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

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## 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

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## 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

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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 of 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.

### 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> 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- $\beta$  secretion [19], and Th3 cells with a variable level of FOXP3 expression [20]. CD8<sup>+</sup>CD25<sup>+</sup> Treg cells are also developed in the thymus, expressing several molecules characteristic of nTregs, namely CD25, FOXP3, CTLA-4, and TNF-receptor. CD8<sup>+</sup>CD28<sup>+</sup> Tregs inhibit priming of CD8<sup>+</sup> and CD4<sup>+</sup> T cells, and antibody-mediated against oral antigens. CD8<sup>+</sup>CD28<sup>-</sup>Tregs can be induced from naïve CD8<sup>+</sup> 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  $\gamma\delta$  T cells are commonly of the CD8<sup>+</sup> + FOXP3<sup>-</sup> 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- $\beta$ , 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<sup>+</sup> Treg cells express on the cell surface high levels of interleukin-2 receptor  $\alpha$  (CD25) and a low level of IL-7 receptor  $\alpha$  (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- $\beta$  [34–36].

According to a number of studies, not all FOXP3<sup>+</sup> 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<sup>+</sup> 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<sup>+</sup> T cells (CD4<sup>+</sup> Tconv), which are activated in presence of TGF- $\beta$ 1 and CD103<sup>+</sup> 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 the CD25, 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<sup>+</sup> 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 as 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<sup>+</sup> and CD8<sup>+</sup> T cells bear a notably different transcriptional profile from that of effector and memory CD4<sup>+</sup> or CD8<sup>+</sup> 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<sup>+</sup> T cell exhaustion likely involves altered inflammatory and tissue microenvironments as well as other populations of



lymphocytes such as CD4<sup>+</sup> 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- $\alpha$  and IFN- $\gamma$  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- $\beta$ , 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<sup>+</sup> T cells [130, 131]. The blocking of IL-10 and the PD1 pathway in a simultaneous manner, synergistically reverses CD8<sup>+</sup> T cell exhaustion and enhances viral control, indicating a role for IL-10 in controlling CD8<sup>+</sup> T cell exhaustion [132]. Depletion of CD4<sup>+</sup> T cells help during pathogen persistence and can contribute to defective CD8<sup>+</sup> T cell responses. Therefore, in HIV

infection, the loss of the CD4<sup>+</sup> T cell response can result in exhausted CD8<sup>+</sup> 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- $\alpha$ , 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<sup>+</sup> 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].

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