] Curotto de Lafaille MA, Lafaille JJ. Natural and adaptive Foxp3+ regulatory T cells: More of the same or a division of labor? Immunity. 2009;**30**:626-635

[13] Komatsu N, Mariotti-Ferrandiz ME, Wang Y, Malissen B, Waldmann H, Hori S. Heterogeneity of natural Foxp3+ T cells: A committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proceedings of the National Academy of Sciences of the United States of America. 2009;**106**(6):1903-1908

[14] Yadav M, Stephan S, Bluestone JA. Peripherally induced Tregs—Role in immune homeostasis and autoimmunity. Frontiers in Immunology. 2013;**4**:232

[15] Thornton AM, Korty PE, Tran DQ, Wohlfert EA, Murray PE, Belkaid Y, Shevach EM. Expression of Helios, an Ikaros transcription factor family member, differentiates thymic-derived from peripherally induced Foxp3+ T regulatory cells. Journal of Immunology. 2010;**lin 184**:3433-3441

[16] Akimova T, Beier UH, Wang L, Levine MH, Hancock WW. Helios expression is a marker of T cell activation and proliferation. PLoS One. 2011;**6**:e24226

[17] Gottschalk RA, Corse E, Allison JP. Expression of Helios in peripherally induced Foxp3+ regulatory T cells. Journal of Immunology. 2012;**188**:976-980

[18] Zeng H, Zhang R, Jin B, Chen L. Type 1 regulatory T cells: A new mechanism of peripheral immune tolerance. Cellular & Molecular Immunology. 2015;**12**:566-571

[19] Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: The role of IL-2, TGF-beta, and IL-10. Journal of Immunology. 2004;**172**:5213-5522

[20] Gol-Ara M, Jadidi-Niaragh F, Sadria R, Azizi G, Mirshafiey A. The role of different subsets of regulatory T cells in immunopathogenesis of rheumatoid arthritis. Art. 2012;**805875**:1-16

[21] Kosten IJ, Rustemeyer T. Generation, subsets and functions of inducible regulatory T cells. Antiinflamm Antiallergy Agents Med Chem. 2015;**13**:139-153

[22] Sakaguchi S. Naturally arising CD4+ regulatory t cells for immunologic self-tolerance and negative control of immune responses. Annual Review of Immunology. 2004;**22**:531-562

[23] Curotto de Lafaille MA, Lafaille JJ, Graca L. Mechanisms of tolerance and allergic sensitization in the airways and the lungs. Current Opinion in Immunology. 2010;**22**:616-622

[24] Belkaid Y, Tarbell K. Regulatory T cells in the control of host-microorganism interactions. Annual Review of Immunology. 2009;**27**:551-589

[25] Demengeot J, Zelenay S, Moraes-Fontes MF, Caramalho I, Coutinho A. Regulatory T cells in microbial infection. Springer Seminars in Immunopathology. 2006;**28**:41-50

[26] Kendal AR, Waldmann H. Infectious tolerance: Therapeutic potential. Current Opinion in Immunology. 2010;**22**:560-565

[27] Yamaguchi T, Sakaguchi S. Regulatory T cells in immune surveillance and treatment of cancer. Seminars in Cancer Biology. 2006;**16**:115-123

[28] Cipolletta D, Kolodin D, Benoist C, Mathis D. Tisular T (Regs): A unique population of adiposetissue- resident Foxp3+CD4+ T cells that impacts organismal metabolism. Seminars in Immunology. 2011;**23**:431-437

[29] Feuerer M, Herrero L, Cipolletta D, Naaz A, Wong J, Nayer A, Lee J, Goldfine AB, Benoist C, Shoelson S, Mathis D. Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nature Medicine. 2009;**15**:930-939

[30] Vudattu NK, Herold KC. Delayed anti-CD3 therapy in a mouse heart transplant model induced tolerance and long-term survival of allograft: Achieving tolerance. Immunotherapy. 2013;**5**:1173-1176

[31] Rouse BT, Sarangi PP, Suvas S. Regulatory T cells in virus infections. Immunological Reviews. 2006;**212**:272-286

[32] Zou W, Regulatory T. Cells, tumour immunity and immunotherapy. Nature Reviews. Immunology. 2006;**6**:295-307

[33] Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, Jiang S, Kuchroo VK, Mathis D, Roncarolo MG, Rudensky A, Sakaguchi S, Shevach EM, Vignali DA, Ziegler SF. Regulatory T cells: Recommendations to simplify the nomenclature. Nature Immunology. 2013;**14**(4):307-308

