Review paper

The role of regulatory T cells and genes involved in their differentiation in pathogenesis of selected inflammatory and neoplastic skin diseases. Part III: Polymorphisms of genes involved in Tregs' activation and function

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Adv Dermatol Allergol 2017; XXXIV (6): 517–525
DOI: https://doi.org/10.5114/pdia.2017.67053

Abstract

Regulatory T cells (Tregs) represent a cell type that promotes immune tolerance to autologous components and maintains immune system homeostasis. The abnormal function of Tregs is relevant to the pathogenesis of several skin diseases like psoriasis, atopic dermatitis, systemic lupus erythematosus, cutaneous T-cell lymphomas, and skin cancer and is also important in rheumatoid arthritis, diabetes and other autoimmune diseases. In this review, we will summarize the role of mutations and/or polymorphisms of genes involved in Tregs development, and functions in the pathogenesis of selected skin diseases.

Key words: Tregs gene polymorphisms, skin diseases.

Introduction

Single nucleotide polymorphisms, frequently called SNPs, are the most common type of the genetic variation among people. SNPs occur normally throughout a person's DNA. They occur once in every 300 nucleotides on average. Of estimated 10 million common SNPs, those with minor-allele frequency constitute at least 5% [1].

The polymorphisms lead to the substitution of SNP depending on where they occur in the structure of the gene. Their location may lead to altered expression or an altered protein function coded by the gene. Polymorphisms in the gene regulatory sequences (promoter, enhancer sequences, and splicing sites) could alter the binding sites of transcription factors and lead to a reduction or increase in the transcription rate of a gene. Polymorphisms in the coding sequences of the gene may lead to changes in the activity of the encoded protein (missense mutations) or absence of protein production (nonsense mutations). Polymorphisms in the non-coding sequences (introns) may modify the formation of m-RNA (m-RNA splicing) and give rise to non-functional transcripts, which may in translation processes produce abnormal proteins [1]

A number of publications describe the relationship of genetic polymorphisms and the formation of skin diseases, but relatively few papers examine polymorphisms associated with the activation and function of T regulatory cells (Tregs) [2–19].

Polymorphisms of the FOXP3 gene

The main gene associated with the differentiation and regulation of Tregs functions is *FOXP3* (fork head BOX P3; locus Xp11.23). FOXP3 is a member of the fork-

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Received: 8.12.2016, **accepted:** 10.01.2017.

winged helix family of transcription factors (named scurfin), and plays an important role in the development and function of naturally occurring CD4 (+) CD25 (+) Tregs. Scurfin activates the promoters of *IL-10, TGF-β1, IL-35, GITR, CTLA4* and *CD25* genes and blocks the promoters of certain cytokines' genes (*IL-2, IL-4,* and *IFN-γ*). This situation favors the formation of Tregs cells with a suppressive phenotype. Activation of FOXP3 transcription occurs upon binding IL-2 or TGF-β1 to their receptors: IL-2R (its part is CD25) or TGF-β1 receptor. Ligation to the receptors causes activation in the Treg cells' IL2-R/JAK /STAT5 or TGF-β1/SMAD/STAT5 signaling pathways. Activation of these pathways leads to production of Tregs specific suppressive cytokines (IL-10, IL-35 and TGF-β1) and increase expression of membrane receptors (GITR, CTLA4 and CD25) [2–12].

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, known as scurfy mice and IPEX (*immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, OMIM – *300292*) in men [2, 3].

A series of FOXP3 gene polymorphisms that may modify its function and affect the formation of Treg cells has been described. They are located in the promoter sequence, as introns and exons [2, 4–12]. There are five single-nucleotide polymorphisms (SNP) in the promoter region of FOXP3: –924A/G (rs2232365), –1383C/T (rs2232364), –2383C/T (rs3761549), –3279C/A (rs3761548) and –3499A/G (rs3761547). By modifying the transcription factor binding sites, promoter polymorphisms vary the rate of gene transcription. For example, polymorphism –3279 C/A affects the binding of transcription factors E47 and C-Myc. The result is a significant reduction in the FOXP3 gene transcription in the homozygotes AA. The association of this polymorphism with psoriasis, asthma, food allergy, allergic rhinitis allergy to airborne allergens and IPEX syndrome in different Asian and European populations has been demonstrated [2–14]. The 3279 C/A (rs3761548) polymorphism in the FOXP3 gene was also associated with the development and intractability of Graves' disease (GD) [7], and with lower anti-dsDNA levels in female systemic lupus erythematosus patients [8].

