Review paper

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

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Abstract

Regulatory FOXP3+ T cells (Tregs) constitute 5% to 10% of T cells in the normal human skin. They play an important role in the induction and maintenance of immunological tolerance. The suppressive effects of these cells are exerted by various mechanisms including the direct cytotoxic effect, anti-inflammatory cytokines, metabolic disruption, and modulation of the dendritic cells function. The deficiency of Treg cells number or function are one of the basic elements of the pathogenesis of many skin diseases, such as psoriasis, atopic dermatitis, bacterial and viral infections. They also play a role in the pathogenesis of T cell lymphomas of the skin (cutaneous T cell lymphomas – CTCL), skin tumors and mastocytosis. Here, in the second part of the cycle, we describe dysfunctions of Tregs in selected skin diseases.

Key words: Treg dysfunction, selected skin diseases.

Introduction

Regulatory T cells (Treg) represent a distinct lineage of T lymphocytes committed to suppressive functions, and play an important role in the induction and maintenance of immunological tolerance. It is estimated that 5–10% of the T cells resident in normal human skin are FOXP3+ Tregs. Certain T cell populations with regulatory potential, such as transforming growth factor β (TGF-β) – producing Th3 cells, IL-10-producing Tr1 cells, and CD4+CD25^{high}FOXP3+CD127^{negative} Tregs have been described, however, their proper functions are not fully understood. The suppressive effects of these cells are mediated by various mechanisms including direct cytotoxic effect, anti-inflammatory cytokines, metabolic disruption, and modulation of DC function [1–4].

Treg cell dysfunction in autoimmune skin diseases may be classified according to three major mechanisms: inadequate number of Tregs, defective function of Tregs and, resistance of T effector cells to Treg-mediated suppression. An inadequate number of Tregs was described in patients with *FOXP3* mutations, which is manifested as IPEX syndrome (immune dysregulation, polyendocrinopathy, enteropathy, X-linked). Defective function of Tregs may occur through the inadequate expression of cell surface molecules that are known to be involved in contact-dependent suppression such as: cytotoxic T lymphocyte antigen 4 (CTLA4), CD39 (ectonucleotidase), lymphocyte activation gene 3 (LAG3), granzyme A and CD95 (FAS) or as a result of a failure to produce the soluble suppressive factors like: TGF-β, IL-10 and IL-35. In addi-

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tion, the composition of the local milieu, including the types of antigen-presenting cells and cytokines (TNF-α, IL-4, IL-6, IL-12, IL-7, IL-15 and IL-21), can influence Treg cells' function. Cell-intrinsic resistance to suppression has been shown in CD4(+) memory T cells and T helper 17 (Th17) cells. Several cytokines, like IL-2, IL-4, IL-7 and IL-15, support the proliferation of effector T cells, despite the presence of Treg cells [1–5].

Treg dysfunction in the pathogenesis of psoriasis

Psoriasis is one of the most common skin diseases, affecting 2–3% of the European population. Its pathogenesis is not fully understood. A characteristic symptom of the disease is chronic skin inflammation with infiltration of the dermis and subcutaneous tissue with CD4(+) T cells, neutrophils and macrophages, activation of mast cells, infiltration of cytotoxic lymphocytes CD8(+) into the epidermis (Munro microabscess) and the abnormal growth of blood vessels (neoangiogenesis). It is estimated that 10–30% of patients develop arthritis, which can cause permanent disability [6–10].

Both CD4(+) Th1, Th17, Th22 and, Th9 subsets and CD8(+) Tc1 and Tc17 subsets with homing potential into the skin play a crucial role in the pathogenesis of psoriasis [6]. The network of secreted cytokines and chemokines lead to the skin inflammation. The skin lesions are characterized by increased expression of pro-inflammatory cytokines such as TNF-α, IFN-γ, IL-6, IL-8, IL-9, IL-12, IL-17, IL-18, IL-20, IL-22 and decreased concentration of anti-inflammatory cytokines – IL-10 and IL-4. It seems that the principal mechanism of psoriatic lesion development is regulated by TNF-α, IL-17 and IFN-γ. It was proved that subcutaneous administration of IFN-γ induces the formation of psoriatic lesions and exogenous IFN-α may trigger psoriasis development. The IFN-γ may potentiate inflammation-promoting activities in psoriasis by regulating the expression of cytokines that contribute to the trafficking of CXCR3+ T cells, including CD8(+) T cells, into the psoriatic lesions. The IL-17 and IFN-γ synergistically stimulate keratinocytes for the synthesis of IL-6, IL-7, IL-8, IL-12, IL-15, IL-18 and TNF-α [6–15].

Many publications indicated that psoriasis patients have an increased number of Tregs (defined as FOXP3(+)) cells in peripheral blood and inflamed skin of the patient and this increase is positively correlated with the disease activity index [16–20]. In contrast, some authors observed a lower percentage of Tregs in peripheral blood, which correlates with disease severity [21]. Nevertheless, a decrease in FOXP3(+) cell number was observed also in the skin samples obtained from psoriasis patients. It was found in the acute, but not in the chronic course of disease [22].

Recent experiments indicate that the number of Tregs is increased in the skin lesions of psoriasis, but these cells have decreased suppressive activity. The functional defects were deduced from the observation that psoriatic CD4+CD25^{high} Treg cells were unable to expand upon polyclonal CD3/CD28 T cell receptor (TCR) stimulation [23]. Another study found that the efficiency of Tregs derived from psoriatic hematopoietic cells is much weaker in controlling the activation of CD4+CD25– cells than it is in case CD4+CD25+ T cells' population of normal individuals [24]. Another publication demonstrated that psoriatic CCR5(+) Tregs cells are numerically, functionally and chemotactically deficient compared to controls and may pose a triple impairment on the ability of psoriatic Tregs to restrain inflammation [25].