[34] Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, Weaver CT. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3− precursor cells in the absence of interleukin 10. Nature Immunology. 2007;**8**(9): 931-941

[35] Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW, Sakaguchi S. Immunologic self-tolerance maintained by CD25(+)CD4(+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. The Journal of Experimental Medicine. 2000;**192**(2):303-310

[36] McHugh RS, Whitters MJ, Piccirillo CA, Young DA, Shevach EM, Collins M, Byrne MC. CD4 (+) CD25 (+) immunoregulatory T cells: Gene expression analysis reveals a functional role for the glucocorticoid-induced TNF receptor. Immunity. 2002;**16**(2):311-323

[37] Lin W, Haribhai D, Relland LM, Truong N, Carlson MR, Williams CB, Chatila TA. Regulatory T cell development in the absence of functional Foxp3. Nature Immunology. 2007;**8**:359-368

[38] Ohkura N, Hamaguchi M, Morikawa H, Sugimura K, Tanaka A, Ito Y, Osaki M, Tanaka Y, Yamashita R, Nakano N, Huehn J, Fehling HJ, Sparwasser T, Nakai K, Sakaguchi S. T cell receptor stimulation-induced epigenetic changes and Foxp3 expression are independent and complementary events required for Treg cell development. Immunity. 2012;**37**: 785-799

[39] Allan SE, Crome SQ, Crellin NK, Passerini L, Steiner TS, Bacchetta R, Roncarolo MG, Levings MK. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. International Immunology. 2007;**19**:345-354

[40] D'Hennezel E, Yurchenko E, Sgouroudis E, Hay V, Piccirillo CA. Single-cell analysis of the human T regulatory population uncovers functional heterogeneity and instability within FOXP3+ cells. Journal of Immunology. 2011;**186**:6788-6797

[41] Huehn J, Siegmund K, Lehmann JC, Siewert C, Haubold U, Feuerer M, Debes GF, Lauber J, Frey O, Przybylski GK, Niesner U, de la Rosa M, Schmidt CA, Bräuer R, Buer J, Scheffold A, Hamann A. Developmental stage, phenotype, and migration distinguish naive- and effector/ memory-like CD4+ regulatory T cells. The Journal of Experimental Medicine 2004;**199**:303-313

[42] Campbell DJ, Koch MA. Phenotypical and functional specialization of FOXP3+ regulatory T cells. Nature Reviews. Immunology. 2011;**11**:119-130

[43] Feuerer M, Hill JA, Mathis D, Benoist C. Foxp3+ regulatory T cells: Differentiation, specification, subphenotypes. Nature Immunology. 2009;**10**:689-695

[44] Koch MA, Tucker-Heard G, Perdue NR, Killebrew JR, Urdahl KB, Campbell DJ. The transcription factor T-beta controls regulatory T cell homeostasis and function during type 1 inflammation. Nature Immunology. 2009;**10**:595-602

[45] Zheng Y, Chaudhry A, Kas A, deRoos P, Kim JM, Chu TT, Corcoran L, Treuting P, Klein U, Rudensky AY. Regulatory T-cell suppressor program co-opts transcription factor IRF4 to control T (H) 2 responses. Nature. 2009;**458**:351-356

[46] Chaudhry A, Rudra D, Treuting P, Samstein RM, Liang Y, Kas A, Rudensky AY. CD4+ regulatory T cells control TH17 responses in a Stat3-dependent manner. Science. 2009; **326**:986-991

[47] Cipolletta D, Feuerer M, Li A, Kamei N, Lee J, Shoelson SE, Benoist C, Mathis D. PPAR-gamma is a major driver of the accumulation and phenotype of adipose tissue Treg cells. Nature. 2012;**486**:549-553

[48] Bettini M, Vignali DA. Regulatory T cells and inhibitory cytokines in autoimmunity. Current Opinion in Immunology. 2009;**21**:612-618

[49] Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR Jr, Muller W, Rudensky AY. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008;**28**:546-558

[50] Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, Ley TJ. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. Immunity. 2007;**27**:635-646

[51] Zhou X, Bailey-Bucktrout SL, Jeker LT, Penaranda C, Martínez-Llordella M, Ashby M, Nakayama M, Rosenthal W, Bluestone JA. Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. Nature Immunology. 2009; **10**:1000-1007

