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Regulatory T cells and their roles in immune dysregulation and allergy

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Abstract

The main function of the immune system is to fight off potential infections, but also to maintain its activity below a level that would trigger self-reactivity. Regulatory T cells (Tregs) such as forkhead box P3$^+$ (FOXP3) Tregs and type 1 regulatory T cells (Tr1) play an essential role in this active process, using several distinct suppressive mechanisms. A wide range of pathologies have been associated with altered Treg cell function. This is best exemplified by the impact of mutations of genes essential for Treg function and the associated autoimmune syndromes. This review summarizes the main features of different subtypes of Tregs and focuses on the clinical implications of their altered function in human studies. More specifically, we discuss abnormalities affecting FOXP3$^+$ Tregs and Tr1 cells that will lead to autoimmune manifestations and/or allergic reactions, and the potential therapeutic use of Tregs.

Keywords

Regulatory T cells; Type 1 regulatory T cells; FOXP3; Allergy; Immune dysregulation

Introduction

The ability of the immune system to control the inflammatory response and prevent reactions against self-antigens (Ags) or harmless environmental molecules is mainly acquired during fetal life and early after birth. Selection and acquisition of both effector and regulatory functions occur in the thymus for T cells and in the bone marrow for B cells and ensure that the naïve or Ag inexperienced newborn repertoire is devoid of the large majority of dangerous auto-reactive T and B cells that could cause harm. Throughout life, peripheral mechanisms of regulation control auto-reactive cells that escaped initial checkpoints and shut down undesired immune responses toward commensal and environmental Ags [1]. Once thought merely as a lack of response, it is now common knowledge that peripheral tolerance consists of active mechanisms in which specialized regulatory T cells (Treg) play a major role. In the last decade, a large body of evidence has unraveled novel features of Treg cells, and it has become possible to isolate them ex vivo or manipulate them in vitro for clinical purposes. This said, several questions remain to be addressed, especially in relation

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to their role in disease and their precise monitoring in vivo in humans. This review focuses on $CD4^+$ Treg cells and summarizes their main characteristics, highlighting important issues that are critical to study their role in pathologies associated with loss of tolerance, such as in immune dysregulation of genetic origin leading to autoimmunity and in allergies.

Different types of human regulatory T cells

$FOXP3^+$ Tregs

A consensus nomenclature has been recently introduced by Abbas and collaborators in order to facilitate understanding [2]. According to this work, Forkhead box P3 $(FOXP3)^+$ Treg cells can be defined as follows: (1) “thymus-derived Treg cells”(tTregs) of thymic origin also known as thymic-derived/’ “natural” Tregs; (2) “peripherally derived Treg cells” (pTregs) which differentiate in the periphery, (3) “$FOXP3^+$ Treg cell” should be used when the origin of the subset is unclear; and (4) “in vitro-induced Treg cells” should be used for all Treg cell populations generated ex vivo.

Thymic-derived Tregs constitute the best studied population of $CD4^+$ Treg cells. Their existence was proven over a decade ago by Sakaguchi and collaborators who showed that tTregs have suppressive properties toward the development of autoimmune manifestations in mice [3]. They can be identified as expressing high levels of the interleukin 2 (IL-2) receptor alpha-chain (CD25), the co-inhibitory receptor cytotoxic T lymphocyte Ag 4 (CTLA4) and the transcription factor FOXP3 [3–6] which is essential for their function (Fig. 1a). In addition, they express low levels of the IL-7 receptor, CD127 [7], and mainly have a naïve CD45RA phenotype, although Ag-experienced memory tTregs are also detectable [8].

tTregs exert their function in different tissues, at sites of inflammation and in close contact with T effector (Teff) cells. Tregs use the same homing molecules used by naïve and Teff cells and thus home to sites of Teff generation and function [9]. More specifically, CCR7 has been shown to be important for Treg cell homing and function to the lymphoid compartment during the initiation of immune responses [10], while CCR4, CCR5, and CXCR3 are more relevant for Treg cell recruitment and function to peripheral sites of inflammation [11].