The cell-surface CD25 expression is critical for maintaining of the immune function and homeostasis. As it was reported for few cases, patients with a different frameshift mutation in the *IL2RA* gene leading to a CD25 null phenotype with clinical manifestations comparable to CD25 deficiency, manifest with severe autoimmune enteritis and viral infections. In patients' blood, $CD4^+FOXP3^+CD127^{low}$CD25null Tregs could be detected. Moreover, increasingly proliferating $CD8^+$ T cells, which failed to respond to pathogens, infiltrated the skin [13].

A polymorphism of the IL2RA gene is observed in several autoimmunological and skin allergic diseases [15–21]. Hoffjan *et al.* analyze polymorphisms of IL-2RA (CD-25) and IL-7RA genes in a case control study in the large German population of atopic dermatitis patients. Authors have found that two polymorphisms of IL-7R gene are the risk factors for AD (rs6897932 in exon 6 and rs987106) [21]. Evaluation of 4 SNP in the IL-2RA gene revealed no significant association for AD studied cohort. IL-7RA (locus 5p13) and IL-2RA (locus 10p13) genes are located in the region associated with AD and asthma. The IL7RA gene encodes IL-7Rα chain receptor for thymic stromal lymphopoietin (TSLP), a crucial mediator of allergic inflammation in the skin [22]. IL-2Rα is essential for developing Tregs, the serum level of soluble IL2Rα is elevated in AD and psoriasis patients [23].

Polymorphisms of the *STAT* genes

The STAT (signal transducer and activation of transcription) genes code cell factors that play a key role in the activation and differentiation of functional T lymphocyte subpopulations: Th1, Th2, Th17 and Tregs. There are 7 types of STAT factors: 1–4, 5a, 5b and 6 [24].

Several studies have shown that *STAT* genes' polymorphisms may play a role in the pathogenesis of inflammatory and allergic skin diseases and also in skin cancer [25–51].

STAT-1 is activated by IL-12, promotes the expression of the transcription factor T-bet, and the development of Th1 cells producing IFN-γ, TNF-α, and TNF-β1 [2, 24].

STAT6 is activated after binding to IL-4 receptor (IL-4R) of two ligands: IL-4 or IL-13. Activation of the STAT6 gene (locus 18q22.2, OMIM) promotes the expression of the transcription factor GATA3, which is involved in increasing synthesis and secretion of IL4, IL-5 and IL-13 and induces the differentiation of Th0 cells to Th2 cells [2, 24, 33].

The STAT3 pathway is activated by IL-6, IL-23 or TGF-β1 and promotes the expression of the transcription factor RORyt, which leads to the secretion of IL-17A, IL-17F, IL-6, IL-22 and TNF-α and to the development of Th17 cells. In contrast, activation of the STAT5 pathway in cells is stimulated by IL-2, activating membrane IL-2R and leads to the activation of FOXP3 synthesis and to the formation of Treg cells [2, 24–31].

Mutation in the *STAT3* gene (locus 17q21.2) is the cause of autosomal dominant Job's syndrome (hyper-IgE syndrome – HIES, OMIM 147 060), which is characterized by impaired immunity involving a decrease in the number of Th17 cells [2, 26–29]. Keratinocytes and bronchial epithelial cells, unlike fibroblasts and endothelial cells, show much higher dependence on Th17 cytokines for their production of anti-staphylococcal factors, including neutrophil-recruiting chemokines and antimicrobial peptides. The result is the increasing incidence of skin infections with *Candida* and *Staphylococcus aureus* and production of excessive amounts of IgE in HIES patients.

