

# The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part I: Treg properties and functions

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## Abstract

Regulatory T cells (Treg) can be divided into two types: the natural cells (tTreg), which arise in the thymus, and the induced cells (iTreg), which are produced in peripheral tissues during immune response. The most recently published studies indicate that the supervisory functions of these cells are weakened in the pathogenesis of autoimmune and neoplastic diseases of the skin. This may be a result of the domination of other immune cells in the skin, such as Th1/Th17/Th22 and Tc1 type in psoriasis and Th2 in atopic dermatitis. The excessive activity of Treg cells can lead to immunosuppression and decrease in the number of Th1 cells, which promote the development and progression of skin cancers. In the case of cutaneous T-cell lymphomas, there are suggestions that tumor progression is associated with the acquisition of the suppressor phenotype of malignant cells. There is genetic background of Treg dysfunction in skin disorders. This article describes the types and functions of Treg cells.

**Key words:** tTreg, iTreg, Breg, FOXP3.

## Properties of regulatory T cells

The main role of the human immune system is to recognize and fight against foreign antigens as well as to build up tolerance to self-antigens. One of the key elements making up the mechanisms that regulate the immune response is a population of T helper cells, called T-regulatory (Treg) lymphocytes. Treg cells are a heterogeneous group of cells responsible for the controlling of the immune system. In the human body these cells perform many tasks. They participate in the formation of tolerance to food and saprophytic bacteria of the gastrointestinal tract, skin, and mucosa. They take part in the tolerance to self-tissues as well as in the formation

of allotransplant tolerance and building up the tolerance to antigens of the fetus during pregnancy. It is executed through interaction of Treg cells with tolerogenic antigen-presenting cells (APC), and inhibition of autoreactive lymphocytes, or even killing of other immune cells. Deficiency and/or dysfunction of Treg cells leads to autoimmune diseases, ageing and allergies. It can cause infertility, pregnancy disorders and transplant rejection. In turn, excessive activity contributes to cancer and an increased susceptibility to infectious diseases [1–18].

There are several regulatory populations in the body, such as human CD8(+) CD28(–) cells,  $\gamma\delta$  T cells, regulatory B cells, myeloid derived suppressor cells, tolerogenic den-

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**Table 1.** Different populations of human immunosuppressive regulatory cells [5–10, 13]

Cell type	Phenotype	Mechanism of immunosuppression
CD4+ regulatory T cells:		
Thymic-derived naturally occurring Treg (tTreg)	CD4+CD25+FOXP3+	Cell-cell contact dependent (CTLA-4/CD80/CD86) and cytokine-dependent (IL-10, TGF-β)
Peripheral induced Tregs (iTreg)	CD4+CD25+FOXP3+	Cell-cell contact (CTLA-4/CD80/CD86) and cytokine-dependent (IL-10, TGF-β)
Tr1 cells	CD4+CD25+FOXP3–IL-10 <sup>high</sup>	Cytokine mediated (IL-10)
Th3 cells	CD4+CD25+FOXP3– TGF-β <sup>high</sup>	Cytokine mediated (IL-10 and TGF-β)
iTreg-35	CD4+CD25+FOXP3–IL-35(+)	Cytokine mediated (IL-35)
CD8+ regulatory T cells:		
T suppressor cells	CD8+CD28–	Cell-cell contact dependent (CTLA-4/CD80/CD86)
IL-10 producing CD8 T cells	CD8+IL-10+	Cytokine mediated (IL-10)
B regulatory cells (Breg)	Breg producing IL-10 Breg producing IL-35	Cytokine mediated (IL-10, IL-35)
NKT cells	CD3+CD4–CD8–CD56+, CD16+, CD94+	Cytokine mediated (IL-10 and TGF-β)
Myeloid derived suppressor cells (MDSC):		
Granulocytic MDSCs	CD11b+Ly6G+Ly6C <sup>low</sup>	Depletion of arginine, NO production, inhibition of proliferation and induction of T cell apoptosis
Monocytic MDSCs	CD11b+Ly6G–Ly6C <sup>high</sup>	
γδT cells	CD80/CD86 CD11a, CD18, CD54	Cell-cell contact dependent (CTLA-4/CD80/CD86) and cytokine dependent (IL-10)

dritic cells, NK-T cells and some cytotoxic T lymphocytes – Table 1 [2, 4, 6, 9, 12, 16–21].

