



Fang-Fang Ji  
Science Editor, Editorial Office  
Baishideng Publishing Group Inc  
*World Journal of Experimental Medicine*

24<sup>th</sup> May 2017

Dear Sirs,

**Treg/Th17 cell balance and Phytohaemagglutinin activation of T lymphocytes in peripheral blood of systemic sclerosis patients.**

**Manuscript NO.: 32948**

Thank you for reviewing the aforementioned paper. We have addressed all the queries raised by the reviewers (listed below):

Comments:	Response:
<b>Reviewer 1:</b>	
The authors have performed an interesting study. It is a good one. They have made some new discovery on the Treg and Th17 cells in patients of systemic sclerosis. There are two issues with the manuscript.	
<b>Comment 1:</b> The discussion is way too long, and it can be cut into half without really losing content.	We support the reviewer's assertion that the discussion section is too long. Therefore, it has been carefully revised and shortened. The redundant paragraphs have been strikethrough and highlighted in yellow.
<b>Comment 2:</b> There need some improvements in English language.	We appreciate the reviewer's opinion. We have examined the whole manuscript with Grammarly. A colleague of us, proficient in English, also has reviewed our manuscript. Accordingly, all the stylistic and grammar errors have been strikethrough and highlighted in yellow and the suggested amendments have been made in red font.

<b>Reviewer 2:</b>	
<p>The study represents an interesting continuum to the research series towards unveiling the immunological profile in SSc. Authors aimed to study the resting and stimulated T reg and Th17 in addition to a number of cytokines including IL-10, TGF B, Il-17 and IL-6. The study confirmed what has been described in literature in addition to illustrating an up-regulated percentage of CD4+CD25-FoxP3+ cells in patients with dcSSc and increased serum levels of IL-17 in lcSSc as opposed to patients with dcSSc phenotype. Authors did a good work. Major revisions are requested. Points to be clarified and revised include:</p>	
<p>1- The introduction section is well presented a part from the issue that authors mentioned conclusions at the end of the introduction while the hypothesis and aim need to be clearly stated without conclusions.</p>	<p>We have removed the conclusions at the end of the introduction section and have clearly stated the aim of our study.</p>
<p>2- Authors should comment on the sample size as being relatively small, scleroderma is a rare disease and better refer to their population data regarding the incidence/prevalence of SSC in their ethnic group if available.</p>	<p>We completely agree with the reviewer's suggestion. Unfortunately, there is no official data concerning the incidence and prevalence of SSc in Bulgarian population. Therefore, we have noted (the paragraph added in red font) the existing data on the incidence and prevalence of SSc for our neighbor countries - Greece and Croatia.</p>
<p>3- The study design wasn't clearly stated, and if authors used any specific check list during performance of the study.</p>	<p>We performed a cross-sectional study. We analyzed data collected from a representative subset of SSc patients and a matching control group, at a specific point in time. In the "Population studied" paragraph of the Materials and methods section, we noted that the assessment of the disease activity was performed using the "Preliminarily Revised EUSTAR Activity Index".</p>
<p>4 - In the methodology section as understood three samples were investigated two from each patient including a control and a stimulated sample in addition to samples from healthy subjects. The author should display in the results section their results</p>	<p>We investigated two but not three samples from each subject (SSc patient or healthy control) - a control sample and a PHA-stimulated one. We showed off only the results for T-cell activation in the PHA-stimulated samples from SSc patients compared to control subjects.</p>

in the patients and controls in the three samples. This wasn't clarified in text or tables, what was the resting T cell profile and cytokine profile in the samples from the patient and interpret this in comparison to healthy subjects at resting and stimulated level. This wasn't clearly displayed neither in text nor in tables.

The results concerning the comparison of the control samples from patients and healthy subjects were statistically insignificant and for this reason have not been indicated, respecting the "Guidelines for Manuscript Preparation and Submission: Basic Study" of WJEM. According to these Guidelines: "Data that are not statistically significant should not be noted."

Regarding the cytokine profile - we measured the cytokine levels only in the sera of all subjects and there is no relation between the investigation of T-cell activation (which is separate stage of the study) and the measurements of the circulating cytokines. Therefore, it could not be displayed in the Results section. We appreciate the reviewer's idea to measure the cytokine levels in unstimulated and stimulated samples of SSc patients and will consider to implement it in our future research.