The possible mechanism by which Tregs exhibit decreased suppressive function is partially due to the pro-inflammatory cytokine milieu in the psoriasis lesions, especially of high levels of IL-6 secreted from endothelial, DCs and Th17 cells. IL-6 inhibits Tregs activity and differentiation and enables infiltrating T effector cells for escape from the suppression [26].

Interesting studies are indicating Treg plasticity and the ability of those cells to transform into pro-inflammatory IL-17 secreting T cells. Bovenschen *et al*. [27] demonstrated that Tregs from patients with severe psoriasis were more prone to differentiate into IL-17-producing cells compared to healthy controls upon stimulation. The authors concluded that a specific population of CD4(+)IL-17A(+)Foxp3(+) cells in the skin lesions contributed to the disease development. The epigenetic phenomena may be involved in this process: the activity of specific miRNAs and specific histone acetylation changes by activating the deacetylase enzyme, what could lead to alteration of the chromatin conformation and opening the RNA polymerases reading frames [28–31].

Zhao *et al.* have found that miR-210 is involved in inhibiting FOXP3 expression and modulating the levels of IL-10, TGF-β, IFN-γ and IL-17 in CD4+ T cells, suggesting that overexpression of miR-210 may contribute to Treg cell dysfunctionality rather than to decreased Treg cell numbers in PV patients [28].

The mechanism of Tregs dysfunction in psoriasis and genetic basis of these changes is still unclear. Some authors indicate that mutations related to defective transcription of FOXP3 play an important role in psoriasis pathogenesis [30]. Transition from the symptomless state to skin lesions in psoriasis is related to disturbance of the balance between Tregs and T-helper cells and with changes of the gene expression profile in the skin [31–33].

Kim *et al.* explored models of disease progression by correlating the gene expression profile in three different phases: initial phase, vertical growth (epidermal hyperplasia) measured by epidermal thickness of lesional skin and radial expansion (the extension of the overall psoriasis area and severity). The authors found that in the initial phase, the increased expression of pro-inflammatory

cytokines: IL-17A, IL-8 and IL-1β is present. In the vertical growth phase of psoriatic plaque, additionally to cytokines hyperexpressed in the initial phase, an increased expression of IL-20 and IL-19 is observed. While in the radial growth of a lesion, the increased expression of IL-20, IL-19, IL-17A, IL-8, IL-1β is associated with a high TNF-α expression and with a lower expression of Tregs' function genes: *FOXP3, CTLA-4, CD69, FAS, IL-9*. The study also revealed that non-lesional skin around the psoriatic plaques is not normal. A low expression of FOXP3, FAS and CD69 is observed also in nonlesional psoriatic patients skin [32, 33]. The work of Keijsers *et al.* indicates that a relatively high FOXP3+/CD4 ratio in symptomless skin of patients with psoriasis suggests the presence of an active immune controlling mechanism, distant from the psoriatic plaque [31].

The number of Tregs in the skin depends on sun exposure, photo(chemo) therapy, skin vitamin D production and supplementation or biologic therapy [34–42]. A low level of vitamin D observed in psoriatic patients may decrease the number of circulatory Tregs, and encourage the inflammatory process in the skin. On the other hand, vitamin D treatment induces the synthesis of the cytokines profile which favor Tregs' activity and can prime tolerogenic dendritic cells able to favor differentiation of such cells from naive T cells [34–40]. Also treatment of patients with infliximab or etanercept increased the Tregs cell number with a more diverse T cell receptor repertoire [41, 42].

Tregs dysfunction in the pathogenesis of atopic dermatitis

Atopic dermatitis (AD), also known as atopic eczema, is a chronic relapsing inflammatory disease of the skin. It affects up to 25% of school-aged children and up to 10% of adults and is the most common skin disease. Pathogenesis of AD is not completely understood. Inflammatory infiltrates are facilitated by a defective filaggrin barrier. They are composed of CD4(+) T lymphocytes expressing the antigen of skin colonization (cutaneous lymphocyte antigen – CLA), eosinophils, histiocytes, dendritic cells (DC): Langerhans cells (LC) and IDEC (inflammatory DC – iDC) and mast cells (MC) [43–48].

There are two phases of AD: acute and chronic. The acute phase is characterized by the increased number of DC in the epidermis, as well as an increased expression of the FcεRI receptor with high affinity for IgE on the surface of TNF-α secreting IDEC. This phase is also characterized by an increased production of IgE by B cells and the influx of Th2 phenotype lymphocytes into the skin. The cytokine milieu of IL-4 and IL-5 and decrease of antimicrobial peptide synthesis facilitates *Staphylococcus aureus* infections observed in the majority of patients. In contrast, chronic phase is characterized by the activation of Th1 cells that produce IFN-γ, TNF-α, IL-8, and IL-12, as well as Th17 and Th22 cells [43–48].

The possible role of FOXP3(+) Tregs in the pathogenesis of AD may be confirmed by the observation of the molecular cause of the IPEX syndrome. Patients with IPEX have severe, multiorgan, autoimmune disease: eczema, type I diabetes, increased IgE levels, eosinophilia, food allergy etc. and exaggerated Th2-type responses [49–51].