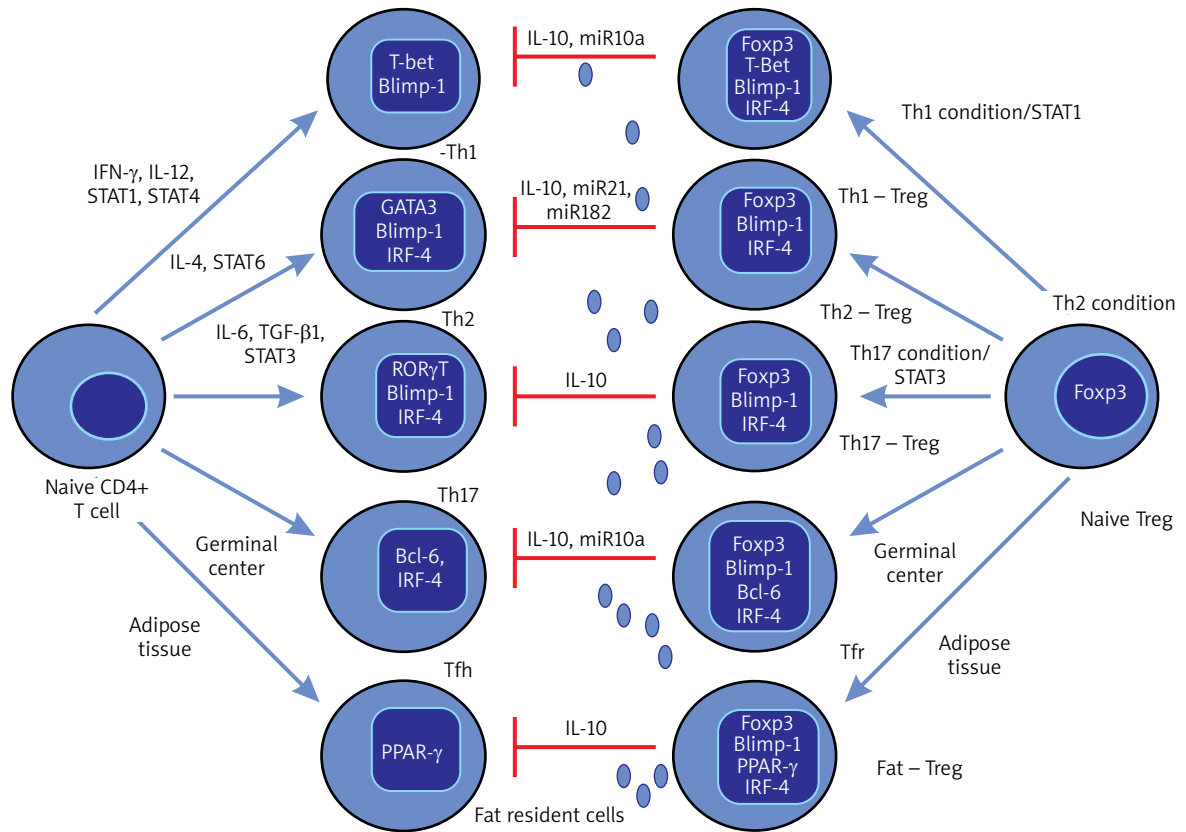
The immunodominant regulatory subset consists of a group of CD4(+) T regulatory cells. These are divided – by the place of formation, effector mechanisms and the profile of cytokines produced – into two main groups – primary regulatory T cells, produced in the thymus and referred to using the symbol tTreg (thymic Tregs), and the secondary, induced regulatory lymphocytes – iTreg (induced/adaptive Tregs). tTregs are secreted in the thymus. After rearrangement of the receptor TCRαβ gene, the clonal autoreactive Tregs are not fully eliminated, as in case of conventional T lymphocytes. Hence, they are capable of recognizing of self-antigens but, in contrast to other T cells, this leads to self-tolerance. Other important phenotypic features of tTregs include a high expression of the CD25 receptor (IL-2R, part of IL-2 receptor) and a constitutive expression of transcription factor forkhead box (FOXP3) protein and production of large amounts of IL-10 and transforming growth factor β (TGF-β).

Population of tTreg is heterogeneous. On the basis of their effector suppressor function in different tissues and expression of specific transcription factors (TF), chemokine receptors and micro RNA signature – various populations of effectors FOXP3(+) Treg cells were distinguished (Figure 1). Naive tTregs could be differentiated into Th1-Treg, Th2-Treg, Th17-Treg, Fat-Treg, and Tfr-Treg. Th1-Treg, Th2-Treg and Th17-Treg suppress Th1, Th2 and Th17 cells, respectively. Fat-Tregs inhibit adipocytes and control

metabolic disorders, while Tfr-Tregs suppress T cells in germinal centers of lymphoid tissue – Tfh cells. These subpopulations of tTreg have different molecular signatures. Th2-Treg express Blimp-1 and IRF-4, chemokine receptors CCR4 and CCR8 and produce miR21 and miR182; Th1-Treg express Tbet, IRF-4, Blimp-1, CXCR3, miR146a and miR10a; IL17-Treg express Blimp-1, and probably IRF-4, CCR6, while the signature of miRNA is unknown; Tfh-Treg express bcl6, Blimp-1, CXCR5, miR10a; Fat-Treg express IRF-4, Blimp-1, and PPAR-γ and the signature of miRNA is unknown [9, 18].

The localization of tTregs in the human body and their trafficking to different tissue are dependent on the expressed homing receptors and adhesion molecules as well as on tissue-specific chemokines gradient. Around 80% of Tregs in the peripheral lymph nodes express CCR7, more than half of Tregs express CD62L (L-selectin), which interact with vascular addressins (CD34, GlyCAM-1). Tregs expressing CCR4, CCR5 and/or CXCR4 accumulate in the inflamed skin, those with CCR6 expression accumulate in the inflamed joints, and Tregs expressing CCR1, CCR2 and CCR9 Treg accumulate in adipose tissue [9]

Induced Treg (iTreg) are formed at the peripheral tissues from mature T lymphocytes after stimulation with antigens, such as non-pathogenic antigens including commensal microbiota, food and fetal antigen cells, presented by APCs in the presence of suppressive cytokines (IL-10, TGF-β1).