The generation of tTregs appears to be dependent on the strength of the binding between TCR and MHC class II peptides in mice [12]. In their work, Hsieh and colleagues cloned Tregs’ TCR into RAG deficient T cells, and subsequently demonstrated that the TCR-peptide avidity required for the generation of tTregs was higher than for the generation of Teff cells. This finding was confirmed in a more recent study [13]. Reducing the strength of the TCR–MHC interaction in mice leads to a decreased negative selection in the thymus, but also to an increase in the numbers of tTregs. This result suggests that the strength of interaction which induces Tregs would fall between that which induces clonal deletion and that which induces a conventional response [14].

$FOXP3^+$ Tregs proliferate very poorly in vitro and do not produce cytokines, with the exception of low transforming growth factor-β (TGF-β) and IL-35 [15]. They are, however, very responsive to IL-2, which acts through its receptor and activation of STAT5. Indeed,

IL-2 is the main factor for Treg survival and maintenance in vivo, and it is required at high doses for their in vitro expansion [16]. Other factors influence tTregs, such as TGF-β [17], thymic stromal lymphopoietin (TSLP) [18], and costimulatory molecules such as CD28 [19].

FOXP3 is a potent repressor of IL-2 production, but upregulates the expression of its receptor (CD25) and of the Treg marker CTLA4 [20]. Interestingly, FOXP3 also induces the expression of anti-inflammatory cytokine IL-10, as described in human tumor-associated Tregs, via a mechanism occurring in cooperation with the transcription factor STAT3 [21]. These data suggest that FOXP3 supports the maintenance of an immunosuppressive milieu. For a long time, FOXP3 was considered to be a master regulator for the development and function of Tregs because its absence in specific KO mice or in the natural mouse mutant, the scurfy mouse, is responsible for massive lymphoproliferation [22] and for a severe autoimmune syndrome. Mutations of FOXP3 lead to a similar phenotype in men [23] (Fig. 1b and see below, “tTregs in immune dysregulation” section). However, the establishment of a mice strain carrying a defective *FoxP3* allele (knocked down by the insertion of a GFP cassette) showed that this gene is essential for the function but not for the development of tTregs. Rather, FOXP3 would potentiate pre-established Treg features, such as responsiveness to IL-2 [24]. Ectopic stable overexpression of FOXP3 by a lentivirus-based method in $CD4^+$ T cells allows the generation of a stable population of human Treg-like cells, starting with nave and memory $CD4^+$ T cells, which are potent suppressor cells [26, 27]. In humans, Treg differentiation is characterized by specific demethylation of over a hundred of loci which become accessible for FOXP3 binding once it is expressed [25]. Importantly, the CpG methylation status of DNA regulates *FoxP3* expression and accesses to its targets [28]. The regulation of FOXP3 expression occurs at both the transcriptional and translational levels. Three conserved non-coding sequences (CNS) have been identified at the *FOXP3* locus [29, 30]. Their epigenetic state of DNA demethylation controls *FOXP3* expression. Specifically, demethylation of the CNS2 (which is also called Treg-specific demethylated region—TSDR-) region is essential for inducing and stabilizing FOXP3 expression as it allows for FOXP3 to access its own locus and create a feedback loop that will ensure its stable expression [30–32]. Activated Teff cells can also express *FOXP3* but this expression is only transient and their TSDR remains methylated. TSDR demethylation is a very specific marker for tTreg, and its quantification is possible from blood and tissue samples [33, 34].