[52] Rubtsov YP, Niec RE, Josefowicz S, Li L, Darce J, Mathis D, Benoist C, Rudensky AY. Stability of the regulatory T cell lineage in vivo. Science. 2010;**329**:1667-1671

[53] Miyao T, Floess S, Setoguchi R, Luche H, Fehling HJ, Waldmann H, Huehn J, Hori S. Plasticity of Foxp3 (+) T cells reflects promiscuous Foxp3 expression in conventional T cells but not reprogramming of regulatory T cells. Immunity. 2012;**36**:262-275

[54] Floess S, Freyer J, Siewert C, Baron U, Olek S, Polansky J, Schlawe K, Chang HD, Bopp T, Schmitt E, Klein-Hessling S, Serfling E, Hamann A, Huehn J. Epigenetic control of the foxp3 locus in regulatory T cells. PLoS Biology. 2007;**5**:e38

[55] Gavin MA, Rasmussen JP, Fontenot JD, Vasta V, Manganiello VC, Beavo JA, Rudensky AY. Foxp3-dependent programme of regulatory T-cell differentiation. Nature. 2007;**445**: 771-775

[56] Yang XO, Nurieva R, Martinez GJ, Kang HS, Chung Y, Pappu BP, Shah B, Chang SH, Schluns KS, Watowich SS, Feng XH, Jetten AM, Dong C. Molecular antagonism and plasticity of regulatory and inflammatory T cell programs. Immunity. 2008;**29**:44-56

[57] Barnes MJ, Powrie F. Hybrid Treg cells: Steel frames and plastic exteriors. Nature Immunology. 2009;**10**:563-564

[58] Wing JB, Sakaguchi S. Multiple Treg suppressive modules and their adaptability. Frontiers in Immunology. 2012;**3**:178

[59] Gratz IK, Rosenblum MD, Abbas AK. The life of regulatory T cells. Annals of the New York Academy of Sciences. 2013;**1283**:8-12

[60] Gratz IK, Rosenblum MD, Maurano MM, Paw JS, Truong HA, Marshak-Rothstein A, Abbas AK. Cutting edge: Self-antigen controls the balance between effector and regulatory T cells in peripheral tissues. Journal of Immunology. 2014;**192**(4):1351-1355

[61] Malek TR. The biology of interleukin-2. Annual Review of Immunology. 2008;**26**:453-479

[62] Barron L, Dooms H, Hoyer KK, Kuswanto W, Hofmann J, O'Gorman WE, Abbas AK. Cutting edge: Mechanisms of IL-2-dependent maintenance of functional regulatory T cells. Journal of Immunology. 2010;**185**:6426-6430

[63] Koreth J, Matsuoka K, Kim HT, McDonough SM, Bindra B, Alyea EP 3rd, Armand P, Cutler C, Ho VT, Treister NS, Bienfang DC, Prasad S, Tzachanis D, Joyce RM, Avigan DE, Antin JH, Ritz J, Soiffer RJ. Interleukin-2 and regulatory T cells in graft-versus-host disease. The New England Journal of Medicine. 2011;**365**:2055-2066

[64] Berin MC, Mayer L. Can we produce true tolerance in patients with food allergy? The Journal of Allergy and Clinical Immunology. 2013;**131**:14-22

[65] Liu AH, Jaramillo R, Sicherer SH, Wood RA, Bock SA, Burks AW, Massing M, Cohn RD, Zeldin DC. National prevalence and risk factors for food allergy and relationship to asthma: Results from the National Health and nutrition examination survey 2005–2006. The Journal of Allergy and Clinical Immunology. 2010;**126**:798-806

[66] Sicherer SH, Wood RA, Stablein D, Burks AW, Liu AH, Jones SM, Fleischer DM, Leung DY, Grishin A, Mayer L, Shreffler W, Lindblad R, Sampson HA. Immunologic features of infants with milk or egg allergy enrolled in an observational study (consortium of food allergy research) of food allergy. The Journal of Allergy and Clinical Immunology. 2010; **125**:1077-1083

[67] Chatila TA, Blaeser F, Ho N, Lederman HM, Voulgaropoulos C, Helms C, Bowcock AM. JM2, encoding a fork head-related protein, is mutated in X-linked autoimmunity-allergic disregulation syndrome. The Journal of Clinical Investigation. 2000;**106**:R75-R81

[68] Jones SM, Burks AW, Dupont C. State of the art on food allergen immunotherapy: Oral, sublingual, and epicutaneous. The Journal of Allergy and Clinical Immunology. 2014; **133**:318-323