Nowadays published studies indicate that the immune dysfunction observed in AD is caused by the impaired number and/or function of Treg cells. However, because of the heterogeneity of these cells and the different cell markers studied, the results of different studies are difficult to compare. Also the plasticity of Treg could play a role in AD pathogenesis. These cells, under the influence of specific cytokines, by epigenetic reprogramming (promoter methylation, histone acetylation, microRNA) are able to differentiate into Th1, Th17 or Th2 cells [3–5, 52–60].

Some authors did not observe an increased number of circulating CD4+CD25highFOXP3+ cells in AD but have found their accumulation in the skin lesions [61, 62]. It might be even more complex as some groups questioned the role of FoxP3+ Tregs and pointed at Tr-1 cells secreting IL-10 and TGF-β, which high levels were observed in AD skin [63].

A study by Stelmaszczyk-Emmel *et al.* in children with atopic allergy indicated that Tregs defined as CD4+CD25(high)CD127–CD71+ were significantly less frequent in comparison to healthy controls. The frequency of Tregs in patients with symptoms of atopic dermatitis and/or food allergy was lower than in patients without these symptoms [64].

In contrast, several other publications indicated that Treg defined as CD25+FOXP3+ cells were increased compared to healthy controls in AD patient's blood and skin. In addition, an elevation of Treg cell numbers correlated significantly with AD severity [65–71].

For example Samochocki *et al*. have found an increased number of Tregs in the blood of AD patients. These cells had upregulated L-selectin (CD62L) and CD134 (OX-40) antigens and the decreased expression of apoptotic CD95 receptor suggesting that they were long-lived apoptosis-resistant cells. The concentrations of IL-10 and TGF-β in the CD4(+) lymphocyte culture supernatants and the sera were decreased in AD patients as compared to controls and negatively correlated with the severity of AD [68].

Lesiak *et al.* have found that in the peripheral blood, the percentage of CD4+CD25^{high}FOXP3+ Tregs was significantly higher when compared with the controls. The authors have also found a high percentage of T cells secreting IL-17 and a low percentage of Treg and mononuclear cells expressing TLR2 and TLR4 in the patient's blood. Authors concluded that the increase in the number of cells secreting IL-17 could be associated with acute lesions.

A decrease in the number of cells expressing TLRs may confirm a role of innate immune defects in susceptibility to bacterial and viral infections complicating the course of AD [69].

Because of transient FOXP3 upregulation in effector cells after stimulation without acquisition of the suppressor cell function, the detection of Tregs based on FOXP3 expression may lead to false positive results. The gold standard of precise analysis of human Tregs is the measurement of the DNA methylation status TSDR (Treg specific demethylated region) in the *FOXP3* locus.

Using this method Roesner *et al.* have not found the differences concerning the Tregs number between AD patients and controls. However, Treg density correlated with the severity of the disease defined by SCORAD. A high number of Treg cells could be observed only in severely affected patients [71].

A study by Hinz *et al.* indicated that both genetic (paternal or maternal atopy) and environmental factors (tobacco smoking in pregnancy, increased levels of IL-13, IL-17, IFN-γ) presumably influenced the development of fetal Tregs. Low cord blood Treg numbers may predict early atopic dermatitis in children [72]. An impairment of T-regulatory cells was also found in atopic mothers [73].

There are also other markers describing highly suppressive effector/memory Tregs – ectonucleotidases (CD39 and CD73) which cleave extracellular adenosine triphosphate (ATP) into adenosine. Adenosine binds to several receptors that are expressed on various cells, including T cells and APCs. CD39+ Tregs suppressed IL-1β, IL-2 and IL-17 expression and proliferation of activated T cells. These cells also downregulated the expression of several costimulatory molecules on DCs, such as CD40, CD80, CD86, and CD83 [3–5, 71, 72, 74]. Conflicting data on the role of CD39 Treg cells in AD patients were published [71, 72]. Zhang et al. have found that CD39 and CD73 expression levels on FOXP3(+) Tregs were higher in patients with severe AD than in the controls, but showed an attenuated suppressive function of the proliferation of autologous T-effectors cells [70].

Roesner *et al.* have found a reduction in CD39+ Treg in comparison to the controls. Authors concluded that it could be an effect of IL-7 activity in inflammatory conditions [71].

A notable feature of Tregs is that they express a variety of skin-homing addressins including CCR4, CCR5, CCR6 and cutaneous lymphocyte-associated antigen (CLA) [1–5, 53, 54, 76–78]. An increased expression of CCR4, CCR5 and CCR6 on Treg in AD skin have been reported [53, 70, 76–79]. A high expression of CCR4, the receptor for TARC/CCL17 in AD skin correlated with the severity of disease and higher IgE titers [76]. Some authors indicated that Treg CCR6(+) cells in AD patients have an immunosuppressive function similar to those in healthy controls, however CCR6(–) Treg cells in AD promote a Th2 immune response [53, 58, 77, 78]. The role of TSLP, TARC/CCL17 and other cytokines in proinflammatory "milieu" on AD skin is presented in Figure 1.

There is emerging evidence to suggest that Tregs can convert themselves to Th2 cells and that this pathway is bi-directional. This phenomenon may be a double-edged sword with important implications not only for subverting Tregs in disease, but also for potential treatments designed to amplify the function of these cells in order to suppress the allergic inflammatory cascade in AD [1, 3–5, 52, 53, 56–60].

The conversion of Treg into Treg secreting Th17 cells increase the inflammatory process in AD skin. Ma *et al.* have found imbalance between Th17 and Treg cells [75]. AD severity score positively correlated with the increase in Th17 cells percentage and Th17/Treg ratio, while negatively correlated with Treg cells percentage [3–5, 61].