A study performed in 2012 in both mice and humans demonstrated that other genes show characteristic DNA demethylation specific for Treg phenotype. These genes include *CTLA4*, *CD25*, glucocorticoid-induced tumor necrosis factor-related receptor (*GITR*), and Eos and can be used to define the Treg population [28]. Previously, the lack of appropriate markers to differentiate tTregs from activated $FOXP3^+$ Teff leads to many controversial results, especially in human studies of different pathologies such as in type 1 diabetes, multiple sclerosis, or rheumatoid arthritis. Depending on which markers were used to detect Treg, the authors would come to different conclusion. The combined use of these epigenetic markers in different pathologic conditions could provide important information on the state of the disease, response to therapy and still unravel new features on the role of Treg cells.

A new line of evidences indicate that tTregs present context-dependent functional diversity. Specific tTreg populations have been linked to the regulation of different T helper (Th) subclasses. As a whole, FOXP3 appears to cooperate with lineage-specific transcription factors in order to skew Treg abilities toward the regulation of diverse types of immune responses [35]. Several studies also indicate that specific subsets of human Treg cells could lose regulatory activity and become memory Teff cells producing IL-17, under specific conditions [36], or could convert to a prevalent Th2 phenotype [37]. In addition, ablation of FoxP3 in mature Tregs enables them to produce Th1 effector cytokines [38]. These observations are of particular importance in understanding the role of Treg cells in disease inflammatory context and the complex interactions with different Th subsets in allergy and autoimmunity.

$FOXP3^+$ Tregs use various distinct mechanisms to exert their suppressive action [39]. They can use perforin and granzyme B to induce cytolysis of CTLs, B, and NK cells [40–42]. However, these studies were performed in mice, and these properties need further investigations in human cells. Mouse and human Tregs can also use the *tumor necrosis factor-related apoptosis inducing ligand* (TRAIL)/death receptor 5 (DR5) pathway to induce cytolysis of T cells [43]. They can produce inhibitory cytokine such as IL-10, IL-35, and TGF-β that can further mediate their suppressive function (reviewed in [39, 44]) and modulate the adenosine nucleosides–adenosine receptor pathway to suppress Teff function in mice and human. The latter mechanism occurs by gap junction transfer of cyclic adenosine monophosphate, a potent inhibitor of proliferation and IL-2, to the target effector cells [45–48]. Finally, in vivo visualization experiments have shown that the absence of Tregs increases the duration of the Teff-dendritic cell (DC) contacts in mice [49, 50]. This process is supposed to be dependent on Treg expression of CTLA4 [51].

Type 1 regulatory cells

Type 1 regulatory T (Tr1) cells are Ag-specific adaptive Tregs that are induced in the periphery upon chronic exposure to Ag in the presence of IL-10. They are defined by their cytokine response, namely their ability to produce high levels of IL-10 and TGF-β, low amounts of interferon γ (IFN-γ) and IL-2, and detectable levels of IL-5, in the absence of IL-4 [52–54]. Unlike nTreg cells, Tr1 cells do not rely on FOXP3 expression for their function [55–57]. Similar to other Treg cells, Tr1 cells are anergic, but their proliferation can be partially restored by IL-2 and IL-15, independently from TCR-mediated activation [52].

To specifically identify Tr1 cells, previous researchers have used a variety of transcriptional and membrane-bound markers. A comparative study of mouse models between Tr1 and tTregs cells showed that Tr1 cells selectively express repressor of GATA-3 (ROG) [58]. In humans, IL-10-producing T cells, which resemble Tr1 cells, selectively express the co-inhibitory molecule ICOS (inducible T cell co-stimulator) [59] or adhesion molecule CD18 [60]. Most recently, the markers LAG3 (lymphocyte-activation gene 3) and CD49b have been identified as cell-specific markers for murine and human Tr1 cells [61]. However, the specific transcription factor for this lineage specification is still unclear.

