BioBacta

Journal of Bioscience and Applied Research
www.jbaar.org

T-lymphocyte subsets ($CD3^+$, $CD4^+$, and $CD8^+$) in Systemic Lupus Erythematosus (SLE): Correlation with Clinical Manifestation

Abeer M. El-Maghraby[1], Yasser B.M. Ali[2], Eman A. El-maadawy[2], Mohamed F. Elshal[2], Iman H Bassyouni[3], Islam M El-Garawani[1], Roba M. Talaat[2]

[1]Zoology Department, Faculty of Science, Menoufia University, Shebin El-Kom 32511, Menoufia, Egypt; dr.garawani@science.menofia.edu.eg;

[2]Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt.dr_abeer_elmaghraby@yahoo.com, eman.anwr@gebri.usc.edu.eg, yasser.ali@gebri.usc.edu.eg, mohamed.elshal@gebri.usc.edu.eg

[3]Rheumatology and Rehabilitation Department, Faculty of Medicine, Cairo University, Cairo, Egypt.iman.bassyouni@kasralainy.edu.eg

Corresponding author: Dr. Roba M. Talaat.

Prof. of Molecular Immunology, Molecular Biology Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City (USC), Egypt. Fax: +2 048 260 1266/68 Tel. +2 048 260 1264/65

E-mail address: roba.talaat@gebri.usc.edu.eg

Running Title: T-lymphocyte regulation in SLE patients

DOI: 10.21608/jbaar.2021.198735

Abstract

AIM: Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that has a multifactorial etiology. T- Lymphocytes are essential in SLE pathogenesis. It plays a crucial role in autoantibody production and the subsequent immune complex formation, which may induce or directly damage multiple organs. This study was carried out aiming to quantify certain T lymphocyte subsets ($CD3^+$, $CD4^+$, and $CD8^+$) in SLE patients and to elucidate if there is a possible influence of disease activity scores and clinical manifestations. **Patients and Methods:** This study included 100 SLE patients with various disease activity scores (SLEDAI) and 100 healthy age and sex-matched controls. The frequency of $CD3^+$, $CD4^+$, and $CD8^+$ was assessed by flow cytometry. **Results:** A significant up-regulation in $CD3^+$ ($P<0.01$), $CD8^+$ ($P<0.001$) coincides with a significant downregulation in $CD4^+$cells ($P<0.001$) were detected in SLE patients compared to controls. A significant up-regulation in $CD4^+$ ($P<0.05$) was demonstrated in active SLE patients compared with the inactive form of the disease. On the other hand, no significant change was observed in the frequency of $CD3^+$ and $CD8^+$T cell subsets between active and inactive patients. Arthritic patients have a significant reduction in $CD3^+$ and $CD4^+$ T cells while those with Vasculities significantly reduce in CD4+, CD8+ compared with SLE patients without these manifestations.

Received: July 26, 2021. Accepted: September 18, 2021. Published: September 29, 2021

Conclusion: The current study results stressed the importance of T cell subsets in SLE disease. They might participate in disease activity and in controlling several manifestations of lupus. Our findings will help design more studies on the role of T cell subsets in SLE pathogenesis which may provide new therapeutic targets for SLE.

Keywords: SLE; T-lymphocyte; $CD3^+$, $CD4^+$, $CD8^+$

---

1. Introduction

Systemic lupus erythematosus (SLE) is characterized by producing a plethora of autoantibodies that potentially drive immune-complex-related inflammation in various tissues and organs (Cheng *et al.*, 2015; Basta *et al*., 2020; Justiz Vaillant *et al.*, 2020). SLE was reported to have a multifactorial etiology. Several genetic, environmental, and other immunoregulatory factors are involved in the pathogenesis of SLE (Mathias and Stohl, 2020; Moore and Putterman, 2020).