*T-Bet (T-BOX expressed in T cells) – Th1-specific T-box transcription factor that controls the expression of the hallmark Th1 cytokine, interferon-gamma. Blimp-1 (B lymphocyte-induced maturation protein 1; positive regulatory domain i-binding factor 1; PRDIBF1) – DNA-binding transcriptional repressor. It exerts its repressive functions through recruitment of histone-modifying enzymes. Bcl-6 (B-cell lymphoma 6) – transcriptional repressor necessary for germinal center formation. IRF-4 (interferon regulatory factor 4) is a transcription factor essential for the development of T helper-2 cells, IL-17-producing Th17 cells, and IL-9-producing Th9 cells. PPAR-γ (peroxisome proliferator-activated receptor γ) – bind chemicals that induce proliferation of peroxisomes, organelles that contribute to the oxidation of fatty acids [adapted from 9, 18].*

**Figure 1.** Differentiation of effector CD4+ T cells and regulatory T (Treg) cells, their function and transcription factors involved

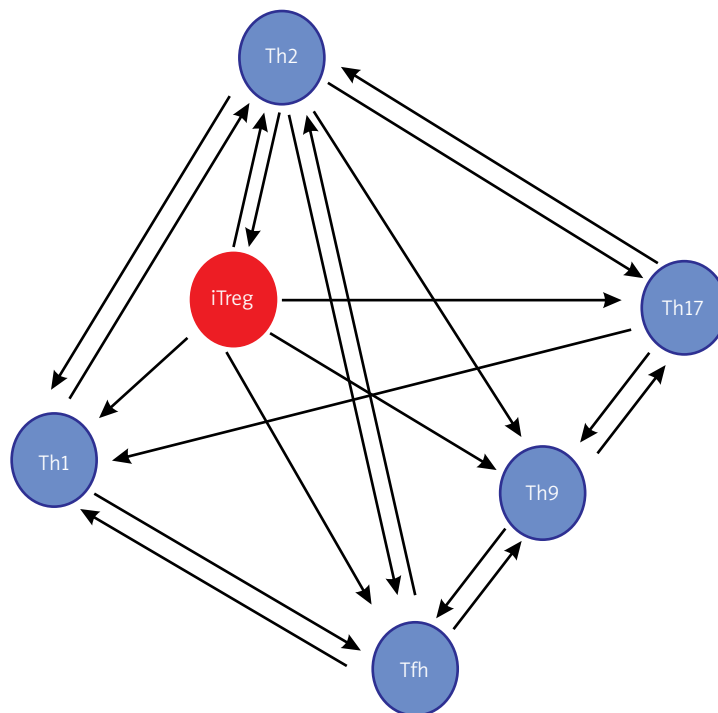
Induced Tregs are divided into cells with and without FOXP3 gene expression. The generation of peripheral iTreg FOXP3(+) naive conventional CD4+ T cells requires triggering of TCR together with the stimulation by IL-2 and TGF-β. The expression of FOXP3 in these cells is much lower and transient than in nTregs [5–9].

FOXP3(–) iTreg are divided into two populations Tr1 and Th3 cells. Tr1 cells predominantly secrete IL-10, and minor amounts of TGF-β1, IL-5 and IFN-γ; Th3 cells predominantly produce TGF-β1 [1–11]. Formation of iTreg cells in the tissues is influenced by immature dendritic cells and immunosuppressive agents such as glucocorticoids, and vitamin D<sub>3</sub> [6, 9, 22–24]. There are reports that also tTregs can be involved in the generation of iTregs in the mechanism called “infectious tolerance”. This mechanism is dependent on the expression of certain integrins on nTregs. The expression of integrin α4β7 induces

mainly Tr1 cells producing IL-10, and the expression of α4β1 integrin induces Th3 cells producing TGF-β1 [5–11].

On the other hand, there are also suggestions that iTreg can be converted into Th1, Th2, Th17 and Tfh (Figure 2). This can be also true in the case of tTregs. Virtually all tTregs are characterized by a stable high expression of FOXP3, but there is some evidence for Treg instability, loss of FOXP3 expression and acquisition of the effector phenotype. The inflammatory cytokines IL-6, in conjunction with IL-1β and IL-23 are capable of induction of RORγT and downregulation of Foxp3, which leads to the formation of so-called exTregs Foxp3(–) cells producing IL-17. It is suggested that epigenetic mechanisms are involved in the lost FOXP3 gene expression in Tregs [6, 9, 11, 25–29].

It has been recently shown that Tregs produce IL-35 cytokine. This new group of regulatory T cells is called iTreg35 [30–33]. Notably, these cells are phenotypic ally



**Figure 2.** Plasticity and flexibility of CD4(+) T helper cell subsets and their multidirectional impact and transformation. iTreg could transform in different cytokines milieu condition into: Th1, Th2, Th17, Th9 and Tfh (follicular) cells. Various effector cells can be mutually converted into each other [adapted from 6, 9, 11, 25–29]

and functionally distinct from other subpopulations of Treg cells described thus far in that they do not express Foxp3 and they mediate immunosuppression via IL-35 and seemingly independent of IL-10, TGF- $\beta$ , the immunomodulatory receptor CTLA-4, or any other currently known Treg cell-associated suppressive molecule. Tregs expressing IL-35 (iTr35) have been shown to inhibit the differentiation of naive CD4+ T cells into Th17 effector cells [30–33].