Tr1 cells are induced in an Ag-specific manner. Once activated through their specific TCR, Tr1 cells secrete IL-10 and TGF-β. These cytokines directly inhibit Teff proliferation, but

also the expression of MHC class II and co-stimulatory molecules on Ag-presenting cells (APCs), thus indirectly suppressing Teff activation. In particular, IL-10 up-regulates the expression of the tolerogenic molecules immunoglobulin-like transcript-3 (ILT3), ILT4 [62, 63], and HLA-G [63, 64] on APCs, thus initiating a regulatory loop. IL-10-producing Tr1 cells have also been shown to directly suppress T cell responses via perforin and granzyme B production [63], cell-contact-dependent mechanisms [65], metabolic disruption [66], and mediate T cell suppression by selectively lysing myeloid cells [67]. Although Tr1 and $FOXP3^+$ Tregs can act using similar mechanisms (e.g., production of TGF-β, perforin, granzyme B,…), the secretion of IL-10 is a hallmark of Tr1 suppressive activity [39, 65].

In addition to $FOXP3^+$ Treg and Tr1, other Treg cells include Th3 cells, γδT cells, and $CD8^+$ Treg cells [68]. The contribution of these cells to clinical tolerance is less well defined.

Regulatory T cells in pathologic conditions

Regulatory T cells and immune dysregulation of genetic origin

Genetic diseases of the immune system have been useful models to study immune functions. Primary immune deficiencies due to mutations of genes encoding for key molecules in lymphocyte functions have greatly improved our understanding of how immune responses are articulated. Similarly mutations in specific genes can also impair regulatory mechanisms [69].

A prototype of genetic disease with lack of peripheral tolerance is the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. Mutations of *FOXP3* lead to the development of dysfunctional tTreg cells resulting in severe autoimmunity with very early onset (Fig. 1b) [70]. The main target organs are skin, intestine, and pancreas, manifesting with severe eczema, intractable diarrhea, and type I diabetes in the first months of life. IPEX syndrome is a life-threatening disease if not quickly recognized. The study of patients with IPEX syndrome helped recognize in humans that FOXP3 mutations do not impair the thymic development of Treg cells but rather their function [71]. The “programming” of tTreg is preserved as evidenced by complete demethylation at the TSDR and detectable *FOXP3* expression, except in rare cases in which the mutation *per se* prevents the protein codification [72].

FOXP3-mutated tTregs have a growing disadvantage in a chimeric situation in which they co-exist with wild-type Treg, as it occurs in carrier mother or after bone marrow transplantations in IPEX patients [73, 74]. In addition, *FOXP3*-mutated tTreg have an unstable phenotype. Usually, tTregs are very poor cytokine producers because *FOXP3* is a potent cytokine repressor. *FOXP3*-mutated cells loose this intrinsic regulation and produce effector cytokines, including IL-17, which is known to contribute to autoimmunity and inflammation [75, 76]. *FOXP3*-deficient tTreg cells are also inefficient at controlling B cell function, another relevant step in the pathogenesis of the disease [77]. Altered *FOXP3* leads to ineffective control of Teff cell proliferation thus to lymphocytosis. Lack of this peripheral arm of the immune system is definitively detrimental and affects multiple cells and functions.

Interestingly, there are other molecules that when hit by genetic alterations can lead to Treg dysfunction. One example is CD25 deficiency (Fig. 1c), which is characterized by clinical manifestations similar to IPEX. Treg cells express high levels of CD25, and they are dependent on exogenous IL-2 for their peripheral survival [78]. In this IPEX-like disease, the impaired Treg function leads to autoimmunity that is associated with a profound immunodeficiency because of the inability of Teff cells to expand properly in response to pathogens. CD25-deficient patients are affected with severe CMV infections that can also induce severe enteropathy and autoimmune manifestations such as alopecia, thyroiditis, and cytopenia. tTreg cells of these patients normally express FOXP3 but they are less responsive to IL-2 and therefore less functional. Importantly, this disease is autosomal recessive and can also manifest in female, unlike IPEX which only affects males. Mutations in *STAT5*, the signaling molecule downstream CD25, can also impinge in the ability of Treg cells to survive in the periphery, to promptly respond to CD25 and TCR activation, and to upregulate FOXP3 expression (Fig. 1d) [79]. However, these patients usually have other symptoms such as growth defect and milder autoimmunity as compared to patients affected with IPEX, suggesting that other regulatory mechanisms could take place or that other pathways can partially replace the STAT5 intracellular defect.