T- and B-lymphocytes are important in SLE pathogenesis; an increased number of circulating plasma memory B cell subsets are linked to disease activity, and B-cell-targeted therapies have shown some clinical improvement (Cassia *et al*., 2017). Moreover, T cells play a crucial role in autoantibody production and the subsequent immune complex formation (Koga *et al.*, 2016; Koga *et al.*, 2017). CD3 are suboptimally synthesized in T cells from patients with SLE. Moreover, reduction of stability and increase in degradation of CD3 in lupus T cells are evident. To replace the deficient CD3 subunits, FcR receptors are reciprocally activated and expressed on lupus T cells (Puliaeva *et al*., 2008).

$CD4^+$ T cells are reported to be essential and sufficient for lupus induction and driving B cell production of autoantibodies. Moreover, these cells might be one of the mechanisms that work to restrict lupus development (Puliaeva *et al*., 2008). $CD4^+$ helper T cells regulate other immune cells' function and play a key role in self-tolerance, elimination of foreign microorganisms, development of the adaptive immune system, and cytokines production that help activate the immune cells (Kuwabara *et al.*, 2017).

CD8+ in patients with SLE has yielded inconsistent results. They play a crucial role in recognizing and removing cells infected by intracellular pathogens and in antitumor response. The binding of surface receptor TCR and MHC-I-bound antigen, found on the professional antigen-presenting cell's (APC) surface, leads to activation [3]. Since stimulation only through the TCR receptor is unable to maintain optimum activation, the second costimulatory signal is essential for full activation and survival of these cells (Murphy *et al.*, 2003; Stritesky *et al.*, 2008; Gaffen *et al.*, 2014; Astry *et al.*, 2015). Other types of Th cells include Treg cells, which participate in the induction and maintenance of peripheral tolerance and actively terminate immune responses (Astry *et al.*, 2015; Koga *et al.*, 2019).

This study was carried out aiming to quantify $CD3^+$, $CD4^+$and $CD8^+$ T-cells population in SLE patients and to elucidate if there is a possible influence on disease activity scores and clinical manifestations.

2. Materials and methods

2.1. Human subjects:

One hundred SLE patients (13 men and 87 women) who fulfilled the American College of Rheumatology (ACR) **(**Hartman et al., 2012; Aringer et al., 2019) criteria for the identification of SLE patients were enrolled in the present study. They all attended the Rheumatology Department at Kasr El-Aini Hospitals, Cairo University, Egypt, for months. Patients were divided according to their disease activity score into two subgroups; low or inactive (SLEDAI score of <6)

and high or active (SLEDAI score ≥6) (Gattoet al., 2020). A physician evaluated patients' disease activity, clinical features, and medication. Clinical manifestations and patient data were gathered retrospectively from hospital records. At the time of blood sample collection, patients with concomitant cancers, diabetes, infections, abnormal lipid profile, and pregnant females were excluded. Demographic and clinical characteristics were collected through a structured interview and physical examination. In parallel, 100 healthy individuals matched for age and gender with the patients recruited with no history of autoimmune diseases or treatment with immunosuppressive agents (15 males and 85 females) served as a control group.

Ethical approval for this study was granted by the local ethics committee of the Ministry of Health, Health, and Human Ethical Clearance Committee guidelines for Clinical Research. Cairo University's local Ethics Committee approved the study protocol. All patients and healthy subjects agreed to be enrolled in this study, and informed consent was obtained from all participants.

2.2. Flow cytometric detection of T-lymphocytes

Venous blood (5ml) was collected in EDTA sterile tubes and centrifuged at 2000 xg for three minutes. Human peripheral blood mononuclear cells (PBMCs) were separated from blood by Ficoll-Hypaque separating media (Biowest SAS, Nuaillé, France). Anti-$CD3^+$, anti-$CD4^+$, and anti-$CD8^+$ monoclonal antibodies labeled with PerCP, FITC, and phyco-erytherin (PE) (BD Bioscience, San Jose, CA), respectively, were used in lymphocyte staining. A four-color BD AccuriTM C6 Plus personal flow cytometer was used to collect data (BD Biosciences, San Jose, CA).

2.3. Statistical analysis

All statistical analyses were performed using SPSS 21.0 (SPSS, Inc., Chicago, IL). Data were statistically presented regarding mean ± standard error (SE), frequencies when appropriate. Univariate analysis and differences between patient and control groups were assessed using Student's T-test for statistical analysis. The correlation between variables was determined using the Person's correlation test. All values showed two-sided with a P-value of <0.05 were considered significant.