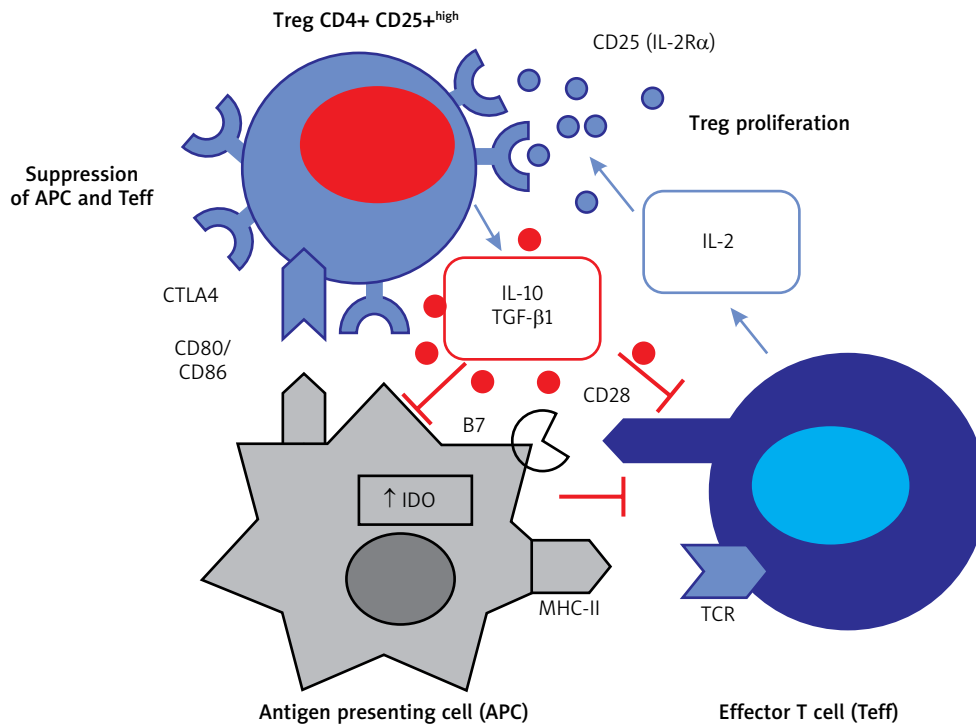
Another group of T cells with a suppressive function are CD8+T suppressor cells. These cells are derived from oligoclonal T cell and they lack CD28 antigen, express FOXP3, GITR, CTL-4, OX-40 and CD62L on the same level as observed in CD4+CD25+ Tregs and also CD122 antigen –  $\beta$  subunit of IL-2 receptor. The mechanisms underlying their suppression mainly include IL-10 and TGF- $\beta$  production and possible cytotoxic T lymphocytes – mediated killing of activated T cells [5, 20, 21].

### Phenotype of Treg

Produced in the thymus tTreg express a high level of IL-2 receptor  $\alpha$  chain (CD25<sup>high</sup>), substantial expression of molecules HLADR, TNF receptor, called GITR (glucocorticoid induced tumor necrosis factor receptor), CTLA-4 (cytotoxic T lymphocyte-associated antigen, CD152) and the constitutive expression of specific transcription factor – Foxp3. A low expression of CD127 (IL-7 receptor  $\alpha$

chain) is often used to give a complete phenotype of human Treg [1–7, 9–11]. The phenotype CD4(+)CD25(+) <sup>high</sup>CD127(-)<sup>low</sup> Foxp3(+) constitutes a small fraction (5–10%) of the total pool of CD4 (+) T-helper lymphocytes. Other molecules that are expressed on activated Foxp3(+) Tregs include the latency-associated peptide (LAP), lymphocyte activation gene-3 (LAG-3), CD39 (plasma membrane-bound ectonucleoside triphosphate diphosphohydrolase), PD-1 (programmed cell death 1, CD279) a receptor of PDL1 (programmed cell death-1 ligand-1) and PDL2 ligands, IL-1 receptor type I and II (CD121a/CD121b) and OX40 (CD134). However, none of these are exclusive to Treg cells [1–7, 9–11].

The IL-2 receptor is composed of  $\alpha$ ,  $\beta$ , and  $\gamma$  chains. The active receptor is a trimer composed of  $\alpha\beta\gamma$  chains and its constitutive expression is essential for the survival of Treg cells. Interleukin 2 (T-cell growth factor) is essential for maintaining tolerance and preventing autoimmunity by Foxp3+ cells. Because Tregs do not produce IL-2, their proliferation and suppressor function depends on exogenous IL-2 produced by T-effector cells (Figure 3). Linking of IL-2 to the receptor induces tyrosine kinase-dependent STAT5 protein expression, increased transcription of cytokine genes (IL-10, IL-35, TGF- $\beta$ 1) and activation of the kinase-dependent MAPK and P13K pathways in Tregs. IL-2 is however a double-sword factor as it stimulates also many effector cells such as B-cells, monocytes,



**Figure 3.** Activation and regulatory function of Treg. Synapse of three cells: Treg lymphocyte, Th responder (effectors) lymphocyte (Teff) and antigen presenting cell (APC) leading to activation of Tregs. Treg cell coming into apposition with an interacting APC–Teff pair through ligation of the TCR on the Treg cell with an MHC class II molecule on the APC. Both the APC and the Treg cell secrete IL-2, which by binding to CD25 expressed on the Treg cell surface and may induce the Treg cell to proliferate, proliferating Treg by secreting IL-10 and TGF-β1 suppress the function of DC and Treg [modified from 5, 50]

mast cells, lymphokine-activated killer cells, natural killer cells, and glioma cells [9].

