DE GRUYTER
OPEN

IL-17 and Th17 cells in systemic sclerosis: a comprehensive review

PAUL BĂLĂNESCU[1], EUGENIA BĂLĂNESCU[2], ANCA BĂLĂNESCU[3]

[1]Internal Medicine Chair, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania
[2]Clincal Immunology Laboratory, CDPC, "Colentina" Clinical Hospital, Bucharest, Romania
[3]Pediatrics Department, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

T cells (especially T helper cells) seem to be strongly associated with systemic sclerosis pathogenesis. Th17-IL-17 axis was proved to be involved in the pathogenesis of multiple autoimmune diseases. By performing a comprehensive research of the literature indexed in PubMed database, the current review summarizes current knowledge related to Th17 and IL-17 in systemic sclerosis. While there is promising data suggesting inhibition of Tregulatory and Th1 signals on one hand and promotion of Th17 and Th2 signals on the other, studies that include prospective and integrated analysis of Tregulatory, Th17, Th1,Th2 (cells and derived cytokines) on the same cohort of Ssc patients are warranted.

Key words: Systemic sclerosis, IL-17A, Th17, IL-17F.

INTRODUCTION

Systemic sclerosis (Ssc) is an autoimmune disease that is characterized by cutaneous and also by interstitial fibrosis that ultimately drives to organ impairment. Two main disease subtypes are described: a limited disease subtype that does not associate extended internal organ fibrosis and a diffuse subtype that is usually associated with a negative prognosis and extended fibrotic changes. The exact mechanisms that explain these fibrotic changes are not completely understood but there is evidence that the immune system plays a key role. Fibrosis seems to be associated with previous vascular changes and endothelial dysfunction. One can conclude that Ssc has a complex physiopathology where autoimmunity associates profound vascular changes and also drives chaotic and apparently uncontrolled collagen synthesis.

T cells (especially T helper cells) seem to be strongly associated with Ssc pathogenesis. There are now identified more than just two T helper (Th) cells subsets as they were firstly defined. Among these relatively new identified Th subsets, Th17 cells seem to play an important and central role in autoimmunity, hence their role was also extensively evaluated in Ssc patients.

Th17 cells were named after the main cytokine they secrete, IL-17A (IL-17). Th17 cells also secrete IL-9, IL-17F, IL-21, IL-22, IL-26 and CCL20 [1]. Th17 cells seem to play a pivotal role between innate and adaptative immune responses, inducing expression of proinflammatory signals that attract and activate neutrophils mainly. Its main cytokine, IL-17A, is secreted as a dimer, either IL-17A-IL-17A homodimer or IL-17A-IL-17F heterodimer. IL-17A and IL-17F share more than 50% similarities within structure although they bind with different affinity to IL-17 receptors on their target cells [2].

Th17-IL-17 axis was proved to be involved in the pathogenesis of multiple autoimmune diseases like systemic and cutaneous lupus erythematosus [3], psoriasis [4]), Still disease [5], inflammatory bowel diseases [6]), rheumatoid arthritis [7], ankylosing spondylitis [8]. In addition there is more and more evidence that therapies that are directed against Th17 main cytokine, IL-17A, are effective and also safe to be used in ankylosing spondylitis and is also the first biologic non-anti-TNFα that has been approved for treating this disease [9].

There are still debates to establish the exact role of Th17-IL-17 axis in Ssc physiopathology. Active efforts of *in vitro* and *in vivo* research are made to establish the implications of Th17 in Ssc pathogenesis. These debates arise also from the conflicting results reported in the literature. Nevertheless these conflicting results could be explained by the different study design and also by the high heterogeneity of this disease. This review aims to critically summarize the existing literature and data regarding Th17 and IL-17 in systemic sclerosis.

DOI: 10.1515/rjim-2017-0027

SEARCH STRATEGY

PubMed database was searched from inception until 9th June 2017 using a search strategy presented in Table 1. Two independent reviewers reviewed abstracts and decided what articles to be included in the analysis. In order to be included in the review the paper should have had systemic sclerosis patient group/subgroup and subsequent analysis of either IL-17 or Th17 cell population. Review papers were excluded from the analysis and also papers referring to other autoimmune diseases and IL-17/Th17 axis. After excluding all irrelevant papers, 29 included articles were read full text and data was extracted. Most papers retrieved were case control studies and only one study prospectively studied IL-17 and Th17 related cytokines.