The bacterial toxins can also influence the function of Tregs in AD [78–81]. It has been demonstrated that *S. aureus* enterotoxin B, acting as a superantigen, inhibits the suppressive function of nTreg [80, 81]. In contrast, antigens from commensal bacterium *Lactobacillus rhamnosus* may activate Tregs and suppress Th1, Th17 and TSLP responses in allergic patients [79]. It has also been suggested that the suppressor function of nTreg is limited mainly to block the function of Th1 cells, which favors the dominance of Th2 responses observed in the acute phase of AD [78, 80, 81].

Reports of some authors indicate that suppression activity of Treg ability to inhibit the proliferation of effector T by Treg lymphocytes are diminished after stimulation with an allergen. In particular, the impairment of the Tregs function during the pollen season was observed in patients with pollen allergy, including the birch pollen allergy [82–87]. It has been shown also that high doses of allergens administered *in vivo* cause an increase in the number of T regulatory cells secreting IL-10 [85].

Recently published data indicated that attenuated inhibitory ability of Tregs on hyper-activated autologous $CD8^+CLA^+$T cells, mediated by TGF-β1, could also play an important role in the pathogenesis of AD [88].

Allergen specific immunotherapy

Allergen specific immunotherapy (SIT) is the only causal treatment of allergic rhinitis and asthma, but still controversial in AD management. Two methods of SIT are currently in use: sublingual (SLIT) and subcutaneous (SCIT). The process of allergens' tolerance development is induced by T cells, B cells, mast cells and basophiles responses. Although quite commonly used – the mechanisms of SIT still remain unclear. Several theories for SIT mechanisms have been suggested so far – one of them considers the induction of Treg population [89]. The switch of the antibody class from IgE to IgG1 and IgG4 is often observed among patients after several months of SIT, which unfortunately does not correlate with clinical im-

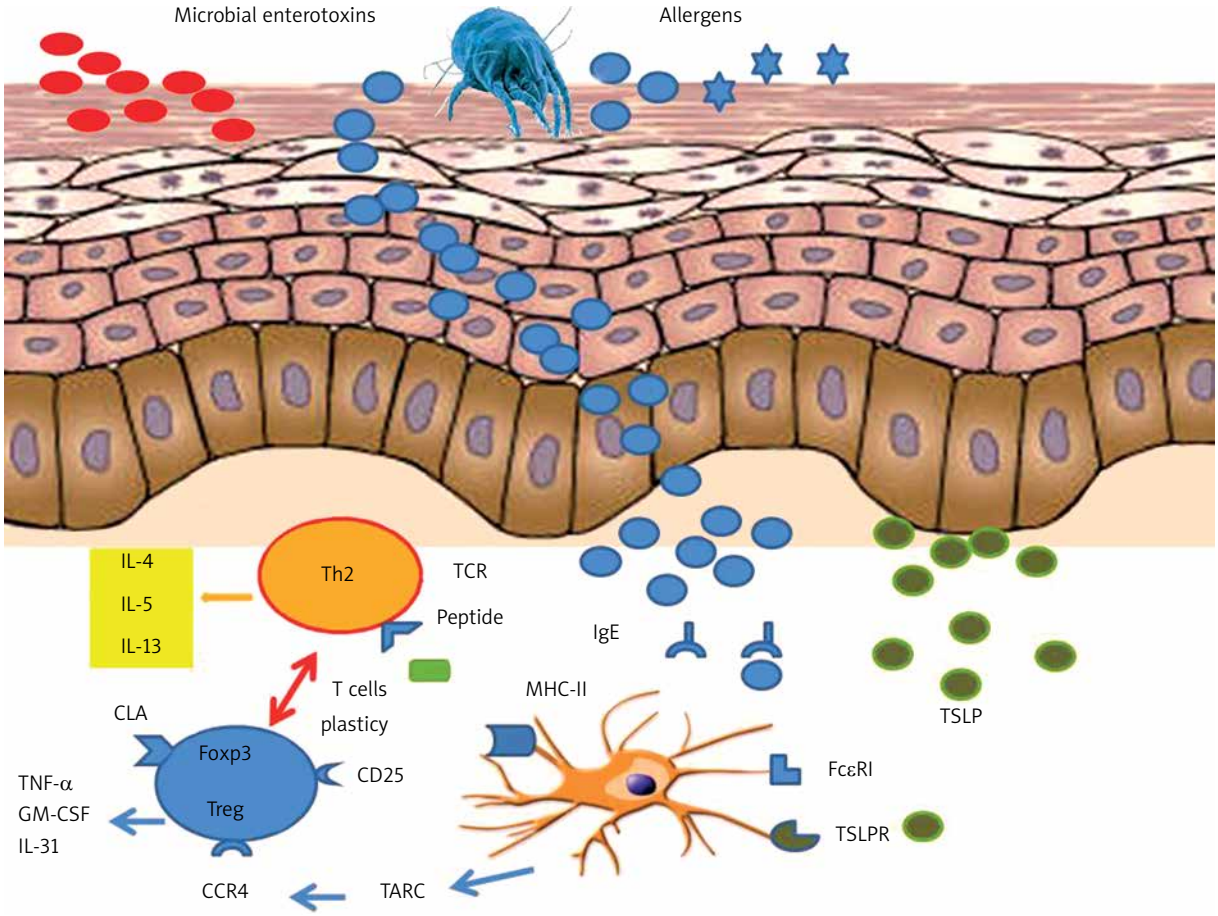

Figure 1. Scheme presenting the "proinflammatory milieu" in the AD skin lesion that may be formed by T cells, dendritic cells and keratinocytes. Dendritic cells play a key role in driving Th2 responses to the allergen and as professional antigen presenting cells (APCs) are also likely to be critical in the development of Tregs (modified from [53])

provement. It can suggest that it is not the main mechanism of tolerance. The studies performed with patients treated with house dust mites immunotherapy (both SCIT and SLIT) revealed an increase in the level of Treg cells, Th1 populations and FOXP3 gene expression, especially in a group that responded well to the treatment. Additional findings were decreased levels of Th2, Th9 and Th17 T cell populations [90, 91]. Similar results were obtained in patients undergoing grass pollen SLIT [92].