5- In the results section some values were explained in terms of means and SD and other were provided in ranges, better mention the ranges, means and SD for all in text.

We appreciate the reviewer's opinion. However, the variables Il-17A and Il-6 have demonstrated non-normal distribution. In this case median, interquartile range [IQR], minimum, and maximum values should be calculated and the Mann-Whitney test should be applied. We have added all the explanations required in the "Statistical analyses" paragraph of the Materials and methods section. A certified biostatistician has evaluated and has reviewed the statistical methods used in the study.

6- How many patients were on immunosuppressive drugs or other DMARDs medications?  
Frequency/percentage this is an important issue and most importantly the authors didn't explain if immunosuppressive therapy in the studied patients had an influence on the reported results regarding T cell profile and cytokine milieu at either baseline or after stimulation.

The exact treatment regimen of every single patient has been displayed in Table 1. The paragraph 6 in the Discussion section thoroughly discusses the role that the immunosuppressive therapy may play in the suppression of the T-cell activation: "On the other hand, the peripheral T cell anergy upon PHA-stimulation in our SSc patients may be due to the immunosuppressive therapy administered. Most of the patients enrolled in the study were under

	<p>treatment with glucocorticoids (GCs)....”</p> <p>As previously described in answer 4, we measured the percentage of Tregs, Th17 and the serum levels of Il-17A, Il-6, Il-10, and TGF- separately from the T-cell activation analysis. Accordingly, “at baseline or after stimulation” are not applicable in this case but only for the whole blood samples investigated for T-cell activation.</p>
<p>7- In spite that figures are quite expressive yet authors should put into consideration insignificant data are as important as positives and should be clarified in the tables with the P value, r value and confidence intervals clearly stated in either situation.</p>	<p>Insignificant data have not been indicated according to the “Guidelines for Manuscript Preparation and Submission: Basic Study” of WJEM (“Data that are not statistically significant should not be noted.”).</p> <p>In all the figures and tables, displayed P values have been clearly stated and reviewed.</p>
<p>8- In the results section concerning the T reg line 7 the authors mentioned ‘meanwhile’ what does this mean in the interpretation of results? the authors should rephrase in a relatively precise way.</p>	<p>We have rephrased the sentence and „meanwhile” has been removed. All the amendments made have been highlighted in the text of the manuscript.</p>
<p>9- Discussion section is very long and might require adjustments following revisions previously stated.</p>	<p>We completely support the reviewer’s assertion that the discussion section is too long. Therefore, it has been carefully revised and shortened. The redundant paragraphs have been strikethrough and highlighted in yellow.</p>
<p><b>Comments in edited Manuscript 32948:</b></p>	
<p>1. Conflict-of-interest statement:</p>	<p>Authors declare no conflict of interests for this article.</p>
<p>2. Institutional review board statement:</p>	<p>All peripheral blood samples were taken from patients and healthy control subjects after informed written consent and ethical permission was obtained for participation in this study. The study was reviewed and approved by the Institutional Review Board of University Hospital Saint Ivan Rilski, Sofia, Bulgaria.</p>
<p>3. Biostatistics:</p>	<p>The statistical methods used in this study were reviewed by Tsvetelina Velikova, MD, PhD from University Hospital Saint Ivan Rilski, Sofia, Bulgaria.</p>
<p>4. Data sharing statement:</p>	<p>No additional data are available.</p>