[69] Torgerson TR, Linane A, Moes N, Anover S, Mateo V, Rieux-Laucat F, Hermine O, Vijay S, Gambineri E, Cerf-Bensussan N, Fischer A, Ochs HD, Goulet O, Ruemmele FM. Severe food allergy as a variant of IPEX syndrome caused by a deletion in a noncoding region of the FOXP3 gene. Gastroenterology. 2007;**132**:1705-1717

[70] Apostolou I, Von Boehmer H. In vivo instruction of suppressor commitment in naive T cells. The Journal of Experimental Medicine. 2004;**199**:1401-1408

[71] Haribhai D, Lin W, Edwards B, Ziegelbauer J, Salzman NH, Carlson MR, Li SH, Simpson PM, Chatila TA, Williams CB. A central role for induced regulatory T cells in tolerance induction in experimental colitis. Journal of Immunology. 2009;**182**:3461-3468

[72] Mucida D, Kutchukhidze N, Erazo A, Russo M, Lafaille JJ, Curotto de Lafaille MA. Oral tolerance in the absence of naturally occurring Tregs. The Journal of Clinical Investigation. 2005;**115**:1923-1933

[73] Curotto de Lafaille MA, Kutchukhidze N, Shen S, Ding Y, Yee H, Lafaille JJ. Adaptive Foxp3+ regulatory T cell-dependent and -independent control of allergic inflammation. Immunity. 2008;**29**:114-126

[74] Josefowicz SZ, Niec RE, Kim HY, Treuting P, Chinen T, Zheng Y, Umetsu DT, Rudensky AY. Extrathymically generated regulatory T cells control mucosal TH2 inflammation. Nature. 2012;**482**:395-399

[75] Niess JH, Leithauser F, Adler G, Reimann J. Commensal gut flora drives the expansion of proinflammatory CD4 T cells in the colonic lamina propria under normal and inflammatory conditions. Journal of Immunology. 2008;**180**:559-568

[76] Hall JA, Bouladoux N, Sun CM, Wohlfert EA, Blank RB, Zhu Q, Grigg ME, Berzofsky JA, Belkaid Y. Commensal DNA limits regulatory T cell conversion and is a natural adjuvant of intestinal immune responses. Immunity. 2008;**29**:637-649

[77] Jiani N, Chai L, You W, Zhou L, Chyi-Song H. T cells and intestinal commensal bacteria-ignorance, rejection, and acceptance. FEBS Letters. 2014;**588**(22):4167-4175

[78] Grainger JR, Askenase MH, Guimont-Desrochers F, da Fonseca DM, Belkaid Y. Contextual functions of antigen-presenting cells in the gastrointestinal tract. Immunological Reviews 2014;**259**:75-87

[79] Kinnebrew MA, Buffie CG, Diehl GE, Zenewicz LA, Leiner I, Hohl TM, Flavell RA, Littman DR, Pamer EG. Interleukin 23 production by intestinal CD103 (+) CD11b (+) dendritic cells in response to bacterial flagellin enhances mucosal innate immune defense. Immunity. 2012;**36**:276-287

[80] Bouladoux N, Hall JA, Grainger JR, dos Santos LM, Kann MG, Nagarajan V, Verthelyi D, Belkaid Y. Regulatory role of suppressive motifs from commensal DNA. Mucosal Immunology 2012;**5**:623-634

[81] Welty NE, Staley C, Ghilardi N, Sadowsky MJ, Igyarto BZ, Kaplan DH. Intestinal lamina propria dendritic cells maintain T cell homeostasis but do not affect commensalism. The Journal of Experimental Medicine. 2013;**210**:2011-2024

[82] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell. 2014; **157**:121-141

[83] Palm NW, Medzhitov R. Pattern recognition receptors and control of adaptive immunity. Immunological Reviews. 2009;**227**:221-233

[84] Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. Science. 2011;**332**:974-977

[85] Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, Glickman JN, Garrett WS. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. Science 2013;**341**:569-573

[86] Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 2013;**504**:451-455

[87] Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013;**504**:446-450

[88] Geuking MB, Cahenzli J, Lawson MA, Ng DC, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity. 2011;**34**:794-806

[89] Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio C-W, Santacruz N, Peterson DA, Stappenbeck TS. Hsieh CH-S. Peripheral education of the immune system by colonic commensal microbiota. Nature. 2012;**478**(7368):250-254

[90] Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. Nature Reviews. Gastroenterology & Hepatology. 2012;**9**:577-589