3. Results

3.1. Characteristic of SLE patients

Demographic and biochemical characteristics of SLE patients and healthy controls were summarized in **Table (1)**. Concerning disease activity, 41 patients were in the active state while 59 were inactive. The comprehensive clinical features are presented in **Table (2)**.

3.2. Flow cytometric detection of T-lymphocytes

Our results in **Figure (1)** showed a significant elevation in percentage of $CD3^+$ ($P<0.01$) and $CD8^+$ ($P<0.001$) lymphocytes in SLE patients compared to healthy controls, with insignificant change in frequency of CD3+ and CD8+ in disease activity. On the contrary, a significant reduction was observed in the percentage of CD4+ T ($P<0.001$) cells in lupus patients in relation to normal. On the other side, there was a significant reduction ($P<0.05$) in the percentage of $CD4^+$ Th cells in active *vs* inactive SLE patients. A negative correlation was observed between the frequency of $CD8^+$ cells and $CD3^+$ ($r= -o.541$; $P<0.01$) and CD4+ ($r= -1.00$; $P<0.001$) **(Figure 2)** in active SLE patients**.**

3.3. Association between T-lymphocytes and SLE clinical manifestations

The association between the clinical manifestations of SLE and detected T- lymphocytes ($CD3^+$, $CD4^+$, and $CD8^+$) was presented in **Table (3).** A significant reduction in $CD3^+$ cells was seen in patients with Arthritis ($P<0.05$) and Haemolytic anemia ($P<0.05$) compared to patients without these

manifestations. A significant elevation in $CD4^+$ cells was observed in patients suffering from Oral Ulcers ($P<0.05$). At the same time, there was a significant reduction ($P<0.05$) in patients with vasculitis and Arthritis ($P<0.05$) compared to patients without those manifestations. Regarding $CD8^+$cells, there was a significant diminution ($P<0.05$) in SLE patients with vasculitis compared to patients without vasculitis.

Table (1): Demographic and biochemical characteristics of controls and SLE patients.

Parameters	**Control group (N=100)**	**SLE group (N=100)**	**P-value**
Age (years)	28.22± 7.90	31.8± 1.0	NS
Gender (female/male)	85/15	87/13	NS
ESR (mm/1st hour)	7.2 ± 0.2	56.4 ± 3.4	P<0.01
WBCs (X1000/µl)	8.5 ± 0.14	8.1 ± 0.4	NS
HGB (g/dl)	14.2 ± 0.14	11.0 ± 0.2	P<0.01
Platelets (X1000/µl)	295.5 ± 6.4	257.1 ± 12.3	P<0.01
Serum Creatinine (mg/dl)	0.93 ± 0.23	0.89 ± 0.7	NS
ALT (IU/L)	22.09 ± 0.6	21.2 ± 1.6	NS
AST (IU/L)	24.8 ± 0.6	23.5 ± 1.5	NS

All data are presented as mean ± standard Error (mean ± SE). NS = not significant.

ESR (erythrocyte sedimentation rate); White Blood Cells (WBCs); HGB (Hemoglobin); Alanine aminotransferase (ALT); Aspartate aminotransferase (AST);

Table (2): Clinical and laboratory characteristics of SLE patients

Demographic data	**Mean ± SD**	**Laboratory Data**	**Mean ± SD**
Age (years)	32.75±10.31	Serum albumin	3.19 ± 0.7
Disease duration (years)	7.97 ±5.73	C3titre	77.4 ± 44.6
Female/Male	87/13	C4 titre	20.6 ± 24.3
SLEDAI	8.37±9.01	Cholesterol	187.5 ± 72.1
ACR criteria of SLE	**No (%)**	Triglyceride	162.97 ± 92.9
Malar rash	73 (72.3)	HDL	47.2 ± 12.9
Photosensitivity	53 (52.5)	LDL	118.3 ± 47.3
Oral Ulcers	63 (62.4)	Consumed C3	34 (33.7)
Arthritis	50 (49.5)	Consumed C4	20 (19.8)
Osteonecrosis	9 (8.9)	**Treatment**	**N (%)**
Serositis	39 (38.6)	Antimalarial drug	89 (94.6)
Glomerulonephritis	62 (61.4)	Cyclophosphamide	62 (65.9)
Neuropsychiatric disorders	13 (14.9)	Azathioprine	67 (71.2)
PanCytopenia	19 (18.8)	Biological	6 (6.3)
Anti-nuclear Ab	71 (70.3)		
Anti-dsDNA Ab	71 (70.3)		
Hemolytic anemia	16 (15.8)		
Leucopenia	34 (33.7)		
Neutropenia	13 (12.9)		
Lymphopenia	31 (30.7)		