5. Institutional animal care and use committee statement:	No animal subjects were involved in our experimental study.
6. Animal care and use statement:	Not applicable
7. Correspondence to: The personal email is not accepted; please offer your institute email. Thank you!	The institutional email of the corresponding author has been provided: dkyurkchiev@medfac.mu-sofia.bg
8. Audio Core Tip:	It has been provided.
<p data-bbox="243 313 617 347">9. Writing requirements:</p> <p data-bbox="292 347 535 380">➤ <b>Background</b></p> <p data-bbox="194 392 795 616">To summarize concisely and accurately the relevant background information so that readers may gain some basic knowledge about your study's relevance and understand its significance for the field as a whole.</p> <p data-bbox="292 1052 617 1086">➤ <b>Research frontiers</b></p> <p data-bbox="194 1086 795 1198">To introduce briefly the current hotspots or important areas in the research field as related to your study.</p>	<p data-bbox="820 347 1404 996">Systemic sclerosis is a generalized debilitating connective tissue disease affecting the skin and internal organs characterized by vasculopathy, fibrosis, and autoimmune alterations. The autoimmune dysregulation in SSc comprises lymphocyte activation that leads to the generation of autoantibodies, abnormal production of cytokines and chemokines, and impairment of the innate immunity. Over the last decade, the accumulating data has shown the central role of T lymphocytes in the pathogenesis of SSc. There is a strong evidence in literature for altered T-cell activation and T helper cells abnormalities in SSc.</p> <p data-bbox="820 1052 1404 1814">There is accumulating data for numerical and functional alterations of Tregs and Th17 cells in patients with SSc. However, a functional heterogeneity exists between the T lymphocytes in the peripheral blood of patients with SSc and the corresponding T cell subsets in skin lesions or internal organs. The cytokine production by T cells affects the function of fibroblasts and endothelial cells, thereby influencing the vascular disease progression and the fibrosis development. Many efforts have been made to identify the cytokine patterns in SSc. Nevertheless important issues remain unresolved, among them, identification of the trigger of the autoimmune response in SSc and the immunological differences between the dcSSc and lcSSc.</p>

➤ **Innovations and breakthroughs**

To summarize and emphasize the differences, particularly the advances, achievements, innovations and breakthroughs, as compared to other related or similar studies in the literature, which will allow the readers to assimilate the major points of your article.

➤ **Applications**

To summarize the practical applications of your research findings, so that readers may understand the perspectives by which this study will affect the field and future research.

➤ **Terminology**

To describe concisely and accurately any terms that may not be familiar to the majority of the readers, but which are essential for understanding your article.

➤ **Peer-review**

To provide the major comments from your peer reviewers that most represent the characteristics, values and significance of your article, and to allow the readers to have an objective point of view regarding your article and research findings.

This is the first study demonstrating an up-regulated percentage of CD4+CD25-FoxP3+ cells in patients with dcSSc as compared to healthy subjects. Another of the original contributions of our research demonstrates a decreased capacity for PHA-induced peripheral T-cells activation in patients with SSc. Regarding the peripheral cytokine profile in SSc, our research group describes for the first time elevated serum levels of IL-17A in the lcSSc as opposed to the dcSSc subset of the disease.

It is likely that the altered percentage of Th17 and CD4+CD25-FoxP3+ cells may play a key role in the disease progression along with the peripheral cytokine profile in SSc patients.

SSc is an abbreviation for Systemic sclerosis as well as lcSSc and dcSSc are abbreviations for the limited cutaneous and the diffuse cutaneous subsets of the disease. Tregs represent the T regulatory lymphocytes (CD4+FoxP3+ cells), a T helper cell subset which is crucial for the establishment of immunological self-tolerance and for the prevention of autoimmunity.

The authors have performed an interesting study. It is a good one. They have made some new discovery on the Treg and Th17 cells in patients of systemic sclerosis.

The study represents an interesting continuum to the research series towards unveiling the immunological profile in SSc. Authors aimed to study the resting and stimulated T reg and Th17 in addition to a number of cytokines including IL-10, TGF B, IL-17 and IL-6.

	The study confirmed what has been described in literature in addition to illustrating an up-regulated percentage of CD4+CD25-FoxP3+ cells in patients with dcSSc and increased serum levels of IL-17 in lcSSc as opposed to patients with dcSSc phenotype. Authors did a good work.
10. References:	The references have been revised as per the suggestions. PMID and DOI have been added in red font in all references where applicable.

We also enclose all the necessary documents as per your instructions. These include:

1. 32948-Revised manuscript (addressing the reviewers' comments; Conflict-of-interest statement; Institutional review board statement; Biostatistics; Data sharing statement; Institutional animal care and use committee statement; Animal care and use statement)
2. 32948-Copyright assignment
3. 32948-Scientific research process
4. 32948-Audio core tip
5. 32948-Institutional review board statement
6. 32948-Institutional animal care and use committee statement
7. 32948-Animal care and use statement
8. 32948-Biostatistics statement
9. 32948-Conflict-of-interest statement
10. 32948-Data sharing statement
11. 32948-Google Scholar
12. 32948-Language certificate

We would like to express our gratitude to the scientific committee and the reviewers for considering and accepting our manuscript.

If you require further details, please do not hesitate to contact us back.

Regards,

Yours sincerely,

Corresponding author:

First author:

Dobroslav Kyurkchiev

Ekaterina Krasimirova