[91] Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity. Annual Review of Medicine. 2011;**62**:361-380

[92] Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends in Microbiology. 2011;**19**:349-359

[93] Honda K, Littman DR. The microbiome in infectious disease and inflammation. Annual Review of Immunology. 2012;**30**:759-795

[94] Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. Science. 2012;**336**:1268-1273

[95] Molloy MJ, Bouladoux N, Belkaid Y. Intestinal microbiota: Shaping local and systemic immune responses. Seminars in Immunology. 2012;**24**:58-66

[96] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell. 2008;**133**:775e87

[97] Buckner JH. Mechanisms of impaired regulation by CD4(þ) CD25(þ)FOXP3(þ) regulatory T cells in human autoinmune diseases. Nature Reviews. Immunology. 2010;**10**:849e59

[98] Dhubana KB. Piccirillo. The immunological and genetic basis of immune dysregulation, polyendocrinopathy, enteropathy, X-linked síndrome. Current Opinion in Allergy and Clinical Immunology. 2015;**15**:525-532

[99] Torgerson TR, Ochs HD. Immune dysregulation, polyendocrinopathy, enteropathy, X-linked: Forkhead box protein 3 mutations and lack of regulatory T cells. The Journal of Allergy and Clinical Immunology. 2007;**120**(4):744-750

[100] Gambineri E, Perroni L, Passerini L, Bianchi L, Doglioni C, Meschi F, Bonfanti R, Sznajer Y, Tommasini A, Lawitschka A, Junker A, Dunstheimer D, Heidemann PH, Cazzola G, Cipolli M, Friedrich W, Janic D, Azzi N, Richmond E, Vignola S, Barabino A, Chiumello G, Azzari C, Roncarolo MG, Bacchetta R. Clinical and molecular profile of a new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: Inconsistent correlation between forkhead box protein 3 expression and disease severity. The Journal of Allergy and Clinical Immunology. 2008;**122**(6):1105-1112

[101] Caudy AA, Reddy ST, Chatila T, Atkinson JP, Verbsky JW. CD25 deficiency causes an immune dysregulation, polyendocrinopathy, enteropathy, X-linked-like syndrome, and defective IL-10 expression from CD4 lymphocytes. The Journal of Allergy and Clinical Immunology. 2007;**119**(2):482-487

[102] Goudy K, Aydin D, Barzaghi F, Gambineri E, Vignoli M, Ciullini Mannurita S, Doglioni C, Ponzoni M, Cicalese MP, Assanelli A, Tommasini A, Brigida I, Dellepiane RM, Martino S, Olek S, Aiuti A, Ciceri F, Roncarolo MG, Bacchetta R. Human IL2RA null mutation mediates immunodeficiency with lymphoproliferation and autoimmunity. Clinical Immunology. 2013;**146**(3):248-261

[103] Bezrodnik L, Caldirola MS, Seminario AG, Moreira I, Gaillard MI. Follicular bronchiolitis as phenotype associated with CD25 deficiency. Clinical and Experimental Immunology. 2014;**175**(2):227-234

[104] Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nature Immunology. 2005;**6**(**11**):1142-1151

[105] Maloy KJ, Powrie F. Fueling regulation: IL-2 keeps CD4+ Treg cells fit. Nature Immunology. 2005;**6**(11):1071-1072

[106] Roncarolo MG, Gregori S, Battaglia M, Bacchetta R, Fleischhauer K, Levings MK. Interleukin-10-secreting type1regulatory T cells in rodent sand humans. Immunological Reviews. 2006;**212**:28-50

[107] Sakaguchi S, Regulatory T. cells. Springer Seminars in Immunopathology. 2006;**28**:1-2

[108] Passerini L, Olek S, DiNunzio S, Barzaghi F, Hambleton S, Abinun M, Tommasini A, Vignola S, Cipolli M, Amendola M, Naldini L, Guidi L, Cecconi M, Roncarolo MG, Bacchetta R. Fork head box protein 3 (FOXP3) mutations lead to increased TH17 cell numbers and regulatoryT-cell instability. The Journal of Allergy and Clinical Immunology. 2011b;**128**:1376-1379

[109] Crespo J, Sun H, Welling TH, Tian Z, Zou W. T cell anergy, exhaustion, senescence, and stemness in the tumor microenvironment. Current Opinion in Immunology. 2013;**25**(2): 214-221

[110] Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nature Reviews. Immunology. 2015;**15**(8):486-499