The transcription factor Foxp3 is crucial for the development and functionality of CD4(+)CD25(+) Tregs. Mutations, which cause loss of Foxp3 function, both in mice and men, result in the absence of Tregs and lead to a phenotype with severe autoimmune disorders [34], known as scurfy mice and IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome) in men. The important function of FOXP3 was also confirmed by studies showing that ectopic expression of Foxp3 in T cells leads to the generation of cells with a regulatory phenotype and a suppressive function [29]. In addition, with regard to the biological function of Foxp3 in Tregs, it was demonstrated that Foxp3 blocks the ability of the Rel-family transcription factors NFAT and NFκB to induce their target genes [35–39], and as a consequence, it acts as a transcriptional repressor of IL-2 synthesis and other cytokine genes (IL-4 and IFN-γ), also induce CD25 and CTLA4 expression and thereby programming a cell not to exert immune stimulatory functions. Foxp3 interacts also with Runx1 transcription factor, crucial for normal haematopoiesis including thymus T cell development and with histone acetyl transferase complex, involved in

epigenetic regulation of gene expression. Recent studies found that Runx1 transcription factor directly binds to 20–30% of Foxp3-dependent genes and controls about 700 genes [6, 9, 35–39].

### Activation of Treg

The activation of Treg cells and start-up of their functions require antigen-specific stimulation of TCR (T-cell receptor); however, once activated, nTreg cells may keep a suppressor phenotype for a long time in a non-specific manner (Figure 3) [1–6, 9].

The T-lymphocytes require two signals to be activated – the first is the recognition by the TCR receptor of an antigen presented by antigen presenting cells (APC), e.g. dendritic cells – DC or macrophages, in the context of MHC protein. Optimal T cell proliferation and acquisition of effector functions require the second signal through other pairs of receptors, with CD28 receptor as one of the most important. Upon binding to its ligands, B7-1 (CD80) and B7-2 (CD86), CD28 enhances T effectors cells (Teff) proliferation by increasing transcription and secretion of IL-2 (Figure 3). Absence of the second signal leads to cell anergy. This state is characterized by impaired ability to

proliferate and produce IL-2, and can be reversed by activation of TLRs (toll like receptors) by bacterial lipopolysaccharide [1–12, 15]. Because Treg cannot produce this cytokine and at the same time they express a high level of IL-2 receptor, their proliferation deprive Teff cells of IL-2. In addition, IL-10 and TGF- $\beta$  produced by Treg suppress proliferation of Teff and activation of DCs (Figure 3).

### Epigenetic regulation of Treg function

Epigenetic modifications modulate gene expression and therefore could substantially influence the differentiation of T cells into various subpopulations. It occurs through changes in chromatin conformation, which creates the formation of the “open transcription frame”, making it easy to attach a RNA transcriptase, or to attach the specific transcription factors to gene promoter regions. Epigenetic changes in chromatin occur by methylation of gene promoters, methylation of gene enhancer regions, by histone acetylation/deacetylation or by action of specific mikroRNAs. Epigenetic changes play an important role in regulation of Treg function, plasticity and differentiation [11, 25–29, 39–45].

Modification of the two DNA sequences rich in CpG island loci ensures the availability of the gene FOXP3 in the case of Treg cells. The active Treg lymphocytes are completely demethylated in the FOXP3 gene promoter, notably in a specific region (TSDR-Treg associated demethylated region, CNS2 located in intron 1), and one of the major factors activating this process is TGF- $\beta$ 1. The majority of thymus-derived Treg possess a completely demethylated TSDR. In humans, TGF- $\beta$ 1-induced i-Treg CD4(+) CD25<sup>low</sup> that transiently express FOXP3 express partially methylated pattern [6]. “Epigenome” of thymus derived tTreg is stable, imprinted in cells and exhibit hypomethylation of Treg specific genes (CTLA4, GITR).

Foxp3 regulates transcription of Foxp3-dependent genes by remodeling the chromatin structure and interacting with histone acetyl transferase (HAT)/histone deacetylase complex (HDAC). Foxp3, when attached to *IL-2* gene, can recruit acetyltransferase (HAT/TIP60). Acetylation of FOXP3 region enhance synthesis of FOXP3 and increases its binding to the promoters of *IL-2* and *IFN- $\gamma$*  gene promoters, which in turn leads to genes silencing and inhibition of IL-2 and IFN- $\gamma$  production. In contrast, when FOXP3 attach to the gene encoding *CD-25* and *CTLA-4*, histone acetylation is stimulated and expression of these genes is up-regulated as well [6, 9, 11, 25–29, 39–45].