Other gene mutations can alter tTreg mechanism of action. Those include adenosine deaminase mutations which are responsible for ADA-severe combined immunodeficiencies (ADA-SCID) impairing an important enzymatic pathway for the function of Treg [47] and Wiskott-Aldrich syndrome in which the mutated WAS protein, an essential component of the T cell synapse, alters a series of events following TCR-mediated signaling in multiple lineages including tTreg cells [80]. Although not described as a monogenic disease, several polymorphisms affecting *CTLA4* have been associated with polyautoimmune symptoms which could be related to impaired function of Tregs (Fig. 1e) [81]. All these examples comprise rare diseases; however, as a group, early immunodysregulatory disorders involve a large number of patients with similar clinical manifestations and pathogenesis even though in many cases the underlying defect is still unrecognized. A quantitative defect of tTreg cells has been found in patients with IPEX-like symptoms of unknown origin with undetectable *FOXP3* or *CD25* mutations [72]. Interestingly, a proportion of them had reduced numbers of tTreg cells as detected on whole blood by measuring TSDR demethylation of *FOXP3*. The few Tregs present exerted normal in vitro suppressive function.

Considering other types of Treg cells, *IL-10* and *IL-10 receptor* (*IL-10R*) gene mutations have been identified in patients with severe early onset ulcerative colitis [82]. Although Tr1 cells have not been investigated in these subjects, it is likely that a profound defect is occurring in this induced Treg subset given the complete dependence (demonstrated both in vitro and in vivo) of Tr1 cell generation from IL-10.

An emerging line of investigation aimed at understanding these disorders comprises a more systematic and efficient genome-wide analysis focused not only on identifying new causative genes but also hypomorphic mutations of already known genes that could determine a wide range of symptoms. In addition, it will be interesting to understand the interplay between the different types of Treg cells and how the genetic impairment of one can be replaced by expansion of another or they have mutual effects. Moreover, novel

insights into the pathogenesis of immune dysregulation will be provided by studying Treg cells and their interaction with innate immunity cells such as innate lymphoid cells (ILCs) in different inflammatory environment.

Regulatory T cells in allergic disease

In addition to self-Ag, immune tolerance must be maintained throughout life against innocuous environmental peptides. Failure to do so may result in allergic reactions, the most important and best described type of which are IgE-mediated allergic diseases. This type of response is initiated when a naïve T cell recognizes a foreign peptide and is activated in the presence of IL-4 and other co-factors which trigger a Th2-type differentiation. Th2 cells release IL-4, IL-5, and IL-13 that stimulate B cells to produce allergen-specific IgE responsible for the acute symptoms. Th2 cytokines can also stimulate eosinophil development and recruitment, mucus production and smooth muscle contraction, and tissue homing of Th2 cells leading to chronic allergic inflammation in the skin and mucosal surfaces [83–85]. In healthy responses, Tregs appear to be essential for maintenance of immune tolerance by preventing the Th2 induction and Th2 cytokine release in response to allergens [54].