All data are presented as mean ± SE. P<0.05(*), P<0.01(**), P<0.001(***)

Table (3): $CD3^+$, $CD4^+$ and $CD8^+$ level in SLE patients with different clinical manifestations

Clinical parameter		**$CD3^+$**	**$CD4^+$**	**$CD8^+$**
Photosensitivity	No	70.81±4.36	48.73±2.93	51.27±2.93
	Yes	71.99±2.95	57.20±3.84	47.71±3.68
Oral Ulcers	No	69.91±4.84	45.32±3.43	54.68±3.43
	Yes	72.47±2.78	**59.16±3.64***	45.91±3.58
Arthritis	No	75.33±3.30	56.03±4.56	46.36±4.1
	Yes	**66.58±3.41***	**53.31±3.53***	52.26±3.55
Serositis	No	71.05±3.35	52.66±3.69	48.92±3.52
	Yes	72.58±3.45	59.06±4.90	48.39±4.61
Neuropsychiatric disorders	No	71.65±2.40	54.87±2.95	48.73±2.75
	Yes	70.56±2.39	54.67±2.82	48.61±2.64
PanCytopenia	No	73.80±2.57	55.47±3.35	47.76±3.21
	Yes	63.74±5.31	52.54±6.70	53.16±4.33
Constitutional symptoms	No	83.35±	43.05±9.19	56.94±9.19
	Yes	71.21±2.45	56.23±3.06	47.74±2.88
Discoid/subacute cutaneous lupus	No	64.47±18.88	47.06±13.28	52.94±13.28
	Yes	72.20±2.33	55.44±3.07	48.41±2.86
Osteonecrosis	No	71.81±2.70	55.53±3.39	48.46±3.19
	Yes	68.53±6.81	48.80±3.07	51.19±3.07
Vasculities	No	71.51±2.61	54.97±3.29	49.05±3.08
	Yes	72.79±6.42	**53.96±0.88***	**46.04±0.88***
Raynaud's phenomena	No	72.08±2.45	54.87±3.06	48.86±2.85
	Yes	60.13±2.33	54.73±3.04	45.27±2.72
Alopecia	No	72.75±2.89	53.79±3.59	48.23±3.12
	Yes	69.31±4.56	57.67±5.27	49.99±5.94
Haemolytic anemia	No	73.89±2.45	54.03±3.22	49.07±3.03
	Yes	**61.32±5.85***	58.88±7.91	46.66±7.29
Leucopenia	No	73.56±2.91	55.44±3.85	46.60±3.45
	Yes	67.19±4.56	53.76±4.99	53.89±4.89
Neutropenia	No	70.95±2.53	54.78±3.17	49.09±2.94
	Yes	84.18±2.34	59.92±3.14	40.08±2.84
Lymphopenia	No	73.56±2.91	55.44±3.86	46.60±3.45
	Yes	67.19±4.56	53.76±4.99	53.89±4.89

All data are presented as mean ± SE. P<0.05(*), P<0.01(**), P<0.001(***)