Clinically this rare primary immunodeficiency syndrome is defined by the triad of symptoms: recurrent abscesses of the skin and the lungs, severe eczema and extremely elevated levels of IgE in the serum. To date, about 230 unique STAT3 mutations have been identified in patients with AD-HIES. The most common hotspot mutations are R382W/Q and V637M [2, 25–32].

Activation of the STAT3 pathway leads to the expression of CD39 and CD73 ectonucleotidases, which are enzymes necessary for the immunosuppressive function of Tregs. Fletcher *et al.* showed that only the CD4(+)CD25^{high}CD39(+) cells, which were predominantly FoxP3(+), suppressed IL-17 production, whereas CD4(+) CD25^{high}CD39(–) T cells produce IL-17 [30]. An examination of T cells from multiple sclerosis patients revealed normal frequency of CD4(+)CD25(+)CD127$^{(low)}$FoxP3(+) cells, but interestingly, a deficit in the relative frequency and the suppressive function of CD4(+)CD25(+)CD127$^{(low)}$ FoxP3(+)CD39(+) Treg cells. Authors concluded that CD39 and CD73 expressing Tregs are involved in the control of the inflammatory autoimmune disease [30].

There were 341 known SNPs of the *STAT3* gene; variants Tyr705Phe and Arg609Ala (both in the SH2 domain) led to a defective phosphorylation of STAT3. Studies of Wjst *et al.* indicated that there was no association of any of 27 *STAT3* variants with asthma, allergic rhinitis or eczema [2, 31]. In addition, neither total and specific IgE and eosinophil count nor any lung function parameter showed any significant association. However, when combining high eosinophil counts and high total IgE levels to an HIES-like trait, four SNPs in the 5-UTR of *STAT3* were slightly over-transmitted [31].

The studies on the cutaneous T cell lymphomas (CTCL) cell lines have demonstrated that antihistamine drugs inhibit the expression of STAT3, thus increasing apoptosis of cancer cells [32]. STAT3 has been proved to be constitutively active in CTCL and can increase survival and resistance to apoptosis in malignant T cells, promote Th2 and Th17 phenotypes in advanced disease and induce expression of miR-21, which is a direct STAT3 target in Sézary cells. Silencing of miR-21 in Sézary cells results in increased apoptosis, suggesting a functional role for miR-21 in the leukogenic process [33, 44]. STAT3 induces synthesis of cytokines (IL-5) involved in eosinophilia and erythroderma observed frequently in CTCL. In addition, STAT3 signaling in CTCL was shown to upregulate VEGF, IL-10 and a suppressor of cytokine signaling-3 (SOCS3) and to interfere with IFN-γ synthesis regulation [36]. STAT3 also upregulates IL-2RA (IL-2 receptor α), what results in increased sensitivity to IL-2 signaling, and has been exploited for CTCL therapy – with denileukin diftitox [39]. Netchiporouk *et al.* summarized that also other STATs are established as playing a central role in the pathogenesis of CTCL [40]. STAT 5 upregulation in early stages can drive the expression of miR-155 oncogene, which targets STAT4 and switches the phenotype from Th1 to Th2. STAT 3 activation at late stages can cause resistance to apoptosis, expression of miR-21 oncogenic microRNA and upregulation of IL-5, IL-10, IL-17 and IL-21 signaling, all leading to the promotion of carcinogenesis [37]. Sibbesen *et al.* [41] have proved that miR-22 expression is down-regulated in CTCL malignant T cells and proposed a molecular mechanism, where aberrant Jak3/STAT signaling leads to STAT3/STAT5 activation and binding to its cognate sequence on the miR-22HG promoter what can then lead to a direct transcriptional repression of the gene. The authors have demonstrated that microRNA can cause the down-regulation of many oncogenes including validated miR-22 targets *MAX, MYCBP, histone deacetylases* (*HDAC4, HDAC6*), *CDK6,* and *NCoA1* genes. Their findings suggest that Jak3/STAT-mediated repression of miR-22 plays a key role in the pathogenesis and progression of CTCL, what can lead to creation of novel therapeutics in CTCL. The role of miRNA in pathogenesis of CTCL is confirmed by other authors [42–44]. STAT4 and STAT6 are inversely regulated in CTCL and loss of STAT4 is a poor prognostic marker for early MF and can be a diagnostic marker for leukemic CTCL. It is known that treatment with HDAC inhibitors is able to restore the balance between STAT4 and STAT6 expression. Further research into the molecular pathogenesis of JAK/STAT signaling in this cancer may enable us to develop effective therapies for our patients [42].