A deregulation of FOXP3$^+$ Tregs seems to play an important role in allergic diseases as well. Some evidence for example indicates that *FOXP3* polymorphisms and impaired tTregs function have been associated with the development of allergy in different populations (Fig. 1f) [86–88]. In addition, monozygotic twin studies in which one of the twins had allergic asthma showed the *FOXP3* locus to be differentially methylated between the twin pairs [89]. In vitro function of FOXP3$^+$ Tregs has been shown to be reduced in subjects with allergic rhinitis and refractive asthma, and Tregs from asthmatic subjects showed deregulation in chemokine signaling pathway [90–97]. Most of the aforementioned studies looked at Tregs in blood. However, some allergic asthma studies looked at tTregs from bronchoalveolar fluid (BALF), an effective way to study tissue tTreg function in allergy. Consistent with previous studies on peripheral blood, asthmatic subjects were found to have lower FOXP3$^+$ Tregs in BALF and their function was impaired [98, 99]. These findings were reversed with corticosteroid treatment and increased with allergen provocation [11, 98–100] showing that migration of functional FOXP3$^+$ Tregs is crucial to the prevention of allergic airway disease. The fact homing of Treg cells toward airway epithelial cells was shown to be impaired in chronic rhinosinusitis with nasal polyposis also supports this concept [101].

To study human FOXP3$^+$ Treg in a humanized mouse model, Martin et al. [102] injected PBMCs from allergic or healthy donors into NOD/SCID mice, which were then challenged with allergens. After activation of Treg by treatment with the CD4-binding, lck-activating recombinant HIV-1 surface protein gp120, they found that PBMCs from healthy but not allergic donors prevented the development of allergen-induced airway inflammation and airway hyper-responsiveness and that treatment with gp120 attenuated both responses. These results further support the concept of a functional tTreg defect in allergic individuals.

Tr1 cells similarly suppress allergen-driven Th2 cytokine production from IL-4-producing Teff cells by releasing IL-10, TGF-β and expressing CTLA4 and PD-1. Their number has also been found to be reduced in peripheral blood from atopic subjects [103]. Furthermore,

Meiler et al. [104] showed in a series of experiments that rapid switch and expansion of IL-10-producing Tr1 cells and the use of multiple suppressive factors represent essential mechanisms in immune tolerance to a high dose of allergens in non-allergic individuals. These studies suggest Tr1 cells also play a role in preventing allergic diseases but more research is needed on the role of this Treg subset.

Because the gut is a preferential tissue for homing of different kind of Treg cells, these cells are considered very important in the pathogenesis of food allergy and in defining new therapeutic interventions to control food allergy. Indeed, innovative therapies suggestive of restoration of tolerance are associated with increased proportion of either $FOXP3^+$ Treg or IL-10-producing cells [105– 109]. In addition, oral immunotherapy against peanut allergen associated with an absence of response to the antigen after 3 months of therapy withdrawal induced a modification of *FOXP3* methylation pattern and of tTreg activation [110].

Possible therapeutic interventions

Tregs are a powerful biological tool for immunomodulation. Much effort by several groups has been devoted to translate Treg therapy into the clinic. Two main approaches can be envisaged: (1) the use of pharmacological compounds that can boost Treg induction or expansion in vivo or (2) the in vitro expansion or in vitro induction, upon good manufacturing practices (GMP) conditions. Limitations have been encountered in both types of approach but the field is rapidly growing [111, 112]. As for in vivo induction, the problem of producing a selective effect on the target Treg cells has been the major challenge. The use of rapamycin has been found to selectively spare Treg cells and suppress Teff [113], and its use in different indications than organ transplantation should be assessed. Recently, novel modified small compounds of the IL-2/IL-2R complex which can partially mimic the effect of IL-2 but being selective for Treg cells are under investigation in preclinical studies and could lead to promising results [114, 115]. The in vivo expansion could represent an advantage over the in vitro expansion; however, both approaches would not allow selective action on Ag-specific Treg cells.

In allergic patients, immunotherapy has been proven to be beneficial and could be a very promising treatment for allergic diseases. It consists in the administration (subcutaneous, epicutaneous, sublingual, oral) of increasing doses of the pathogenic allergen, during a long period of time. The rational of this chronic Ag exposure is the induction of specific T cells which are not pathogenic but rather tolerogenic. After 1 year of allergen-specific immunotherapy, tTregs from these patients were increased numerically and functionally and showed specific demethylation of the *FOXP3* locus [107]. However, the number of patients who remain tolerant to the Ag once the immunotherapy has stopped is still limited. Several complementary approaches are ongoing in order to render this strategy more efficacious, such as the parallel administration of anti-IgE Ab, allowing a faster Ag dose escalation [116].