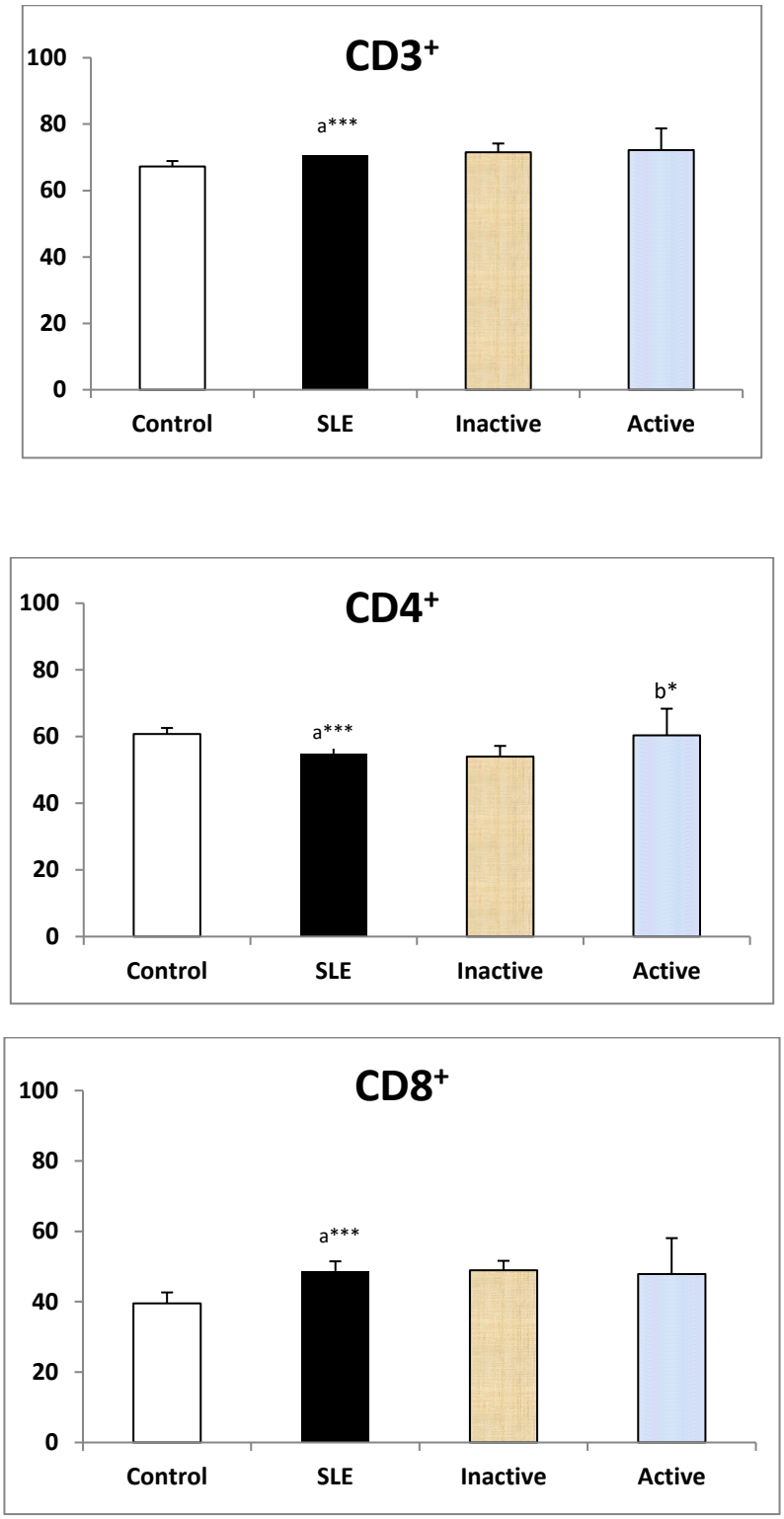

Figure 1: Percentage of $CD3^+$, $CD4^+$, and $CD8^+$ in controls and SLE patients (active and inactive) all data are presented as mean ± SE. "a" significant from the control group, "b" significant from inactive group. $P<0.05$(*), $P<0.01$(**), $P<0.001$(***).