STAT5 activation is necessary for Tregs development [45–48]. Activation of IL2R complex by IL-2 leads to phosphorylation of the cytoplasmic domain of IL-2Rβ by kinases JAK1 and JAK3 and to recruitment and activation of STAT5 and rapid conversion of progenitors into natural FOXP3(+) Tregs. The *FOXP3* gene includes regulatory sequences for STAT5. It has been shown that a mutation of the gene for human STAT5 leads to immune disorders associated with the lack of expression of CD25 and FOXP3 and a decrease in the number of Tregs [45].

Studies using techniques, such as genome scans, have revealed that the gene polymorphism of the JAK/STAT affects the risk of developing basal cell carcinoma [46]. Inhibitors against MEK1/2, STAT5 and STAT6 were able to decrease survival in combination with radiotherapy in head and neck cancer lines [47]. There is no data concerning the role of polymorphisms of genes STAT in mastocytosis. However, hyper expression of STAT5 in neoplastic mast cells in mastocytosis was documented [48, 49].

STAT6 gene polymorphisms have been revealed as associated with asthma, atopic dermatitis, and increased levels of IgE in allergic diseases. The polymorphism in intron 2 (rs324011) showed an association with total IgE in AD patients [50]. It has been also demonstrated that gene polymorphism STAT6 shows an association with decreased expression of Tregs genes (*FOXP3, GITR*) in fetal blood of children with early atopic dermatitis (AD) occurring before the age of 3 [51].

Polymorphisms of the *CTLA4* gene

CTLA4 is known as a surface receptor of Treg cells, which, through the activation of CD28 on Th1/Th2 cells, can inhibit the differentiation and the synthesis of the produced cytokines. By interaction with antigen presenting cells, it inhibits the activation of effector T cells [52]. Its mutation in the mouse can lead to the formation of lethal lymphoproliferative syndrome with proliferation of the Th2 phenotype [53]. In humans, mutations in CTLA4 result in CTLA-4 autosomal dominant haploinsufficiency or impaired ligand binding and finally in disrupted T and B cell homeostasis and a complex immune dysregulation syndrome. Patients with this mutation presented with a complex, autosomal dominant immune dysregulation syndrome characterized by hypogammaglobinemia, recurrent infections and multiple autoimmune clinical features. Whereas Treg cells were generally present at elevated numbers in these individuals, their suppressive function, CTLA-4 ligand binding and trans-endocytosis of CD80 were impaired [2, 54].

A number of *CTLA4* gene polymorphisms related to psoriasis, atopic dermatitis, malignant melanomas of the skin, and certain lymphomas, were described [55–68]. It has been shown that certain haplotypes of polymorphism *CTLA4* (49 AG/60 CT) increase the risk of AD [56]. The polymorphism of the *CTLA4 C60T* gene or the haplotypes 49 AG/60 CT can reduce the risk of psoriasis and skin tumors (basal and squamous cell carcinomas, BCS and SCC) [58–62].

In advanced stages of mycosis fungoides, an increased expression of CTLA4 was observed. The role of the *CTCLA4* gene polymorphism in the pathogenesis of lymphoma has been indicated by Monne *et al.*, which showed that the risk of non-Hodgkin lymphoma is increased in patients with genotype + 49AA *CTLA4* gene [65]. This has not been confirmed in studies of lymphoma conducted by Liu *et al.* [63].

It has been shown that the polymorphism of *CTLA4* gene can influence the number and function of Tregs. Jonson *et al.* have observed that people with a genotype + 49GG *CTLA4* gene have reduced numbers of Treg cells [68].