Table 1
PubMed search strategy and number of papers retrieved

Search strategy	PubMed results
(“scleroderma, systemic” [MeSH Terms] OR (“scleroderma” [All Fields] AND “systemic” [All Fields]) OR “systemic scleroderma” [All Fields] OR (“systemic” [All Fields] AND “sclerosis” [All Fields]) OR “systemic sclerosis” [All Fields]) AND (“interleukin-17” [MeSH Terms] OR “interleukin-17” [All Fields] OR “IL 17” [All Fields])	127 papers retrieved
(“scleroderma, systemic” [MeSH Terms] OR (“scleroderma” [All Fields] AND “systemic” [All Fields]) OR “systemic scleroderma” [All Fields] OR (“systemic” [All Fields] AND “sclerosis” [All Fields]) OR “systemic sclerosis” [All Fields]) AND (“Th17 cells” [MeSH Terms] OR (“Th17” [All Fields] AND “cells” [All Fields]) OR “Th17 cells” [All Fields] OR “Th17” [All Fields])	149 papers retrieved
(“scleroderma, systemic” [MeSH Terms] OR (“scleroderma” [All Fields] AND “systemic” [All Fields]) OR “systemic scleroderma” [All Fields] OR “scleroderma” [All Fields] OR “scleroderma, localized” [MeSH Terms] OR (“scleroderma” [All Fields] AND “localized” [All Fields]) OR “localized scleroderma” [All Fields]) AND (“interleukin-17” [MeSH Terms] OR “interleukin-17” [All Fields] OR “IL 17” [All Fields]) retrieved 66 hits	66 papers retrieved
(“scleroderma, systemic” [MeSH Terms] OR (“scleroderma” [All Fields] AND “systemic” [All Fields]) OR “systemic scleroderma” [All Fields] OR “scleroderma” [All Fields] OR “scleroderma, localized” [MeSH Terms] OR (“scleroderma” [All Fields] AND “localized” [All Fields]) OR “localized scleroderma” [All Fields]) AND (“Th17 cells” [MeSH Terms] OR (“Th17”[All Fields] AND “cells” [All Fields]) OR “Th17 cells” [All Fields] OR “Th17” [All Fields])	66 papers retrieved

DISCUSSION

Circulatory IL-17 levels in Ssc patients

IL-17 was firstly identified to be involved in Ssc pathogenesis even before Th17 cell subset was described. Using a case-control study design, Kurasawa *et al.* [10] found on a relatively small Ssc patient group higher IL-17 serum levels compared to controls. In addition, early Ssc patients had higher IL-17 expression. They also described IL-17 + cells in Ssc skin lesions and IL-17 expression from bronchoalveloar fluid lavage of patients with pulmonary fibrosis. They demonstrated that *in vitro* IL-17 could overstimulate Ssc patients derived fibroblasts and also healthy controls derived fibroblasts. Nevertheless they did not find increased collagen synthesis from fibroblasts stimulated with IL-17.

It was not until Th17 cells were discovered few years later that studies focused more on associations between Ssc clinical characteristics and IL-17 expression. There are studies that found higher IL-17 serum levels in Ssc patients compared to controls and also that higher modified Rodnan scores (score that evaluates the extent of cutaneous fibrosis) associated with higher circulatory IL-17 levels [11]. However, when a larger cohort comprising 444 Ssc patients was analyzed [12], serum IL-17 levels were lower compared to healthy controls even after adjustment for potential confusing factors (age and disease duration). Also it was firstly concluded in Ssc patients that IL-17 and also other cytokines have specific dynamics with disease duration. Those conflicting results in the literature could come from different sample sizes (it is known that Ssc is a rare disease and in order to have more patients in the analysis one should focus upon having a multicenter study). Also there could be differences arising from controls being used, study design (cross sectional approaches *versus* prospective approaches) and also from the fact that there Ssc patients were analyzed from different stages of evolution. Principal component analysis performed by Higgs *et al*. also showed that IL-17 and IL-17 associated gene pathway with Ssc phenotype [13]. Studies on pediatric localized scleroderma also showed increased serum IL-17A levels and also an association with early and more

active disease severity [14]. Other rather small studies showed lower or no difference of IL-17A and IL-23 levels between Ssc patients and controls but higher IL-21 levels. Also IL-17A seems to be associated with pulmonary dysfunction [15] and also with a more active disease course [16].