The possibility of “producing” Treg from conventional T cells by in vitro induction has been the ambitious aim of many laboratories. From their discovery, Tr1 cells were known for being easily generated in vitro in an Ag-specific manner. This has been widely demonstrated in an allogenic system [117]. Up to now, two major proof-of-concept (POC) clinical trials

have been performed with Ag-specific Tr1 cells and both considered the contribution of Ag-specific stimulation once the cells were re-injected in vivo. In a first trial, Crohn's disease patients in which ovalbumin (OVA)-specific Tr1 cells were established in vitro and then infused in the patients who were fed with OVA in order to favor the gut homing of the infused Ag-specific cells and possibly their maintenance [118]. In a different POC trial, donor-derived Tr1 cells specific for the HLA Ag of the host were infused in patients affected with hematologic malignancies who were transplanted with haploidentical hematopoietic stem cells [119]. Safety was established and this Tr1 cell therapy improved the outcome of the transplant providing fast immune reconstitution and inducing tolerance across the allogeneic barrier. In both trials, the number of cells injected was a critical issue, and larger clinical trials in more homogenous cohort of patients are required to better assess the right cell dose to be administered.

A manufacturing protocol for establishing Ag-specific FOXP3$^+$ Treg has been recently published [120] and could be applied in the future. However, polyclonal FOXP3$^+$ Treg cells have been successfully used by different groups, as cell therapy in the context of allogeneic transplantation in order to prevent GVHD [121, 122]. In these protocols, Treg isolation was achieved by purification with beads-conjugated antibodies and cells were either infused fresh or upon a short period of expansion in vitro. In both cases, the purity of the cell population and therefore the stability and potency of suppression were critical. Encouraging positive results were obtained, although there have been concerns on the possible general suppression generated by Treg cells infusion. This issue could be avoided by administration of Ag-specific cells. Beside the allo-Ag system that has already been explored, differentiation of Treg cells toward a variety of different Ag could be now envisaged to cure patients with autoimmunity such as rheumatoid arthritis or patients affected with specific allergies.

An additional in vitro strategy for either Tr1 or FOXP3$^+$ Treg generation consists in IL-10 or *FOXP3* gene transfer into conventional T cells, respectively [27, 123]. Results obtained in vitro and in vivo in murine models of xeno GVHD show the feasibility of both approaches. While such a strategy would be mandatory in case of genetic mutations (for example, in IPEX patients), a large application in different pathologies remain to be assessed.

In conclusion, the therapeutic use of Treg cells could be achieved in several different ways, and there are good premises supporting the fact that implementation of Treg cell therapy will be of great benefit, alone or in combination with pharmacological compounds, in order to boost immune regulation, similarly to what has been achieved boosting immune responses with vaccinations.