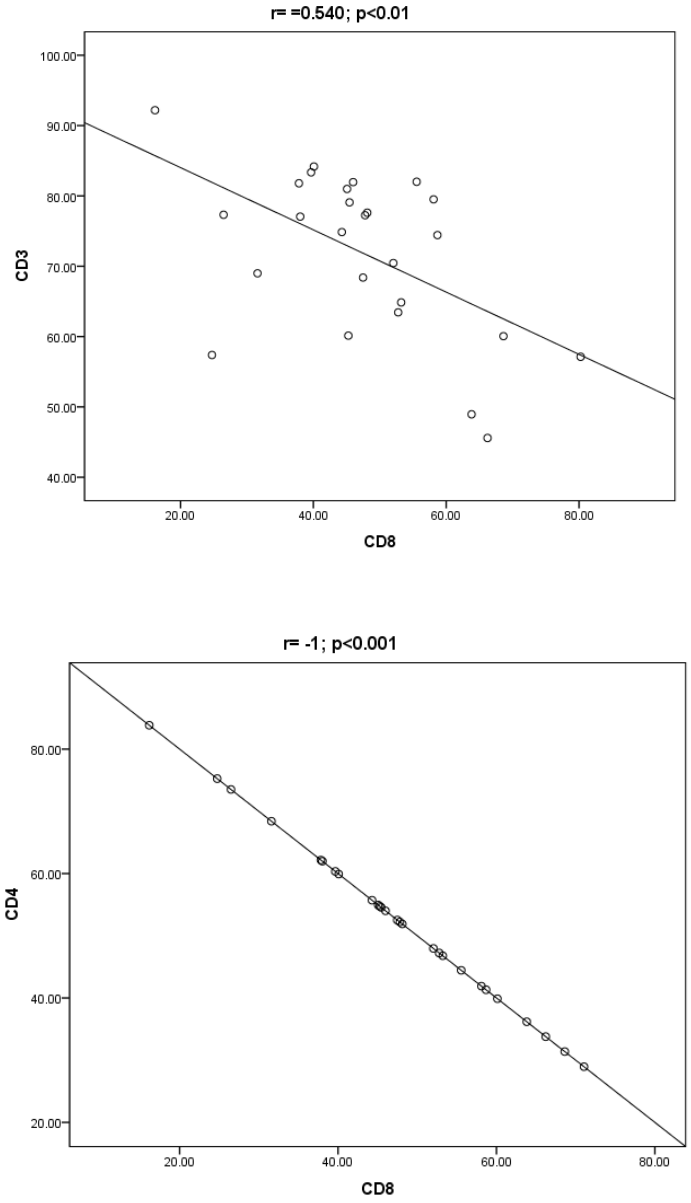

Figure 2: Correlation between different T cell subsets in active SLE patients.

4. Discussion

SLE is characterized serologically by B cell hyperactivity and a panoply of autoantibodies against nuclear, cytoplasmic, and cell surface antigens. Since these autoantibodies are mainly of the IgG1 subclass, T lymphocytes are likely essential in providing help to the autoantibody-producing B cells **(Manjili and Payne, 2016)**. Evidence for T cell involvement in disease pathogenesis is illustrated by the association of SLE with particular major histocompatibility complex (MHC) class II alleles and affinity maturation of IgG autoantibody production **(Kailashiya*et al.*, 2019)**. It is thought that T helper (Th) cells drive the production of pathogenic anti-DNA autoantibodies in SLE, and it has been shown, in vitro, that some of these are CD47 CD87. These, i.e., double-negative (DN) T cells, have been shown to express either the αβ T cell receptor (TCR) or γδ TCR (**Abdirama *et al.*, 2021)**.

Autoreactive $CD4^+$ T cells are implicated in the pathogenesis of SLE by promoting autoantibody production by B cells and directly propagating organ damage in inflamed target organs **(Suárez-Fueyo*et al.*, 2016; Abdirama *et al.*, 2021)**.

$CD8^+$ cells play a key role in recognizing and removing cells infected by intracellular pathogens and in antitumor response. Since stimulation only through the TCR receptor is unable to maintain optimum $CD8^+$ activation, the second costimulatory signal is essential for full activation and survival of these cells **(Jandl and King, 2016)**. The best-known costimulatory signal is provided by the interaction of CD28 molecules presented on the T lymphocyte as well as the CD86 and CD80 molecules expressed on the APC's surface. Adequate signal power delivered to naive T $CD8^+$ results in the proliferation and differentiation of two cell types. One of these is cytotoxic T lymphocytes (CTL), which undergo apoptosis after reaching maturity and fulfilling their effector function **(Yap *et al.*, 2010; Talaat *et al.*, 2015)**. The second type is T $CD8^+$ memory cells, both central and effector. Their continuous presence in the circulation is essential to control another potential exposure to the same antigen faster and more effectively **(Su*et al.*, 2012)**.