IL-17 and Th17 cells in rodent models of Ssc

When it comes to Th17-IL-17 axis, there are some notable differences between human studies and *in vivo* studies from rodent models. In rodent models (bleomycine induced fibrosis), IL-17A was proved to be indispensable for cutaneous and pulmonary fibrosis [17], but not all *in vitro* studies (please see below) replicated these findings. The effects of IL-17A associated murine fibrosis seem to be driven by IL-21 and IL-21R as secreting IL-21 Th17 cell count is higher in bleomycin induced murine models compared to controls. IL-21 mediated fibrosis is most probably performed in a positive feedback manner. IL-21 promotes murine Th17 differentiation. As such, Th17 secretes IL-17A and IL-21 that will promote more Th17 development, maturation and fibrosis [18]. Moreover IL-17A and Th17 related cytokines correlated with murine pulmonary and cutaneous fibrosis and IL-21 associated with murine circulating Th17 cells stimulating *in vitro* Th17 proliferation and development [19]. Differences with *in vitro* models could be explained by the well-known species variations and also by different *in vivo* and *in vitro* responses.

Flow cytometry findings regarding Th17 in Ssc patients

The next logical step in the analysis of Th17-IL-17 axis was to perform flow-cytometry studies assessing Th17 cell distribution and expression in Ssc patients. Radstake *et al*. [20] found that Ssc patients had more activated T cells compared to healthy controls and that circulatory Th17 were more frequent in Ssc patients. Also they found that there is a high expression of IL-17A, IFN-γ and TGF-β in limited Ssc patients, but also different expression patterns in early and late diffuse Ssc patients, with IFN-γ expression lowering alongside with disease duration of diffuse Ssc supporting also inhibition of Th1 signals. These data suggest that within Ssc patients there is a distinct immune dysregulation and that disease subsets can be also distinguished by these particular patterns.

Fenoglio *et al*. [21], Papp *et al*. [22] and Truchetet *et al*. [23] found a higher number of circulating Th17 cells (that express CCR6) in Ssc patients compared to healthy controls. In addition they demonstrated that in Ssc patients there is a skew in the immune response towards Th17 driven inflammation rather than a Th1 response. Also T regulator (Treg) population was also evaluated and they found that they were less compared to healthy controls and that they had less suppressive action. There was also found a direct correlation between up-regulation of Th17 responses and decrease in Treg action. These data suggest that the imbalance between Th17 and T regs by promotion of the first and inhibition of the latter could be the pathogenic mechanism triggering autoimmunity in Ssc patients. Also Ssc patients have an increased number of memory T cells compared to healthy controls [22]. Th17 alongside with Th22 have pulmonary and cutaneous homing [23]. Larger Ssc group flow cytometry studies (135 Ssc patients) confirmed these findings [24], but also described in late Ssc patients an increase in Th2 cells in addition to Th17. As for circulating T reg cells there were no differences from healthy controls. Also lack of correlation between elevated circulating Th17 cells and IL-17A serum levels was also noticed [25] most probably due to the fact that IL-17 is mainly expressed locally. Circulating Th17 cells were significantly higher in diffuse Ssc patients compared to limited patients and also higher in early disease compared to late stages of Ssc.