[111] Lang KS, Recher M, Navarini AA, Harris NL, Löhning M, Junt T, Probst HC, Hengartner H, Zinkernagel RM. Inverse correlation between IL-7 receptor expression and CD8 T cell exhaustion during persistent antigen stimulation. European Journal of Immunology. 2005;**35**(3):738-745

[112] Wherry EJ. T cell exhaustion. Nature Immunology. 2011;**12**(6):492-499

[113] Kahan SM, Wherry EJ, Zajac AJ. T cell exhaustion during persistent viral infections. Virology. 2015;**479**:180-193

[114] Speiser DE, Utzschneider DT, Oberle SG, Munz C, Romero P, Zehn D. T cell differentiation in chronic infection and cancer: Functional adaptation or exhaustion? Nature Reviews. Immunology. 2014;**14**(11):768-774

[115] Ahmadzadeh M, Johnson LA, Heemskerk B, Wunderlich JR, Dudley ME, White DE, Rosenberg SA. Tumor antigen-specific CD8 T cells infiltrating the tumor express high levels of PD-1 and are functionally impaired. Blood. 2009;**114**(8):1537-1544

[116] Zajac AJ, Blattman JN, Murali-Krishna K, Sourdive DJ, Suresh M, Altman JD, Ahmed R. Viral immune evasion due to persistence of activated T cells without effector function. The Journal of Experimental Medicine. 1998;**188**(12):2205-2213

[117] Crawford A, Angelosanto JM, Kao C, Doering TA, Odorizzi PM, Barnett BE, Wherry EJ. Molecular and transcriptional basis of CD4+ T cell dysfunction during chronic infection. Immunity. 2014;**40**:289-302

[118] Bucks CM, Norton JA, Boesteanu AC, Mueller YM, Katsikis PD. Chronic antigen stimulation alone is sufficient to drive CD8+ T cell exhaustion. Journal of Immunology. 2009;**182**: 6697-6708

[119] Blackburn SD, Shin H, Haining WN, Zou T, Workman CJ, Polley A, Betts MR, Freeman GJ, Vignali DA, Wherry EJ. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. Nature Immunology. 2009;**10**:29-37

[120] Baitsch L, Baumgaertner P, Devêvre E, Raghav SK, Legat A, Barba L, Wieckowski S, Bouzourene H, Deplancke B, Romero P, Rufer N, Speiser DE. Exhaustion of tumor-specific CD8(+) T cells in metastases from melanoma patients. The Journal of Clinical Investigation. 2011;**121**(6):2350-2360

[121] Gros A, Robbins PF, Yao X, Li YF, Turcotte S, Tran E, Wunderlich JR, Mixon A, Farid S, Dudley ME, Hanada K, Almeida JR, Darko S, Douek DC, Yang JC, Rosenberg SA. PD-1 identifies the patient-specific CD8(+) tumor-reactive repertoire infiltrating human tumors. The Journal of Clinical Investigation. 2014;**124**(5):2246-2259

[122] Im SJ, Hashimoto M, Gerner MY, Lee J, Kissick HT, Burger MC, Shan Q, Hale JS, Lee J, Nasti TH, Sharpe AH, Freeman GJ, Germain RN, Nakaya HI, Xue HH, Ahmed R. Defining CD8+ T cells that provide the proliferative burst after PD-1 therapy. Nature. 2016;**537**:417-421

[123] He R, Hou S, Liu C, Zhang A, Bai Q, Han M, Yang Y, Wei G, Shen T, Yang X, Xu L, Chen X, Hao Y, Wang P, Zhu C, Ou J, Liang H, Ni T, Zhang X, Zhou X, Deng K, Chen Y, Luo Y, Xu J, Qi H, Wu Y, Ye L. Follicular CXCR5-expressing CD8+ T cells curtail chronic viral infection. Nature. 2016;**537**:412-428

[124] Brooks DG, McGavern DB, Oldstone MB. Reprogramming of antiviral T cells prevents inactivation and restores T cell activity during persistent viral infection. The Journal of Clinical Investigation. 2006;**116**(6):1675-1685

[125] Petrovas C, Price DA, Mattapallil J, Ambrozak DR, Geldmacher C, Cecchinato V, Vaccari M, Tryniszewska E, Gostick E, Roederer M, Douek DC, Morgan SH, Davis SJ, Franchini G, Koup RA. SIV-specific CD8+ T cells express high levels of PD1 and cytokines but have impaired proliferative capacity in acute and chronic SIVmac251 infection. Blood. 2007; **110**(3):928-936