### Mechanisms of suppression by Treg

Figure 4 presents different suppression mechanisms of effector cells caused by Tregs and indicate the cell receptors, ligands and proteins involved in the mechanisms of direct and indirect suppression of T effector cells and APCs by Treg [46–50].

Tregs can induce immunosuppression by influencing a variety of cell types such as CD4+ and CD8+ T cells, natural killer cells and APC. They can exert their suppressive activity through a variety of mechanisms including direct cell-cell contact, IL-2 deprivation, production of the immunosuppressive cytokines IL-10 and TGF- $\beta$ , cytotoxicity, and modulation of the function of antigen-presenting cells [6, 9, 46–50].

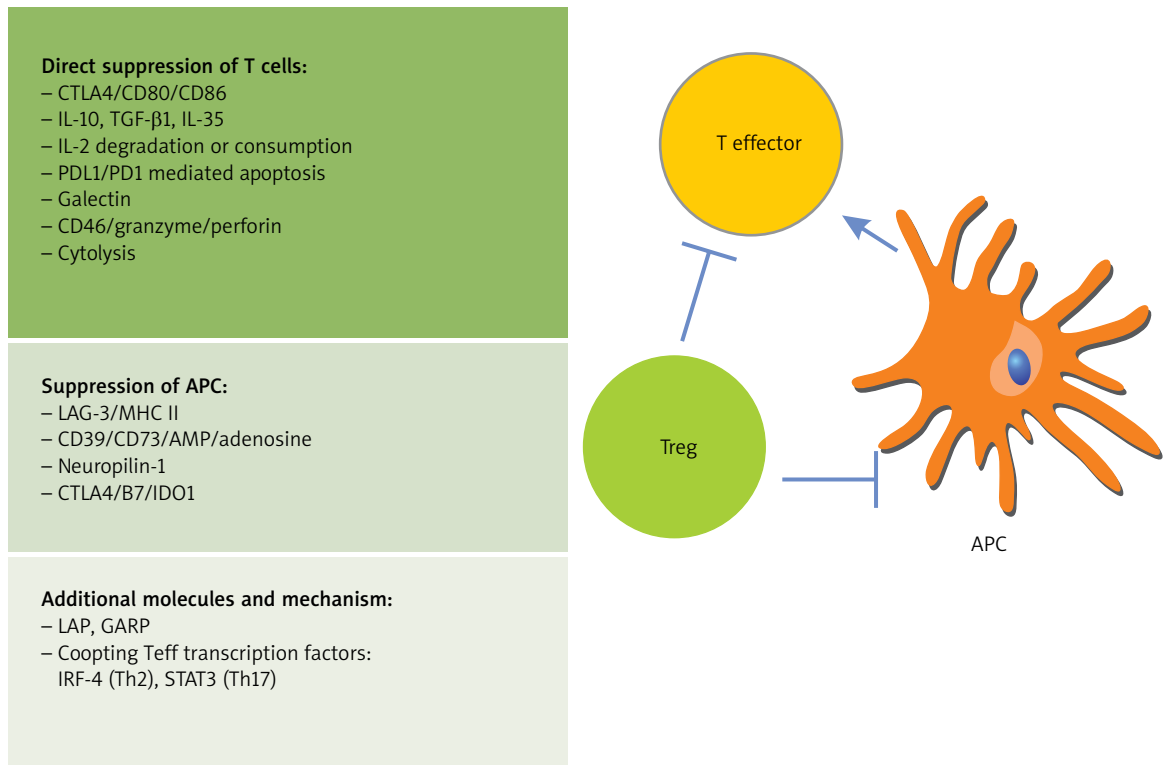
### Suppression by cell-cell contact

Several *in vitro* studies have demonstrated that CD4+CD25+ Tregs suppress proliferation and IFN- $\gamma$  production by effector cells through a direct cell-cell contact dependent stimulation between Tregs and effector cells, possibly mediated by expression on their surface of GITR, CTLA-4 and PD-1. The receptor CTLA-4 (CD152) on Tregs is homologous to CD28 molecule expressed on T effectors and both CD152 and CD28 compete for B7 receptors on APCs. Since CTLA-4 has a 40-100-fold higher affinity to B7 than CD28 molecules, Treg cells bind to DC stronger than T effector cells. It leads to the blockade of the activation signal for T cells dependent on B7. CD4 (+)CD25(+) T<sub>resp</sub> cells without receiving the second costimulatory signal enter a state of anergy, which reduces their ability to IL-2 production, and finally leads to apoptosis [46–52].

The next mechanism of affecting the effector T cell activation by Treg is based on the modulation of DC function. Ligation of CD80/CD86 (B7) on DCs to CTLA-4 on the suppressor cells results in expression and activation of indoleamine 2,3-dioxygenase (IDO), a catabolic enzyme involved in tryptophan degradation. Reduced tryptophan concentration in a culture medium has been reported to be associated with decreased activation of T cells and T cell deletion. These results indicate that CTLA-4 plays a functionally significant role in Treg suppressive activity. On the other hand, CTLA-4 knockout mice appear to have fully functional cells that express the Treg-specific transcription factor FOXP3 [51, 53].

GITR is one of the molecular markers of peripheral iTregs, highly expressed on their surface, but also in activated T cells. The activation of GITR by the GITRL present on dendritic cells leads to impairment of the function of Treg. In general, GITR activation has four distinct effects on Treg/effector cell interplay: (1) transient inhibition of Treg regulatory activity, (2) decreased sensitivity of effector T cells to Treg suppression, (3) killing of Tregs (at least within solid tumors), and (4) increased proliferation and expansion of the Treg compartment [51–54].