All these studies outline a profound imbalance between Th17 and Tregs in Ssc patients with Th17 activation. Treg phenotype associates high plasticity as there are intermediate Treg cells that are characterized by an intermediate phenotype between Treg and Th17. Such cells are upregulated in Ssc patients [25]. It is therefore suggested that at least some Treg cells, under particular signals could "switch" towards Th17 phenotype [26]. Interestingly, specific treatment for localized scleroderma (polymerized collagen injections in the lesion site) partially reverse Th17/T reg ratio, restoring the balance and inhibiting proinflammatory cytokines [27].

Pathogenesis of IL-17 and Th17 in Ssc lesions

Nakashima *et al*. [28] provided the first insight regarding implications of Th17 and IL-17 in Ssc. They found that circulating IL-17A but not IL-17F levels were higher compared to healthy controls.

Also IL-17A mRNA from Ssc lesional skin was higher compared to healthy skin. However IL-17F mRNA in Ssc skin was undetectable. Ssc patients seem to have a Th17 immunologic response skewed towards IL-17A rather than IL-17F. IL-17F + cells were lower in Ssc skin compared to healthy controls and no coexpression of IL-17A and IL-17F was found [29]. Surprisingly, IL-17A seems to have antifibrotic effects by decreasing collagen synthesis as proved by Nakashima *et al*. [28]. The authors also proposed a mechanism for IL-17A action in fibrotic skin. The main player in collagen synthesis is TGF-β. At the same time, TGF-β *via* Smad-3 inhibits IL-17R expression on fibroblast, thus inhibiting IL-17A signaling. The authors sustain that higher circulating IL-17A levels are due to a negative feedback mechanism, secondary to decrease/desensitization of IL-17R [28]. These findings are also supported by further *in vitro* studies that showed that IL-17A decreased collagen synthesis, but promoted matrix metalloproteinase 1 and IL-8 synthesis by the fibroblast from Ssc derived lesions [30]. IL-17A promotes its action by binding to IL-17R. IL-17R is a heterodimer being comprised of IL-17RA and IL-17RC. Activation of IL-17R determines further activation of intracellular AKT kinase, NF-kβ, c-JUN/JNK and ERK1/2. In addition Th17 cells promote similar answers and signal transduction pathways *via* IL-17A and TNF-α when they interact with Ssc fibroblasts *in vitro* [30]. It is not clear whether MMP-1 and IL-8 display a direct fibrotic effect, but it is clear that fibroblasts can escape from Th17 action by decreasing their receptivity to their signals (mainly IL-17A).

IL-17A positive cells are more frequent in Ssc lesional skin compared to normal skin. Interestingly, among IL-17+ lymphocytes there are also IL-17 + mastocytes in the dermis from Ssc lesional skin [23]. Th17 cells and positive IL-17 cells were inversely associated with fibrosis skin score (modified Rodnan skin score) stressing upon the antifibrotic effect of IL-17A in Ssc patients and in counterbalance with TGF-β.

Nevertheless IL-17A has effect, at least *in vitro*, upon another important pathogenic feature of Ssc-vascular dysfunction through microangiopathy. Vascular thickening (through collagen deposition in small arterial and arterioles wall) and endothelial dysfunction is characteristic for Ssc patients [31]. IL-17A exhibits a complex effect upon vascular smooth muscle cells derived from Ssc lesional skin. Firstly, IL-17A determines proliferation of these cells. In addition, IL-17A activates vascular smooth muscle cells via ERK 1/2 pathway determining increased collagen synthesis. Interestingly, IL-17A also determines migration of dermal vascular smooth cells, mechanism that is usually characteristic of atherosclerosis lesions [32]. IL-17A also modifies endothelial cell phenotype, inducing *in vitro* upregulation of adhesion molecules like chemokine (C-Cmotif) ligand 20 (CCL-20), intercellular adhesion molecule 1 (ICAM-1), chemokine (C-X-C motif) receptor 4 (CXCR-4) and vascular cell adhesion molecule 1 (VCAM-1) *via* ERK 1/2 pathway and thus promoting both recruitment and migration of leukocytes through the endothelial wall [33]. Therefore, IL-17A seems to preferentially use ERK1/2 pathway in dermal fibroblasts, vascular smooth cells and endothelial cells suggesting a common pathogenic effect through this particular signaling pathway, but studies are warranted in order to fully elucidate the complex signaling pathways used by IL-17A through IL-17R upon effector cells in Ssc patients.