Further studies with sensitized mice revealed that the level of Treg cells correlates with the efficacy of SIT [93]. The response of Treg cells may vary according to the SIT type. Nevertheless, it seems to be confirmed that the number of Treg cells in SCIT is significantly higher than in SLIT [94].

A novel tool in immunotherapy is T cell epitope based immunotherapy – the administration of synthetic peptides representing immunodominant T cell epitopes that may alter T cell responses to allergen through an increase in the Tregs number. It may be considered as significant enhancement of immunotherapy with no burden of systemic side effects [95]. There are reports on successful treatment with T cell epitope peptides in animals undergoing SIT with cedar pollen, egg and milk allergens [96–98]. Further studies are needed to ensure safety and efficacy of this future treatment.

The gene expression profile related to immunological mechanisms that might be involved with Treg cells in immunotherapy

The data concerning the gene expression profile in patients treated with immunotherapy are scarce. There were described significant differences in gene expression related to known mechanisms of T-lymphocyte differentiation and activation of mast cells in the whole genome analysis in patients treated with insect venom immunotherapy (VIT) [99]. The gene expression pattern characteristic of effective VIT was present in all patients with successful VIT but absent in all subjects with VIT failure. Moreover, the same gene expression profile was present in the majority (88%) of patients in the maintenance phase. The analysis of differentially expressed genes confirms the involvement of immunologic pathways.

The genes differentially expressed between patients with success and failure of VIT included genes involved in known mechanisms of immunotherapy such as *FcεRI, JAK-STAT, MAPK*, and *Wnt*, calcium signaling pathways, cell signaling or transcription [100]. However genes for *IL-10, IL-4* and *osteopontin* were not differentially expressed. This does not exclude significant differences in RNA expression in subpopulation of cells, like Treg, but they could not be demonstrated in this prediction model. In patients with success of VIT, the *TWIST2* gene coding transcription factor, which promotes the production of IL-10 and decreases the synthesis of IL-4, and also proinflammatory cytokines, such TNF-α and IL-1β [101], were upregulated. This phenomenon may be responsible for the differences in cytokine levels and cell subtypes typical for immunotherapy [99]. The downregulation of *PRLR* (prolactin receptor gene) may indicate a shift toward Th1. There was a decrease in prolactin during sublingual immunotherapy [102] which induces IL-4 dependent IgE and IgG1 response and therefore, the development of Th2 lymphocytes [101, 103]. Another overexpressed gene in patients with success of VIT is claudin (*CLDN1).* This gene product is a structural component of tight junctions and plays a role in adhesion and migration of dendritic cells [101, 104]. It is known that TGF-β increases the expression of *CLDN1*. A higher expression of *CLDN1* in dendritic cell may be related to the role of these cells in Treg differentiation [100, 103].

The role of Treg in the pathogenesis of skin T cell lymphomas

Cutaneous T cell lymphomas (CTCL) is a heterogeneous group of lymphoproliferative malignant disorders in which pathological cell derives from CD4(+) CD45RO(+) T memory cells. Despite extensive knowledge about the function and differentiation of cells, leading to the generation of numerous cell lines, the pathogenesis of CTCL is not fully understood. The profile of Th1 cytokines', including IFN-γ, IL-12 and IL-2, dominant at the beginning of the disease,-decreases with the progression, at the expense of Th2 cytokines: IL-4, IL-5, IL-13 and also IL-10 in the skin and in the peripheral blood. On the other hand, the role of CD8(+) T cells in the skin is reduced. At the initial stages of MF, this reduction is responsible for antitumor activity [60, 61].

Figure 2 presents changes in the number of normal and malignant T cell and Treg cells in disease progression. In patch stage lesions, there is a small population of malignant T cells that primarily are located in the epidermis and non-malignant T cells that preferentially are located in the upper dermis. Importantly, the benign lymphocytic infiltrates contain a relatively large proportion of Tregs that potentially suppress infiltrating immune cells as well as the malignant T cells. In the plaque stage, both the number of malignant and non-malignant T cells increases but the proportion of Tregs in the benign lymphocytic infiltrates remains fairly constant. However, in the tumor lesions, there is a decrease in the number of infiltrating non-malignant T cells. There is a steep decrease in the proportion of Tregs within the benign lymphocytic infiltrates and a large increase in the number of malignant T, which are now primarily found in the dermal compartment. In plaque and patch stage lesions, a population of the malignant T cells might express low levels of FOXP3 but this expression seems to be almost absent in tumor stage lesions [105–116].

Wang and Ke, on the basis of the role in pathogenesis of systemic lymphomas, divided the Tregs into 4 groups: suppressor Tregs, which suppress anti-tumor CD8+ cytotoxicity, malignant FOXP3+Tregs, direct-tumor killing Tregs which increase anti-tumor cytotoxicity (all involved in pathogenesis of CTCL) and incompetent Tregs (found e.g. in angioimmunoblastic T cell lymphoma) [112].