Most autoimmune diseases are associated with an increase in T CD8+CD28− (CD8+CD57+) cells, which exhibit highly cytotoxic activity and can be related to more severe disease manifestations **(Jin and Dong, 2013; Strzępa and Szczepanik, 2013)**. Quantitative changes in the CD8+CD57+ population were observed, among others, in multiple sclerosis, type 1 diabetes, Graves' disease, and rheumatoid arthritis. The decreased number of CD8+CD28− T-cells correlates with clinical response to abatacept in patients with rheumatoid arthritis **(Shah *et al.*, 2010; Talaat *et al.*, 2015)**. Some researchers have reported that lymphocytes with CD8+CD28− phenotype show regulatory properties. So far, few studies addressing the size of the CD8+CD28− subpopulation in patients with SLE have been conducted. It has been shown that the number of CD8+CD28− cells might be reduced or unchanged compared to the control group (**Tang *et al.*, 2019; Yazdani *et al.*, 2020).**

5. Conclusion

In conclusion, the current study reported a significant downregulation in $CD4^+$ cells with a substantial upregulation of $CD3^+$ and $CD8^+$ frequency in SLE patients. We stressed the importance of these subsets in several disease manifestations. Despite the conflicting reports in T lymphocytes frequencies between SLE patients and control subjects, the therapeutic potential of this cell population holds a great promise for the future employment of effective treatment modalities using the T cells population. Further functional research is needed to validate our findings.

Received: July 26, 2021. Accepted: September 18, 2021. Published: September 29, 2021