Yang *et al*. [34] provided interesting insights by adding prospective follow-up of Th17 cells in Ssc patients. Patients that had advanced Ssc had less lymphocytes in the lesioned skin compared to the skin from early Ssc patients. Also the number of Th17 cells and T regs decreased alongside with disease duration. Th17 circulating number was higher in active and stable disease. Under immunosuppressive treatment the number of Th17 cells decreased. They also showed *in vitro* that IL-17A and Th17 cells promote fibroblast proliferation and collagen production contrary to findings of Nakashima *et al*. and Brembilla *et al*. [28, 30].

Similar with findings in rodents Th17 cells seem to contribute to disease progression (and fibrosis) *via* IL-21 and IL-21R through indirect Th2 signals (at least in the early stages of disease). Th2 signals appear markedly upregulated in the later stages of disease. Moreover, Ssc skin (mainly in epidermis) have higher expression for receptors of interleukins secreted by Th17 (IL-17RA, IL-21RA and IL-22RA) being thus more susceptible to Th17 actions. Interestingly, IL-22 and IL-22R were inversely correlated to TGF-β and it is speculated that IL-22 could play a protective role against fibrosis in Ssc patients. Also, IL-22 and IL-22RA mRNA positively correlate with modified Rodnan skin scores [35].

Another important aspect to discuss is the implication of Th17 cells in pulmonary manifestations of Ssc patients. Although there were no differences

reported between Th17 cell count from broncho-alveolar fluid between patients with and without pulmonary fibrosis [36], IL-17A was higher in exhaled breath concentrate from diffuse Ssc patients when compared to healthy controls and limited Ssc patients. Also IL-17A from the exhaled breath concentrate inversely correlated with DLCO values [37]. Molecular profiling of lung tissue using microarray mRNA expression studies proved that pulmonary hypertensive derived fibroblasts had higher expression for IL-17, IL-6 and IL-8 expressed genes [38]. Ssc patients that were positive for anti-topoisomerase I antibodies had higher Th17 circulating cell count and lower Th1 circulating cell count compared to Ssc patients negative for anti-topoisomerase I antibodies. Ssc patients positive for anti-topoisomerase I antibodies also associate more frequently interstitial lung disease [39]. These data combined with insights from murine models suggest an implication of Th17 in pulmonary manifestations of Ssc. However, the exact role of Th17 in Ssc pathogenesis is not clear. To date a direct action of Th17 promoting fibrosis has not yet been proven in Ssc patients. It seems that fine tuning of secreted cytokines alongside with Th17/Treg balance and influence upon other T cells with receptor sensitivity modulation could be the key for fibrosis regulation in Ssc patients. Activation of ERK1/2 pathway in effector cells via Th17 secreted cytokines seems to promote proliferation and activation of these cells.

CONCLUSIONS

There is an obvious interest and proof about Th17 implication in Ssc but there are still some questions that are not answered. It is not yet clear if Th17 have a direct profibrotic action. Also the fine tuning between Treg/Th17/Th1/Th2 and maybe other Th subsets is not well described in Ssc patients yet. We have some promising data that suggest inhibition of Treg and Th1 signals on the one hand and promotion of Th17 and Th2 signals on the other. This fine tuning could also be a marker for Ssc course and this interesting aspect needs to be further studied. Studies that include prospective and integrated analysis of Treg, Th17, Th1, Th2 (cells and derived cytokines) on the same cohort of Ssc patients are warranted.

Conflict of interest: The authors declare no conflict of interest.

Limfocitele T (în special limfocitele T helper) par a fi asociate cu patogeneza sclerodermiei. Axa Th17/IL-17 s-a dovedit a fi implicată în patogeneza mai multor boli autoimune. Acest articol sumarizează, după ce a fost realizată o căutare extensivă a articolelor în PubMed, datele existente privind limfocitele Th17 şi IL-17 în sclerodermie. Există date promiţătoare privind supresia populaţiei limfocitelor T reglatoare şi Th1 pe de o parte, şi stimularea răspunsurilor mediate de către limfocitele Th17 şi Th2, pe cealaltă parte. Sunt însă necesare studii prospective cu analiza integrată a populaţiilor limfocitare pe acelaşi grup de pacienţi cu sclerodermie.