Those subgroups have been found to get different roles in the prognosis and Tregs-based immunotherapies.

In patients with CTCL, where Tregs act as suppressor or malignant Tregs, the anti-tumor cytotoxicity can be suppressed and the decreased numbers of those Tregs are associated with good prognosis. In other lymphomas, when Tregs serve as tumor killers (also in some CTCL) and/or incompetent Tregs, the anti-tumor cytotoxicity can be enhanced and an increased number of such Tregs can be associated with good prognosis [112]. The problem is that in the same type of CTCL e.g. Sezary syndrome, the malignant cells can reveal the different suppressor phenotype what can cause a different prognosis and might require different treatments [113].

Following these ideas, one theory on the pathogenesis of CTCL assumes that tumor cells escape from immune surveillance and at the same time keep suppressor properties [105, 106, 113]. It has been shown that the number of Tregs, in the tumors infiltrates is higher in the early stages of CTCL (e.g. in mycosis fungoides, MF), and decreases in advanced forms (SS) [106–113]. In contrast, other studies indicate that the resistance to drugs observed in late stages of CTCL is associated with the activation of mutations signaling pathway JAK/STAT, impaired secretion of immunosuppressive cytokines IL-10, TGF-β1, and overexpression of immunoregulatory proteins – CTLA-4, PD-L1 and Foxp3, which promotes the immunosuppression of the immune system [111–113]. The percentage of malignant T cells, identified as FOXP3+, is variable in these patients. In some patients, practically all of the malignant T cells express FOXP3, whereas in other patients, FOXP3 is only expressed in a sub-population of malignant T cells indicating heterogeneity in the malignant population. Taken together, these studies suggest that SS patients in general have a low density of benign Tregs but that the malignant T cells in some SS patients express FOXP3 and that these patients accordingly have

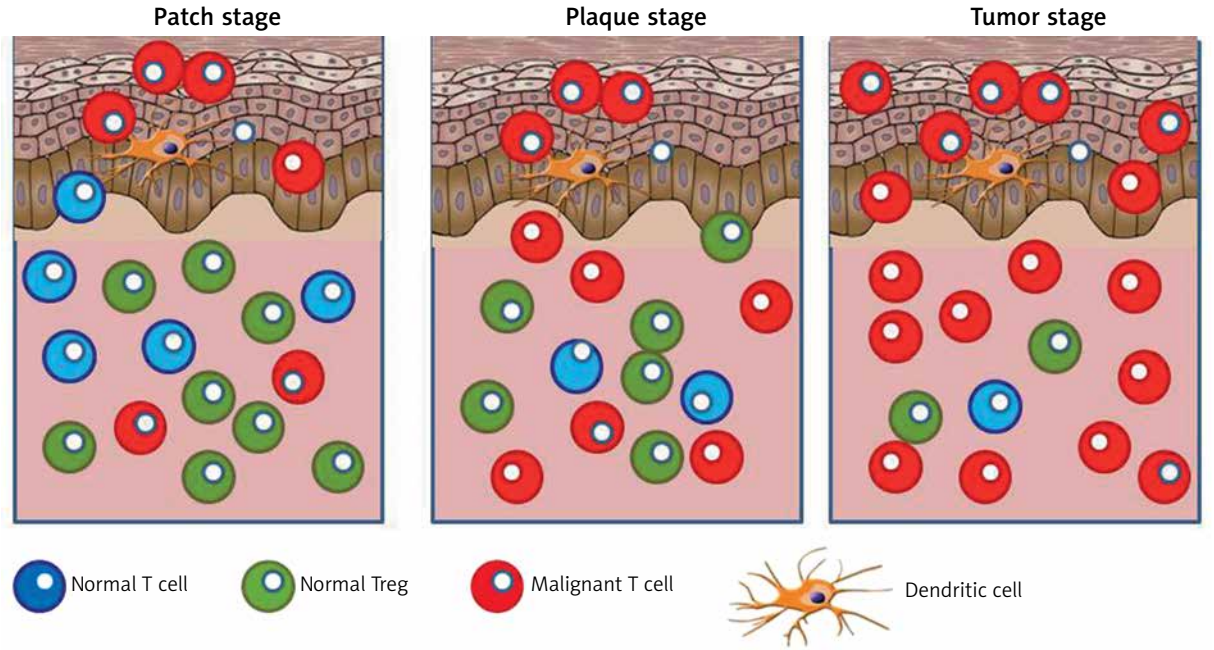

Figure 2. Schematic illustration of changes in the numbers of malignant and non-malignant T cells in MF skin lesions during disease progression

relatively high numbers of FOXP3+ cells in their skin and blood (Figure 2).

Another interesting problem is the differential diagnosis between CTCL and severe AD, because of similarity of skin manifestations, especially in erythroderma. Hanafusa *et al.* have revealed that the frequency of Tregs was significantly higher in AD skin lesions compared to those in CTCL, which could be helpful in the differential diagnosis of these two diseases in the future [117–119]. Understanding the relationship of Tregs to CTCL might be crucial in the improvement of the treatment efficacy. For example, it is known that retinoids promote the generation of CD4+ Foxp3+ Tregs, raising the question of an induction of Tregs by bexarotene. Knol *et al.* have established that the frequency of CD4+CD25(high) Treg cells was not significantly different before starting bexarotene and after 6 months of treatment in CTCL patients (MF and SS). Also the functional assays demonstrated that Foxp3 expressing CD4+CD25+^{high} T cells were capable of suppressing autologous CD4(+)CD25(–)T cell proliferation [114].