Biography

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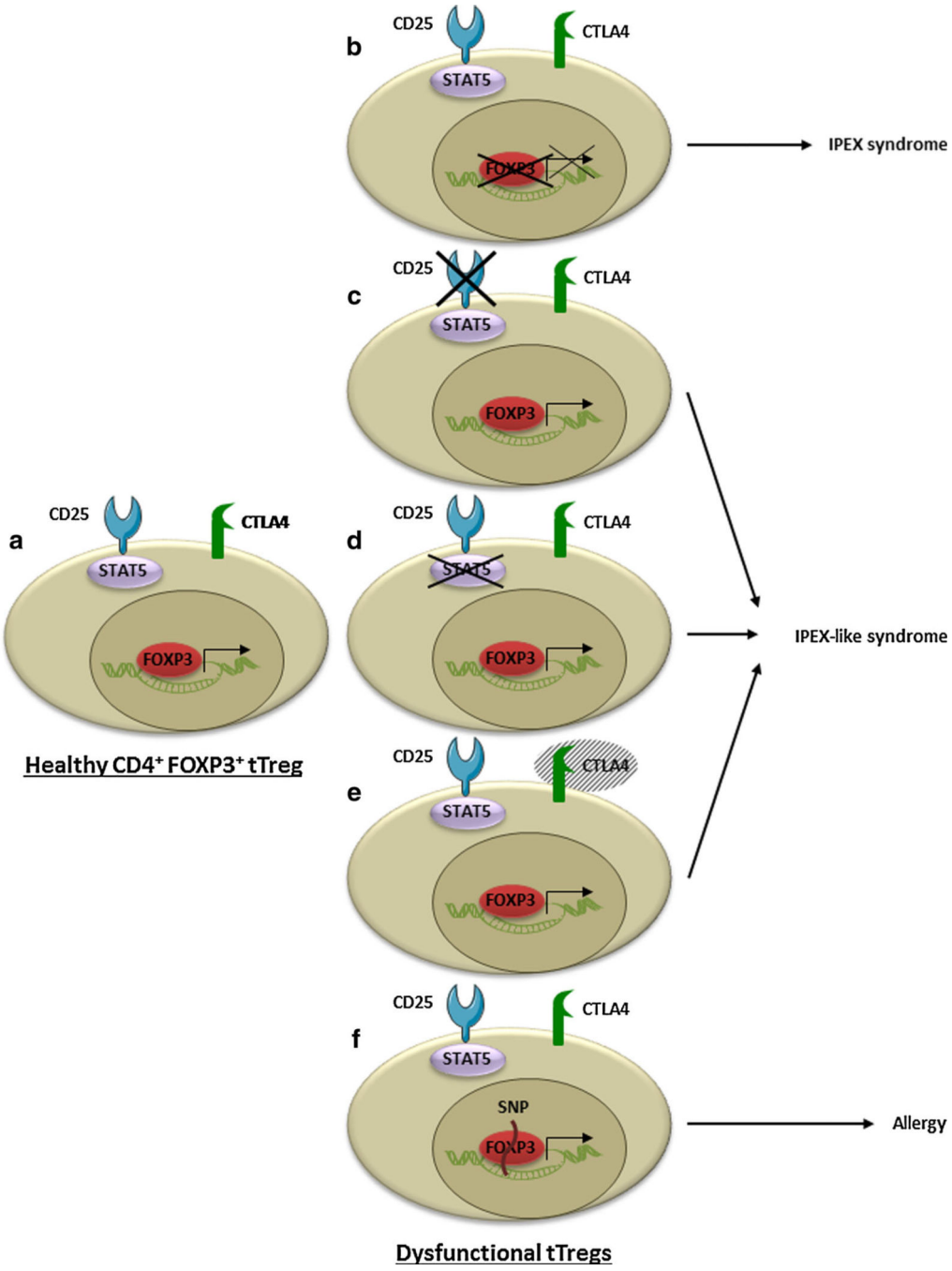

Fig. 1.

FOXP3$^+$ cells in healthy and pathologic conditions. *a* Healthy FOXP3$^+$ regulatory T cells (Tregs) express the IL-2 receptor CD25, CTLA4 and the transcription factors STAT5 and FOXP3. Genetic abnormalities of these molecules are associated with Treg dysfunction: *b* *FOXP3* mutations are causative for the immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome. *c*, *d* Mutations of *CD25* and *STAT5* are responsible for the development of immunodeficiency with autoimmunity related to IPEX. *e* Polymorphisms of *CTLA4* have been associated with polyautoimmune pathologies. *f*

Polymorphisms of the *FOXP3* gene have been observed in allergic patients, suggesting a causative link