Correspondence to: Paul Bălănescu, MD, PhD,
Internal Medicine Chair University of Medicine and Pharmacy,
"Colentina" Clinical Hospital, 19-21 Ştefan cel Mare Street, Bucharest, Romania
E-mail: plbalanescu@gmail.com

REFERENCES

1. GRAEBER KE, OLSEN NJ. *Th17 cell cytokine secretion profile in host defense and autoimmunity*. Inflamm Res. 2012; **61** (2):87-96.
2. HYMOWITZ SG, FILVAROFF EH, YIN JP, LEE J, CAI L, RISSER P, *et al. IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding*. EMBO J. 2001; **20**:5332–41.
3. TANASESCU C, BĂLĂNESCU E, BĂLĂNESCU P, OLTEANU R, BADEA C, GRANCEA C, *et al. IL-17 in cutaneous lupus erythematosus*. Eur J Intern Med. 2010; **21** (3):202-7.
4. BLAUVELT A. *T-helper 17 cells in psoriatic plaques and additional genetic links between IL-23 and psoriasis*. J Invest Dermatol. 2008; **128** (5):1064-7.
5. CHEN DY, CHEN YM, LAN JL, LIN CC, CHEN HH, HSIEH CW. *Potential role of Th17cells in the pathogenesis of adult-onset Still's disease*. Rheumatology (Oxford). 2010; **49** (12):2305-12.