The authors wrote that their results did not exclude a role for bexarotene in the control of regulatory T cells in CTCL but further studies are necessary [114]. Authors have also found that CD4+CD25+^{high} Treg cells in SS but not in MF were significantly increased, compared to healthy donors before treatment. However, the authors also emphasized that the interpretation of that result should be very careful [114] because the prevalence of CD4+CD25+^{high} Tregs seems to be age-dependent [120] and there are various views on the problem. There are studies revealing differences in CD4(+)CD25(+)Treg percentage between CTCL patients and healthy controls with a lower percentage of CD4+CD25+^{high} Tregs in blood of healthy controls than in SS, as well as studies revealing no differences at all [107, 108]. Those incompatibilities can be caused by differences in methodology used in those studies. The knowledge concerning Tregs in CTCL can be also helpful in the treatment choice in some cases. For example, it is known that extracorporeal photopheresis alone or in combination therapy might be effective in leukemic CTCL patients whose malignant T cells have a CD4(+) CD25(–) Foxp3(+) phenotype [121].

The role of Treg cells in the tumors of the skin

Treg cells by inhibiting the effector function of many cells may cause immune suppression of the immune system, which can encourage the growth of tumor cells [3, 5]. In many tumors, a correlation between the tumor stage and an increased percentage of Treg cells has been noted. Tregs inhibit the effector cells, which are responsible for tumor lysis. Increased percentages of Treg cells have been observed in patients with melanoma, lung, breast and ovarian cancers [122–127]. However, the role of these cells in the development of cancer is not fully understood. Basal cell carcinoma (BCC) is an immunogenic neoplasm. The tumor tissue is infiltrated by regulatory CD4+, CD25+, Foxp3+ T cells and immature dendritic cells. The immune response seems to play a major role in spontaneous and pharmacologically induced (imiquimod) BCC regression [123].

It has been shown in immunosuppressed patients with organ transplants that the progression of SCC is associated with a reduction in the number of FOXP3 + cell infiltration around the tumor. It is also known that an

aggressive tumor growth is associated with an increase in the number of Tc22 and Treg cells [124, 125]. In turn, impairment of Tregs function observed in the elderly is associated with a higher incidence of BCC and SCC [126]. Studies of Kaporis *et al*. [123] showed that the increase in the CD4 + CD25 + FOX3P+ infiltration of the surrounding of BCC, promotes tumor growth by the development of Th2 response in the tumor environment and by reducing the activity of Th1 cells [123].

Tregs in mastocytosis

Mastocytosis is a heterogeneous group of rare myeloproliferative disorders characterized by the pathological accumulation of MC in different tissues. Lesions are observed in the skin (cutaneous mastocytosis – CM) or in internal organs, particularly bone marrow, liver, spleen and lymph nodes (systemic mastocytosis – SM). The pathophysiology of the disease is not yet completely understood. It is believed that mechanisms responsible for the proliferation and maturation of MC play the main role in the process. Somatic activating mutation of c-KIT oncogene, encoding the receptor for stem cell factor (SCF) is observed in mastocytosis. This mutation, especially D816V, is considered the main factor-inducing systemic mastocytosis. In the case of cutaneous mastocytosis, its etiopatomechanism is not completely understood. It is believed that an important potential role is played by local MC of the skin and epidermal growth factors, cytokines, and chemotactic factors which stimulate/inhibit the proliferation of MCs and their migration into tissues. Based on histopathology of the skin biopsies obtained from patients with mastocytosis, it has been concluded that the predominant mast cell infiltration is accompanied by lymphocyte subpopulations predominantly CD4(+)CD25(+), suggesting involvement of these cells in the pathogenesis of cutaneous mastocytosis. Nevertheless, the role of Treg cells in the pathogenesis of mastocytosis has not been studied yet and remains unclear [128–130].

Evidence of possible MC-Treg interaction, through a variety of molecular mechanisms, comes from different experiments with animals and of the human disease model. MCs secrete IL-2 which may promote the formation and proliferation of Tregs. MC surface ligand ICOSL activate ICOS (inducible costimulator), expressed on activated T cells and switch these cells to IL-10 secreting Tregs. IL-10 secreting Tregs can suppress migration and expansion of MCs and inhibit focal mastocytosis and polyposis in a model of colon polyposis.

On the other side, MCs may directly or indirectly sustain the availability of inflammatory signals that locally inhibit Treg suppression. The cytokine IL-6, released by innate and adaptive cells on activation, is known to break Treg anergy and suppression and to transform Tregs into Th17 cells. IL-17 increases the production of SCF by keratinocytes, which in turn is a critical mediator of mast cell activation, expansion and maturation. In many diseases, the plasticity of Treg–mast cell interactions which regulate immune response and play the role in tumor formations, was indicated. Conventional, anti-inflammatory FOXP3+ nTregs, through direct contact OX40/OX40L-mediated crosstalk, suppress histamine degranulation by mast cells, thus controlling systemic anaphylaxis, one of the major threats in mastocytosis. Crosstalk between Tregs cell membrane bound TGF-β1 and TGF-β1R inhibit degranulation of MC and increase secretion of IL-6, which diminishes suppressive activity of Treg and their conversion to proinflammatory Th17 cells. Tregs infiltrating skin allografts produce granzyme B to directly suppress T cell response and secrete IL-9 to recruit tolerogenic mast cells and establish tolerance to alloantigens. IL-10 secreting Tregs can suppress migration and expansion of MCs and inhibit focal mastocytosis and polyposis in a model of colon polyposis [131–136].