[126] Yamamoto T, Price DA, Casazza JP, Ferrari G, Nason M, Chattopadhyay PK, Roederer M, Gostick E, Katsikis PD, Douek DC, Haubrich R, Petrovas C, Koup RA. Surface expression patterns of negative regulatory molecules identify determinants of virus-specific CD8+ T-cell exhaustion in HIV infection. Blood. 2011;**117**(18):4805-4815

[127] Woo SR, Turnis ME, Goldberg MV, Bankoti J, Selby M, Nirschl CJ, Bettini ML, Gravano DM, Vogel P, Liu CL, Tangsombatvisit S, Grosso JF, Netto G, Smeltzer MP, Chaux A, Utz PJ, Workman CJ, Pardoll DM, Korman AJ, Drake CG, Vignali DA. Immune inhibitory molecules LAG-3 and PD-1 synergistically regulate T-cell function to promote tumoral immune escape. Cancer Research. 2012;**72**(4):917-927

[128] McKinney EF, Lee JC, Jayne DR, Lyons PA, Smith KG. T-cell exhaustion, co-stimulation and clinical outcome in autoimmunity and infection. Nature. 2015;**523**:612-616

[129] Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence in vivo. Nature Medicine. 2006;**12**:1301-1309

[130] Ejrnaes M, Filippi CM, Martinic MM, Ling EM, Togher LM, Crotty S, von Herrath MG. Resolution of a chronic viral infection after interleukin-10 receptor blockade. The Journal of Experimental Medicine 2006;**203**:2461-2472

[131] Ng CT, Oldstone MB. Infected CD8α- Dendritic cells are the predominant source of IL-10 during establishment of persistent viral infection. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:14116-14121 [PubMed: 22893686]

[132] Said EA, Dupuy FP, Trautmann L, Zhang Y, Shi Y, El-Far M, Hill BJ, Noto A, Ancuta P, Peretz Y, Fonseca SG, Van Grevenynghe J, Boulassel MR, Bruneau J, Shoukry NH, Routy JP, Douek DC, Haddad EK, Sekaly RP. Programmed death-1-induced interleukin-10 production by monocytes impairs CD4+ T cell activation during HIV infection. Nature Medicine. 2010;**16**:452-459

[133] Brooks DG, Ha SJ, Elsaesser H, Sharpe AH, Freeman GJ, Oldstone MB. IL-10 and PD-L1 operate through distinct pathways to suppress T-cell activity during persistent viral infection. Proceedings of the National Academy of Sciences of the United States of America. 2008;**105**:20428-20433

[134] Yi JS, Du M, Zajac AJ. A vital role for interleukin-21 in the control of a chronic viral infection. Science. 2009;**324**:1572-1576

[135] Sprent J. Central tolerance of T cells. International Reviews of Immunology. 1995;**13**:95-105

[136] Salojin KV, Zhang J, Madrenas J, Delovitch TL. T-cell anergy and altered T cell receptor signaling: Effects on autoimmune disease. Immunology Today. 1998;**19**:468-473

[137] Zha Y, Marks R, Ho AW, Peterson AC, Janardhan S, Brown I, Praveenv K, Stang S, Stone JC, Gajewski TF. T cell anergy is reversed by active Ras and is regulated by diacylglycerol kinase-a. Nature Immunology. 2006;**7**:1166-1173

[138] Harris JE, Bishop KD, Phillips NE, Mordes JP, Greiner DL, Rossini AA, Czech MP. Early growth response gene-2, a zinc-finger transcription factor, is required for full induction of clonal anergy in CD4+ T cells. Journal of Immunology. 2004;**173**:7331-7338

[139] Anandasabapathy N, Ford GS, Bloom D, Holness C, Paragas V, Seroogy C, Skrenta H, Hollenhorst M, Fathman CG, Soares LGRAIL. An E3 ubiquitin ligase that inhibits cytokine gene transcription is expressed in anergic CD4+ T cells. Immunity. 2003;**18**:535-547

[140] Seroogy SM, Soares L, Ranheim EA, Su L, Holness C, Bloom D, Fathman CG. The gene related to anergy in lymphocytes, an E3 ubiquitin ligase, is necessary for anergy induction in CD4 T cells. Journal of Immunology. 2004;**173**:79-85

[141] Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nature Immunology. 2010;**11**(1):7-13

[142] Steinman R, Hawiger D, Nussenzweig M. Tolerogenic dendritic cells. Annual Review of Immunology. 2003;**21**:685-711