PD1 (programmed death 1, member of the B7, CD80) are cell surface receptors present on T-cells, B cells, myeloid cells and cutaneous mast cells (MC). There are two ligands for PD1: PDL1 (B7-H1 molecule), and PDL2 (B7-DC). PDL1 is present on activated Treg, macrophages, myeloid DC, B-cells, epithelial cells and malignant MC and many others. PDL2 is present in DC, monocytes, and



LAG-3 – lymphocyte activation gene 3, involved in lymphocyte activation. binds to HLA class-II antigens, expresses in activated T and NK cells. GARP (glycoprotein a repetitions predominant = LRRC32) – LRRC32 associates with latent TGF- $\beta$  dimers on the surface of regulatory T cells and platelets and regulates TGF- $\beta$  bioavailability. CD39/AMP – vascular ATP diphosphohydrolase, apyrase, regulate the number of Treg. Nrp-1 – neuropilin 1 = vascular endothelial growth factor-165 receptor; VEGF165R, inhibit proliferation of T cells with CD3 expression. LAP – latency-associated peptide involved in Treg activation, the presence of LAP prevents TGF- $\beta$  from activating its receptor. IRF-4 – transcription factor essential for the development of T helper-2 (Th2) cells, IL17-producing Th17 cells, and IL-9 producing Th9 cells [modified from 48].

**Figure 4.** Molecules and mechanisms implicated in suppression mediated by human T regulatory cells. The possible mechanisms of suppression by regulatory T cells (Tregs). Tregs mediate their suppressive action by direct cell-cell contact mediated by CTLA-4 on both effector T cells as well as antigen-presenting cells (APCs), such as dendritic cells (DCs). Tregs produce soluble immunosuppressive cytokines, such as IL-10 and TGF- $\beta$ , and IL-35 suppresses DC maturation, making DCs tolerogenic. Moreover, Tregs can kill effector T cells by expression of perforin and granzyme A or induce galectin mediated apoptosis

malignant MC. The interaction between PD1 and PDL1 leads to the inhibition of Th1 and Tc1 cells, decreases production of their cytokines (IFN- $\gamma$  and IL-2), suppresses T-cell migration, proliferation, and secretion of suppressive (IL-10) or cytotoxic mediators, and consequently restricts tumor cell killing. The PDL1-PD1 axis protects the host from overactive T-effector cells not only in cancer, but also during microbial infections and is important in fetus tolerance. The effect of PD-1 activation may act on the immunological system in dual mechanisms, promoting apoptosis in antigen specific T effector cells while simultaneously reducing apoptosis in regulatory T cells [54–57].

Another mechanism of the suppressor function of Treg is CD39/CD73 pathway. CD39 (vascular ATP diphosphohydrolase, apyrase) is a surface cell enzyme which with hydrolyse extracellular ATP to ADP and AMP, next CD73 (ecto-5-nucleotidase) degrade AMP to adenosine. Ninety percent of Foxp3<sup>+</sup> Tregs are CD39<sup>+</sup> but this enzyme

is also expressed on natural killer (NK) cells, monocytes, DC and B and subsets of activated T cells. Adenosine produced by Tregs downregulates NF- $\kappa$ B activation in Teff cells, thereby reducing the release of a broad spectrum of proinflammatory cytokines and chemokines [58].

#### Suppressive soluble factors produced by Tregs

The main suppressive factors produced by Tregs are IL-10, TGF- $\beta$ 1 and IL-35.

Interleukin 10 is secreted by Treg and anergic T-cells. It inhibits the differentiation of Th1 cells and secretion of IFN- $\gamma$  and IL-2. It also affects the suppression of DC and macrophages by inhibition of the MHC II expression and reduction of their ability to present antigen. In addition, IL-10 inhibits secretion of pro-inflammatory cytokines, such as IL-1, IL-6, IL-12 and TNF- $\alpha$  by these cells. Some autoimmune diseases, such as colitis ulcerosa are related to IL-10 deficiency [49].



Transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) inhibits the proliferation of T cells and NK cells, the formation of Tc and affects the formation of Tregs. TGF- $\beta$ 1 downregulates MHC II class expression on APC and, as IL-10, reduces their ability to present antigen. It also inhibits the expression of costimulatory molecules on DC. On the other hand, DCs secrete TGF- $\beta$ , which induces Foxp3 in naive T cells, driving differentiation of naive T cells into iTregs [5, 46, 47, 59, 60].