6. DE NITTO D, SARRA M, CUPI ML, PALLONE F, MONTELEONE G. *Targeting IL-23 and Th17-cytokines in inflammatory bowel diseases*. Curr Pharm Des. 2010; **16** (33):3656-60.
7. ZHANG L, LI YG, LI YH, QI L, LIU XG, YUAN CZ, *et al. Increased frequencies of Th22 cells as well as Th17 cells in the peripheral blood of patients with ankylosing spondylitis and rheumatoid arthritis*. PLoS One. 2012; **7** (4):e31000.
8. RAYCHAUDHURI SP, RAYCHAUDHURI SK. *IL-23/IL-17 axis in spondyloarthritis-bench to bedside*. Clin Rheumatol. 2016; **35** (6):1437-41.
9. LUBRANO E, PERROTTA FM. *Secukinumab for ankylosing spondylitis and psoriatic arthritis*. Ther Clin Risk Manag. 2016; **12**:1587-92.
10. KURASAWA K, HIROSE K, SANO H, ENDO H, SHINKAI H, NAWATA Y, *et al. Increased interleukin-17 production in patients with systemic sclerosis*. Arthritis Rheum. 2000; **43** (11):2455-63.
11. MURATA M, FUJIMOTO M, MATSUSHITA T, HAMAGUCHI Y, HASEGAWA M, TAKEHARA K, *et al. Clinical association of serum interleukin-17 levels in systemic sclerosis: is systemic sclerosis a Th17 disease?* J Dermatol Sci. 2008; **50**(3):240-2.
12. GOURH P, ARNETT FC, ASSASSI S, TAN FK, HUANG M, DIEKMAN L, *et al. Plasma cytokine profiles in systemic sclerosis: associations with autoantibody subsets and clinical manifestations*. Arthritis Res Ther, 2009; **11** (5):R147.
13. HIGGS BW, ZHU W, RICHMAN L, FIORENTINO DF, GREENBERG SA, JALLAL B, *et al*. *Identification of activated cytokine pathways in the blood of systemic lupus erythematosus, myositis, rheumatoid arthritis, and scleroderma patients*. Int J Rheum Dis. 2012; **15** (1):25-35.
14. TOROK KS, KURZINSKI K, KELSEY C, YABES J, MAGEE K, VALLEJO AN, *et al. Peripheral blood cytokine and chemokine profiles in juvenile localized scleroderma: T-helper cell-associated cytokine profiles*. Semin Arthritis Rheum. 2015; **45** (3):284-93.
15. OLEWICZ-GAWLIK A, DANCZAK-PAZDROWSKA A, KUZNAR-KAMINSKA B, GORNOWICZ-POROWSKA J, KATULSKA K, TRZYBULSKA D, *et al. Interleukin-17 and interleukin-23: importance in the pathogenesis of lung impairment in patients with systemic sclerosis.* Int J Rheum Dis. 2014; **17** (6):664-70.
16. BĂLĂNESCU P, LĂDARU A, BĂLĂNESCU E, NICOLAU A, BĂICUŞ C, DAN GA. *IL-17, IL-6 and IFN-γ in Systemic Sclerosis Patients*. Rom J Intern Med. 2015; **53** (1):44-9.
17. OKAMOTO Y, HASEGAWA M, MATSUSHITA T, HAMAGUCHI Y, HUU DL, IWAKURA Y, *et al. Potential roles of interleukin-17A in the development of skin fibrosis in mice.* Arthritis Rheum. 2012; **64** (11):3726-35.
18. LEI L, HE ZY, ZHAO C, SUN XJ, ZHONG XN. *Elevated frequencies of CD4(+) IL-21(+) T, CD4(+) IL-21R(+) T and IL-21 (+) Th17 cells, and increased levels of IL-21 in bleomycin-induced mice may be associated with dermal and pulmonary inflammation and fibrosis*. Int J Rheum Dis. 2016; **19** (4):392-404.
19. LEI L, ZHONG XN, HE ZY, ZHAO C, SUN XJ. *IL-21 induction of CD4+ T cell differentiation into Th17 cells contributes to bleomycin-induced fibrosis in mice.* Cell Biol Int. 2015; **39** (4):388-99.
20. RADSTAKE TR, VAN BON L, BROEN J, HUSSIANI A, HESSELSTRAND R, WUTTGE DM, *et al. The pronounced Th17 profile in systemic sclerosis (SSc) together with intracellular expression of TGFbeta and IFNgamma distinguishes SSc phenol-types*. PLoS One. 2009; **4** (6):e5903.
21. FENOGLIO D, BATTAGLIA F, PARODI A, STRINGARA S, NEGRINI S, PANICO N, RIZZI M, *et al. Alteration of Th17 and Treg cell subpopulations co-exist in patients affected with systemic sclerosis*. Clin Immunol. 2011; **139** (3):249-57.
22. PAPP G, HORVATH IF, BARATH S, GYIMESI E, SIPKA S, SZODORAY P, *et al. Altered T-cell and regulatory cell repertoire in patients with diffuse cutaneous systemic sclerosis*. Scand J Rheumatol. 2011; **40** (3):205-10.
23. TRUCHETET ME, BREMBILLA NC, MONTANARI E, ALLANORE Y, CHIZZOLINI C. *Increased frequency of circulating Th22 in addition to Th17 and Th2 lymphocytes in systemic sclerosis: association with interstitial lung disease*. Arthritis Res Ther. 2011; **13**(5):R166.
24. ALMANZAR G, KLEIN M, SCHMALZING M, HILLIGARDT D, EL HAJJ N, KNEITZ H, *et al. Disease manifestation and inflammatory activity as modulators of Th17/Treg balance and RORC/FoxP3 Methylation in Systemic Sclerosis*. Int Arch Allergy Immunol. 2016; **171**(2):141-54.
25. RODRÍGUEZ-REYNA TS, FURUZAWA-CARBALLEDA J, CABIEDES J, FAJARDO-HERMOSILLO LD, MARTÍNEZ-REYES C, DÍAZ-ZAMUDIO M, *et al. Th17 peripheral cells are increased in diffuse cutaneous systemic sclerosis compared with limited illness: a cross-sectional study*. Rheumatol Int. 2012; **32** (9):2653-60.
26. LIU X, GAO N, LI M, XU D, HOU Y, WANG Q, *et al. Elevated levels of CD4(+)CD25(+)FoxP3(+) T cells in systemic sclerosis patients contribute to the secretion of IL-17 and immunosuppression dysfunction*. PLoS One. 2013; **8** (6):e64531.
27. FURUZAWA-CARBALLEDA J, ORTÍZ-ÁVALOS M, LIMA G, JURADO-SANTA CRUZ F, LLORENTE L. *Subcutaneous administration of polymerized type I collagen downregulates interleukin (IL)-17A, IL-22 and transforming growth factor-β1 expression, and increases FoxP3-expressing cells in localized scleroderma*. Clin Exp Dermatol. 2012; **37** (6):599-609.
28. NAKASHIMA T, JINNIN M, YAMANE K, HONDA N, KAJIHARA I, MAKINO T, MASUGUCHI S, *et al. Impaired IL-17 signaling pathway contributes to the increased collagen expression in scleroderma fibroblasts*. J Immunol. 2012; **188** (8):3573-83.
29. LONATI PA, BREMBILLA NC, MONTANARI E, FONTAO L, GABRIELLI A, VETTORI S, *et al. High IL-17E and low IL-17C dermal expression identifies a fibrosis-specific motif common to morphea and systemic sclerosis*. PLoS One. 2014; **9** (8):e105008.
30. BREMBILLA NC, MONTANARI E, TRUCHETET ME, RASCHI E, MERONI P, CHIZZOLINI C. *Th17 cells favor inflammatory responses while inhibiting type I collagen deposition by dermal fibroblasts: differential effects in healthy and systemic sclerosis fibroblasts*. Arthritis Res Ther. 2013; **15** (5):R151.
31. FRECH TM, REVELO MP, DRAKOS SG, MURTAUGH MA, MARKEWITZ BA, SAWITZKE AD, LI DY. *Vascular leak is a central feature in the pathogenesis of systemic sclerosis*. J Rheumatol 2012; **39**:1385-91.