[143] Van Gisbergen K, Paessens L, Geijtenbeek T, van Kooyk Y. Molecular mechanisms that set the stage for DC-T cell engagement. Immunology Letters 2005;**97**(2):199-208

[144] Schwartz R. Models of T cell anergy: Is there a common molecular mechanism? The Journal of Experimental Medicine. 1996;**184**(1):1-8

[145] Schwartz RH. T cell anergy. Annual Review of Immunology. 2003;**21**:305-334

[146] Bour-Jordan H, Esensten J, Martinez-Llordella M, Penaranda C, Stumpf M, Bluestone J. Intrinsic and extrinsic control of peripheral T-cell tolerance by costimulatory molecules of the CD28/B7 family. Immunological Reviews. 2011;**241**(1):180-205

[147] Fife B, Bluestone J. Control of peripheral T-cell tolerance and autoimmunity via the CTLA-4 and PD-1 pathways. Immunological Reviews. 2008;**224**:166-182

[148] Greenwald R, Boussiotis V, Lorsbach R, Abbas A, Sharpe A. CTLA-4 regulates induction of anergy in vivo. Immunity. 2001;**14**(2):145-155

[149] Zarek P, Huang CT, Lutz ER, Kowalski J, Horton MR, Linden J, Drake CG, Powell JD. A2A receptor signaling promotes peripheral tolerance by inducing T-cell anergy and the generation of adaptive regulatory T cells. Blood 2008;**111**(1):251-259

[150] Lokesh A, Kalekar LA, Mueller DL. Relationship between CD4 regulatory T cells and Anergy in vivo. Journal of Immunology. 2017;**198**:2527-2533

[151] Kalekar LA, Schmiel SE, Nandiwada SL, Lam WY, Barsness LO, Zhang N, Stritesky GL, Malhotra D, Pauken KE, Linehan JL, O'Sullivan MG, Fife BT, Hogquist KA, Jenkins MK, Mueller DL. CD4 (+) T cell anergy prevents autoimmunity and generates regulatory T cell precursors. Nature Immunology. 2016;**17**:304-314

[152] Knoechel B, Lohr J, Zhu S, Wong L, Hu D, Ausubel L, Abbas AK. Functional and molecular comparison of anergic and regulatory T lymphocytes. Journal of Immunology. 2006;**176**(11):6473-6483

[153] Lechner O, Lauber J, Franzke A, Sarukhan A, von Boehmer H, Buer J. Fingerprints of anergic T cells. Current Biology 2001;**11**(8):587-595

[154] MacKenzie D, Schartner J, Lin J, Timmel A, Jennens-Clough M, Fathman CG, Seroogy CM. GRAIL is up-regulated in CD4+ CD25+ T regulatory cells and is sufficient for conversion of T cells to a regulatory phenotype. The Journal of Biological Chemistry. 2007;**282**(13): 9696-9702

[155] Venuprasad K. CBL-B and ITCH: Key regulators of peripheral T-cell tolerance. Cancer Research. 2010;**70**(8):3009-3012

[156] Dejaco C, Duftner C, Grubeck-Loebenstein B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. Immunology. 2006;**117**(3):289-300

[157] Osorio F, LeibundGut-Landmann S, Lochner M, Lahl K, Sparwasser T, Eberl G, Reis e Sousa C. DC activated via dectin-1 convert Treg into IL-17 producers. European Journal of Immunology. 2008;**38**(12):3274-3281

[158] Yin B, Ma G, Yen C-Y, Zhou Z, Wang GX, Divino CM, Casares S, Chen SH, Yang WC, Pan PY. Myeloid-derived suppressor cells prevent type 1 diabetes in murine models. Journal of Immunology. 2010;**185**(10):5828-5834

[159] Zappia E, Casazza S, Pedemonte E, Benvenuto F, Bonanni I, Gerdoni E, Giunti D, Ceravolo A, Cazzanti F, Frassoni F, Mancardi G, Uccelli A. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. Blood. 2005; **106**(5):1755-1761

[160] Ferreira GB, Gysemans CA, Demengeot J, da Cunha JP, Vanherwegen AS, Overbergh L, Van Belle TL, Pauwels F, Verstuyf A, Korf H, Mathieu C. 1, 25-Dihydroxyvitamin D3 promotes tolerogenic dendritic cells with functional migratory properties in NOD mice. Journal of Immunology 2014;**192**(9):4210-4220