The polymorphism of the *GITR* gene

GITR (glucocorticoid-induced tumor necrosis factor receptor-related protein) is one of the molecular markers of Tregs, which is highly expressed on the surface of these cells. Activation of GITR by the GITRL presented on dendritic cells leads to impairment of the Tregs function [69, 70]. Tomizawa *et al.* [70] demonstrated that the *GITR* gene polymorphisms play a role in regulating the number of Tregs and affect the severity of clinical symptoms of autoimmune thyroid disease. Some interesting work on the expression of genes involved in the regulation of the Tregs function in cord blood demonstrated that the *IL-10* gene polymorphism and STAT6 are associated with a reduced expression of mRNA of *FOXP3* and *GITR* genes in children who developed AD before the third year of age [51]. No data concerning the role of the *GITR* gene polymorphism in the pathogenesis of psoriasis, CTCL and mastocytosis can/could be found in PubMed/literature available.

The polymorphisms of selected cytokine genes and skin diseases

Cytokines play an obvious role in the regulation of proliferation and function of the main regulatory cells. It especially applies to IL-2, IL-6, IL-10 and TGF-β1.

Interleukin-2 (IL-2) is considered to be the main factor in the growth and differentiation of Tregs, both natural and induced. This cytokine, by activation of the receptor, leads to activation of the JAK/STAT5 pathway and subsequently to an increase in membrane synthesis of FOXP3 and IL-2 receptor (CD25 antigen) [71]. There are few studies published on the gene polymorphism of IL-2 and psoriasis, AD and basal cell carcinoma, but there is nothing in literature concerning IL-2 polymorphisms in CTCL or mastocytosis [72–77]. Studies of the IL2 (CD25) gene polymorphisms did not show its relationship with AD [74]. Sobjanek *et al.* hade revealed the association of *IL-2* –330 GG genotype and allele G with an increased risk of BCC [75, 76]. A similar observation was made in patients with nasopharyngeal, breast and urinary bladder cancer, non-Hodgkin's lymphoma and neuroendocrine tumors of the pancreas and gastrointestinal tract. The authors also noted a link between more advanced and multiple tumors and –330 GG genotype. These results strongly suggest that –330 T/G *IL-2* polymorphisms affect BCC susceptibility and the clinical course of this disease, at least in the Polish population. Finally, authors have noted significantly lower concentrations of IL-2 in patients with more advanced diseases and multiple tumors [76].

Interleukin 6 (IL-6) is a glycosylated polypeptide of molecular weight 21–28 kDa, composed of four long α-helices connected by loops. It is a typical secretory protein, which is produced together with the N-terminal signal peptide. The gene encoding IL-6 is located on chromosome 7, and comprises five encoding segments (exons). The promoter sequences of this gene can bind transcription factors such as NF-κB, AP-1, C/EBP and CREB. They regulate gene transcription of IL-6 in a manner dependent on the cell type and an activating agent, wherein a CREB repressor and the other factors are activators of transcription [78]. IL-6 promotes the development of autoimmune response and weakens the suppressive mechanisms of the immune response as a result of the fact that it significantly affects Tregs. The studies on mice had suggested that IL-6 impairs the suppressive activity of Tregs, weakens the sensitivity of the effector cells to the suppressive function of Tregs and inhibits de novo

production of Treg cells in the acquired mechanism of response [79]. It was also shown that Tregs may lead to the increase in IL-6 secretion by mast cells via TGF-β, which is secreted by Tregs. IL-6, which in turn can be produced by mast cells, monocytes, macrophages, fibroblasts, lymphocytes and endothelial cells, is involved also in hematopoiesis, induce inflammatory response and modulate the immune response process of carcinogenesis. The correlation has been shown between an increase in the IL-6 level and the involvement of internal organs in mastocytosis [78–80].

It is also known that the polymorphism –174 G/C (rs1800795) and/or –597G/A of the *IL-6* gene has been shown to affect this cytokine expression and has a connection with psoriasis, systemic mastocytosis and BCC [81–93].

Boca *et al.* have found that minor alleles homozygous genotype of both IL-6 (rs1899795 CC) and IL-12B SNP rs6887685 and absence of HLA-cw6 was associated to a 96.2% decreased risk of psoriasis [88].