Interleukin 35 (IL-35) belongs to the IL-12 family – a group of heterodimeric cytokines that are composed of one of five subunits (p19, p28, p35, p40, and Epstein-Barr virus-induced gene 3 (Ebi3)) that come together in various combinations to form IL-12, IL-23, IL-27, and IL-35. IL-35 has the ability to direct suppression of effector T cell responses. It is also able to expand regulatory responses by propagating infectious tolerance and generating a potent population of IL-35-expressing Tregs. The expression of IL-35 has been identified in a population of IL-35-induced CD4+ Tregs, what is defined as iTr35 cell [30]. IL-35 receptor is composed of IL-12R $\beta$ 2 and gp130, which are also associated with the IL-12 and IL-27 receptors, respectively. The binding of IL-35 to its receptors in iTr35 cell activate STAT1 pathway and increase the expression of target genes: including *p35* and *Ebi3*, promoting increased IL-35 expression and iTr35 cell formation. iTr35 has been shown to inhibit the differentiation of naive CD4+ T cells into Th17 effector cells, and mice lacking *Ebi3* have a significant increase in the production of IL-17. The loss of IL-35 has also been shown to be associated with the development and exacerbation of disease, including many inflammatory diseases such as encephalomyelitis and inflammatory bowel diseases. Recombinant rIL-35 reduces the frequency and severity of arthritis and causes a decrease in the inflammatory immune responses. As opposed to these inflammatory diseases, tumor models have shown that IL-35 contributes to tumorigenesis. These effects are mediated through both immune-directed and tumor-directed effects, as IL-35 can act to suppress tumor-infiltrating lymphocytes that may have anti-tumor activity, as well as potentially supporting the proliferation of tumor cells by promoting angiogenesis [5, 9, 30–33].

In autoimmune diseases, such as systemic lupus erythematosus (SLE), a high level of plasma IL-35 in active SLE patients was observed with a low level of IL-35 receptor (gp130) on CD4+ Th cells. These results raise the possibility that the level of IL-35 expression in SLE patients is not sufficient to induce the production of CD4(+)CD25(+) <sup>high</sup>CD127(–)Tregs, and subsequently suppress the release of inflammatory cytokines and chemokines upon inflammation [32].

Recent studies are beginning to explore other cellular sources of IL-35, in other regulatory cells such as regulatory B cells (Bregs) and CD8(+) Tregs. Interleukin-10 (IL-10) and IL-35 producing regulatory B cells suppress au-

toimmune diseases, and increased numbers of Breg cells prevent host defense to infection and promote tumor growth and metastasis by converting resting CD4+ T cells to regulatory T cells [61–63].

Furthermore, CD4+CD25+ Tregs can be activated to express granzyme A and kill activated CD4+ and CD8+ T cells through a perforin-dependent mechanism and induce galectin mediated apoptosis [5, 8–11, 46–50].

### The role of skin DC in formation and function of Treg

The DCs play an active role in tolerance under steady state conditions through several mechanisms which are dependent on IL-10, TGF- $\beta$ , retinoic acid, indoleamine-2,3,-dioxygenase along with vitamin D. Several of these mechanisms are employed by DCs in induction of regulatory T cells which are comprised of Tr1 regulatory T cells, natural and inducible FOXP3+ regulatory T cells and Th3 regulatory T cells. It appears that certain DC subsets are highly specialized in inducing regulatory T cell differentiation and in some tissues the local microenvironment plays a role in driving DCs towards a tolerogenic response. DCs are a complexed cell population in the skin consisting of epidermal Langerhans cells (LC) and dermal DCs, which differ in their anatomic location, antigen recognition, processing machinery, and migratory capacity. Cutaneous DCs (LCs as well as dermal DCs) function as sentinels that survey invading agents and transmit the information into immune responses by taking up exogenous antigens. Different DC subpopulations may sequentially present skin-acquired antigens, possibly serving as a regulatory mechanism of cell-mediated immunity and adding further complexity to established concepts. Nevertheless, cutaneous DCs are involved in several pathologies (including infections, inflammatory disorders, or skin cancers) and play a pivotal role in regulating the balance between immunity and peripheral tolerance. However, it is widely accepted that cutaneous DC in an immature state may have tolerogenic properties resulting in the induction or expansion of Tregs. It has been shown that immature dendritic cells type-1 (DC1) can induce Treg cells secreting IL-10 and TGF- $\beta$ 1 and also Th2 cells, whereas mature cells DC1 can stimulate production of Th1 and Tc1 cells. Other type of dendritic cells called DC2 promotes Th2 cells [13–15, 63–67].

One of the DC antigens which influence Treg cells formation is CD39 (vascular ATP diphosphohydrolase, apyrase) found that individuals with a single intronic variant (rs11517041) of the apyrase gene, homozygous for the T allele had the lowest number of CD39+ activated CD4+ T-regulatory cells. Heterozygotes were intermediate, and C homozygotes had the highest level of this enzyme, suggesting that this is a quantitative trait locus (QTL) for expression of this gene. Authors concluded that this mutation is a candidate mechanism in which cis-acting variation regulates the expression of a key marker in-



dividual cells and therefore determines the number of cells expressing this molecule influencing Treg formation [62]. The DCs have also the ability to inhibit lymphocyte suppressor GITR-L. This molecule is a ligand to Treg receptor of TNF- $\alpha$  (GITR). The studies on mice and the experiments with human cells *in vitro* have demonstrated that blocking GITR receptor and/or CTLA-4 with the corresponding antibodies causes impairment of DCs suppression of Treg cells. The first clinical trials with tolerogenic DCs have recently been conducted and more tolDC trials are underway. It is worth noting there that the macrophages (regulatory macrophages M-regs) also have the regulatory functions [63–67].

The role of Tregs in the pathology of selected skin diseases will be the clue of Part II of the article.

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### Conflict of interest

The authors declare no conflict of interest.

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