In contrast, proinflammatory Tregs, which fail to secrete IL-10, produce IL-17 and promote MC recruitment and expansion. Mast cells produce huge amounts of TNF-α which in an autocrine manner may also enhance Tregs conversion into IL-17–producing cells (Figure 3).

Interesting data, which indicate a potential role of Tregs in systemic mastocytosis, have been published by Rabenhorst *et al.* Authors demonstrated that adult patients with mastocytosis show increased serum levels of PD-L1 (programmed death-1 ligand). Levels of PD-L1 correlate with the severity of the disease and serum tryptase levels. Furthermore, PD-L1 levels are enhanced in supernatants of cell lines that carry the KIT mutation D816V. These findings suggest the possible usefulness of PD-L1 levels as a diagnostic marker indicating the disease progression in adult patients with mastocytosis [137]. Programmed death-1 (PD-1) is a PD-L1 receptor and is a key immune checkpoint receptor expressed on activated T cells, B cells, dendritic cells and cutaneous MCs. After ligation with PD-1, PD-L1 and PD-L2 deliver a signal that inhibits TCR-mediated activation of IL-2 production and T cell proliferation. In humans, PD-L1 is expressed on dendritic cells, monocytes, and activated Treg cells, whereas expression of PD-L2 is restricted to dendritic cells and monocytes. Upregulation of PD-L1 may allow cancers to evade the host immune system [138–140].

Kataoka *et al.* have found the expression of PD1 in the skin of mastocytosis patients and conclude that this MC receptor PD-1 could be a marker for human cutaneous mastocytosis and regulate the growth of human PD-1-positive mastocytosis cells. Currently tested PD-1 antibody could be considered treatment of mastocytosis patients [141].

In conclusion, as it is revealed in the review, the role of Tregs in inflammatory and neoplastic skin diseases is important but not completely understood. Further

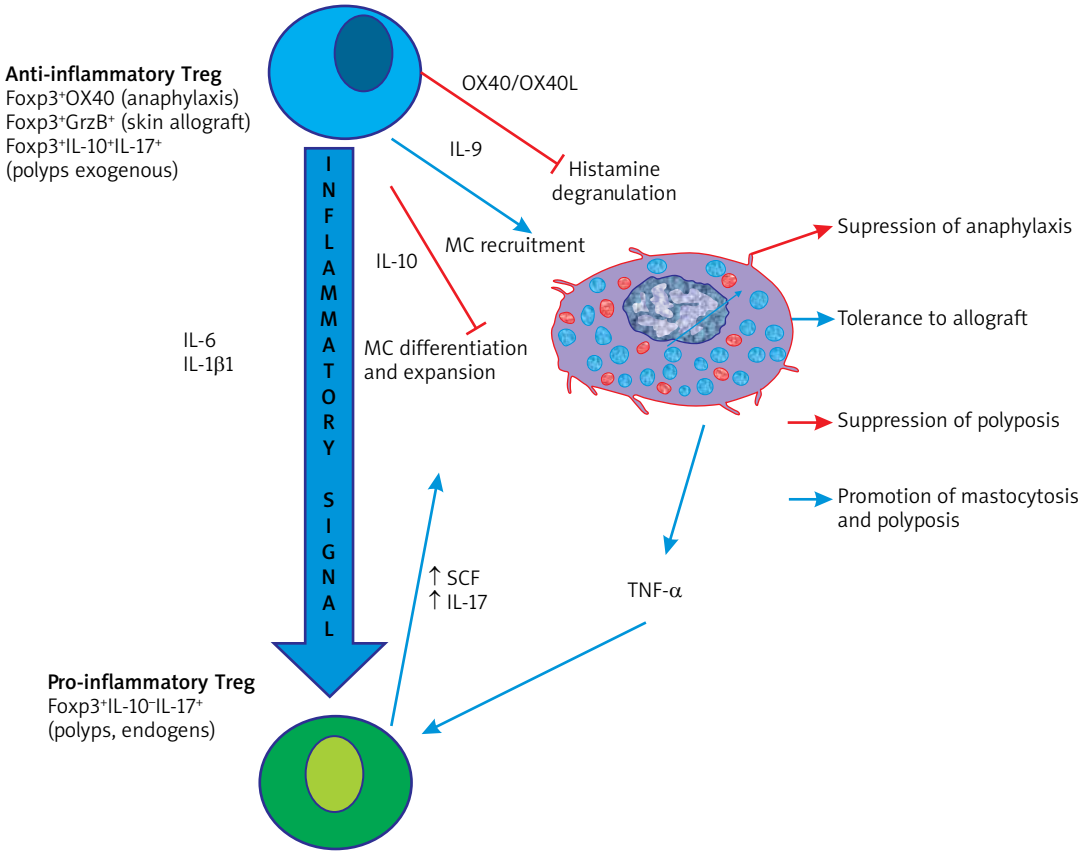

Figure 3. The role of mast cells in Treg activation and suppression

research should be performed, especially that in some diseases knowledge on the Tregs status may be nowadays, the basis of new successful treatments which modulate the Tregs number and function. For example, mogamulizumab (KW-671) is a new defucosylated anti-CCR4 monoclonal antibody which reduces the numbers of CCR4+ malignant T cells and CCR4+ Treg cells in cutaneous T cell lymphoma [142]. New strategies based on *ex vivo* proliferation and transplantation of autologous Tregs in autoimmune and allergic diseases have been recently developed [143].

The difficulties in understanding the role of Tregs in dermatoses can be even higher because of possible polymorphisms of genes involved in regulation of Tregs, which will be discussed in the third part of the article.

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Conflict of interest

The authors declare no conflict of interest.

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