Rausz *et al.* have found no association of mastocytosis and the IL6-174G/C polymorphism, however, analysis of the IL6R Asp358Ala polymorphism showed that carriers of the AA genotype had a 2.5-fold lower risk of mastocytosis than those with the AC or CC genotypes [85]. Ruiz-Padilla *et al.* have found that IL-6 -174G/G genotype confers a higher risk of failure in therapeutic response to Lefludomid in Mexicans patients with rheumatoid arthritis [82].

Transforming growth factor beta (TGF-β1) is a peptide with a broad activity playing a pleiotropic role in proliferation, differentiation, and migration of cells involved in the regulation of apoptosis and stimulation of the synthesis of extracellular matrix proteins and angiogenesis. TGF-β1 is the primary regulator of the immune response along with anti-inflammatory and immunosuppressive agents. It inhibits the differentiation of Th1 cells, Th2 cells, and cytotoxic B lymphocytes. TGF-β1 stops the secretion of immunoglobulin, inhibits the synthesis of pro-inflammatory cytokines (IFN-γ and IL-2) and can induce, in the absence of pro-inflammatory cytokines IL-6 and IL-1β, the formation of iTregs in peripheral tissues [94]. TGF-β1 may play a dual role: a suppressor by inhibiting the proliferation of tumor cells and inducer, by stimulating angiogenesis, and immunosuppression in the pathogenesis of cancer. The high expression of TGF-β1 and IL-10 promotes the dominance and Treg suppression of cytotoxic lymphocytes in the advanced stages of squamous cell cancer and lymphomas [94–96].

Interleukin-10 (IL-10) is a cytokine secreted by Th2 lymphocytes, macrophages, monocytes, MC and dendritic cells. IL-10 is known as a very strong anti-inflammatory and immunosuppressive agent, inhibiting T cell proliferation and synthesis of a number of cytokines e.g. IL-2. At the transcriptional level, *IL-10* acts by inhibiting the transcription factor NF-κB. This results in inhibition of the synthesis of IL-2, TNF-α, IL-6, IL-1α, IL-5 and IL-4. There are numbers of variants of the *IL-10* gene promoter – differing by single nucleotides or dinucleotide length of repetitive sequences [2]. The polymorphic variant – 1082G promoter of the *IL-10* gene is associated with increased transcriptional activity. Its relationship to psoriasis, AD, Hodgkin lymphoma, BCC and cancer has been examined [76, 97–111]. Sobjanek *et al.* [76] have demonstrated a significant association between *IL-10* –1082 GA (medium expression) genotype and susceptibility to BCC. Secondly, the –1082 GG high expression genotype, was significantly less frequent among BCC patients. Finally, the presence of the low expression of A allele correlated with a sevenfold higher risk of BCC occurrence. Statistically, a higher serum concentration of IL-10 in BCC patients was also recorded. These findings are similar to those of Howell *et al.*, who reported that *IL-10* genotypes associated with a high level of IL-10 expression *in vitro* were protective in cutaneous melanoma, while low expression genotypes were found to constitute risk factors for more advanced and poorer prognosis disease stages [111].

Future therapies concerning Tregs

In some autoimmune diseases, such as type I diabetes, treatment with autologous Tregs cells *in vitro* started to be administered. Another promising way of treatment involving the Tregs is administration of autologic tolerogenic DC [112, 113]. Perhaps in the future, such therapy may also be introduced in psoriasis, connective tissue diseases, bullous and vascular diseases.

In conclusion, abnormal differentiation and functions of regulatory cells, either naturally produced in the thymus or formed in the peripheral tissue, have been revealed in inflammatory and neoplastic skin diseases. Probably, the cause of these disorders can be inherited by those people who have specific genetic polymorphisms, which can then lead to the modification of the expression of genes important for the phenotype and function of Tregs. However, still little is known about the mechanisms regulating Tregs function, proliferation and differentiation. If we want to treat dermatological patients with autologic transplants of Tregs cultivated *in vitro* (as it happens in type I diabetes [114, 115]), we should still study the pathological mechanisms of immunologic skin system dysregulations.

Acknowledgments

The article is financed by the Polish Ministry of Science and Higher Education grant 02-0066/07/253.

Conflict of interest

The authors declare no conflict of interest.

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