32. LIU M, YANG J, XING X, CUI X, LI M. *Interleukin-17A promotes functional activation of systemic sclerosis patient-derived dermal vascular smooth muscle cells by extracellular-regulated protein kinases signalling pathway*. Arthritis Res Ther. 2014; **16** (6):4223.
33. XING X, YANG J, YANG X, WEI Y, ZHU L, GAO D, *et al. IL-17A induces endothelial inflammation in systemic sclerosis via the ERK signaling pathway*. PLoS One. 2013; **8** (12):e85032.
34. YANG X, YANG J, XING X, WAN L, LI M. *Increased frequency of Th17 cells in systemic sclerosis is related to disease activity and collagen overproduction*. Arthritis Res Ther. 2014; **16** (1):R4.
35. ZHOU Y, HOU W, XU K, HAN D, JIANG C, MOU K, *et al. The elevated expression of Th17-related cytokines and receptors is associated with skin lesion severity in early systemic sclerosis.* Hum Immunol. 2015; **76** (1):22-9.
36. MELONI F, SOLARI N, CAVAGNA L, MOROSINI M, MONTECUCCO CM, FIETTA AM. *Frequency of Th1, Th2 and Th17 producing T lymphocytes in bronchoalveolar lavage of patients with systemic sclerosis*. Clin Exp Rheumatol. 2009; **27** (5):765-72.
37. ROLLA G, FUSARO E, NICOLA S, BUCCA C, PERONI C, PARISI S, *et al. Th-17 cytokines and interstitial lung involvement in systemic sclerosis.* J Breath Res. 2016; **10** (4):046013.
38. HSU E, SHI H, JORDAN RM, LYONS-WEILER J, PILEWSKI JM, FEGHALI-BOSTWICK CA. *Lung tissues in patients with systemic sclerosis have gene expression patterns unique to pulmonary fibrosis and pulmonary hypertension*. Arthritis Rheum. 2011; **63** (3):783-94.
39. FAVA A, CIMBRO R, WIGLEY FM, LIU QR, ROSEN A, BOIN F. *Frequency of circulating topoisomerase-I-specific CD4 T cells predicts presence and progression of interstitial lung disease in scleroderma.* Arthritis Res Ther. 2016; **18** (1):9.

Received June 20, 2017