6. Conflict of interest: The authors declare that they have no conflict of interest.

7. References

- Abdirama D, Tesch S, Grießbach AS, von Spee-Mayer C, Humrich JY, Stervbo U, Babel N, Meisel C, Alexander T, Biesen R, Bacher P, Scheffold A, Eckardt KU, Hiepe F, Radbruch A, Burmester GR, Riemekasten G, Enghard P. Nuclear antigen-reactive CD4+ T cells expand in active systemic lupus erythematosus, produce effector cytokines, and invade the kidneys. Kidney Int. 2021 Jan;99(1):238-246. DOI: 10.1016/j.kint.2020.05.051. Epub 2020 Jun 24. PMID: 32592813.
- Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrøm K, Massarotti E, McCune J, Ruiz-Irastorza G, Sanchez-Guerrero J, Schneider M, Urowitz M, Bertsias G, Hoyer BF, Leuchten N, Tani C, Tedeschi SK, Touma Z, Schmajuk G, Anic B, Assan F, Chan TM, Clarke AE, Crow MK, Czirják L, Doria A, Graninger W, Halda-Kiss B, Hasni S, Izmirly PM, Jung M, Kumánovics G, Mariette X, Padjen I, Pego-Reigosa JM, Romero-Diaz J, Rúa-Figueroa Fernández Í, Seror R, Stummvoll GH, Tanaka Y, Tektonidou MG, Vasconcelos C, Vital EM, Wallace DJ, Yavuz S, Meroni PL, Fritzler MJ, Naden R, Dörner T, Johnson SR. 2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus. Arthritis Rheumatol. 2019 Sep;71(9):1400-1412. DOI: 10.1002/art.40930. Epub 2019 Aug 6. PMID: 31385462; PMCID: PMC6827566.
- Astry B, Venkatesha SH, Moudgil KD. Involvement of the IL-23/IL-17 axis and the Th17/Treg balance in the pathogenesis and control of autoimmune arthritis. Cytokine. 2015 Jul;74(1):54-61. DOI: 10.1016/j.cyto.2014.11.020. Epub 2015 Jan 13. PMID: 25595306; PMCID: PMC4457562.
- Azab NA, Bassyouni IH, Emad Y, Abd El-Wahab GA, Hamdy G, Mashahit MA. CD4+CD25+ regulatory T cells (TREG) in systemic lupus erythematosus (SLE) patients: the possible influence of treatment with corticosteroids. Clin Immunol. 2008 May;127(2):151-7. DOI: 10.1016/j.clim.2007.12.010. Epub 2008 Mar 4. PMID: 18299252.
- Basta F, Fasola F, Triantafyllias K, Schwarting A. Systemic Lupus Erythematosus (SLE) Therapy: The Old and the New. Rheumatol Ther. 2020 Sep;7(3):433-446. DOI: 10.1007/s40744-020-00212-9. Epub 2020 Jun 2. PMID: 32488652; PMCID: PMC7410873
- Becker-Merok A, Eilertsen GØ, Nossent JC. Levels of transforming growth factor-beta are low in systemic lupus erythematosus patients with active disease. J Rheumatol. 2010 Oct;37(10):2039-45. DOI: 10.3899/jrheum.100180. Epub 2010 Aug 3. PMID: 20682675.
- Cassia M, Alberici F, Gallieni M, Jayne D. Lupus nephritis and B-cell targeting therapy. Expert Rev Clin Immunol. 2017 Oct;13(10):951-962. DOI: 10.1080/1744666X.2017. 1366855. Epub 2017 Aug 18. PMID: 28800401.
- Cepika AM, Soldo Jureša D, MorovićVergles J, Malenica B, Santak M, Kapitanović S, Mayer M, Anić B, Sentić M, Gagro A. Decrease in circulating DNA, IL-10 and BAFF levels in newly-diagnosed SLE patients after corticosteroid and chloroquine treatment. Cell Immunol. 2012 Mar-Apr;276(1-2):196-203. DOI: 10.1016/j.cellimm.2012.05.009. Epub 2012 May 29. PMID: 22703694.
- Chen M, Chen X, Wan Q. Altered frequency of Th17 and Treg cells in new-onset systemic lupus erythematosus patients. Eur J Clin Invest. 2018*;*48*(*11*):e13012.*
- Cheng HB, Chen RY, Wu JP, Chen L, Liang YH, Pan HF, Pan ZF, Zhang QH, Li Q, Du TX, Lv YM,

Shi JQ. Complement C4 induces regulatory T cells differentiation through dendritic cell in systemic lupus erythematosus. Cell Biosci. 2015 Dec 23;5:73. DOI: 10.1186/s13578-015-0052-8. PMID: 26705467; PMCID: PMC4690337.

- Dai H, He F, Tsokos GC, Kyttaris VC. IL-23 Limits the Production of IL-2 and Promotes Autoimmunity in Lupus. J Immunol. 2017 Aug 1;199(3):903-910. DOI: 10.4049/jimmunol.1700418. Epub 2017 Jun 23. PMID: 28646040; PMCID: PMC5526729.
- Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. Ann N Y Acad Sci. 2010 Jan;1183:211-21. DOI: 10.1111/j.1749-6632.2009.05133.x. PMID: 20146717; PMCID: PMC2914500.
- Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. Clin Immunol. 2009 Jan;130(1):27-33. DOI: 10.1016/j.clim.2008.08.018. Epub 2008 Oct 8. PMID: 18845487; PMCID: PMC2660866.
- Fattorossi A, Battaglia A, Buzzonetti A, Ciaraffa F, Scambia G, Evoli A. Circulating and thymic CD4 CD25 T regulatory cells in myasthenia gravis: effect of immunosuppressive treatment. Immunology. 2005 Sep;116(1):134-41. DOI: 10.1111/j.1365-2567.2005.02220.x. PMID: 16108825; PMCID: PMC1802400.
- Gaffen SL, Jain R, Garg AV, Cua DJ. The IL-23-IL-17 immune axis: from mechanisms to therapeutic testing. Nat Rev Immunol. 2014 Sep;14(9):585-600. DOI: 10.1038/nri3707. PMID: 25145755; PMCID: PMC4281037.
- Gatto M, Saccon F, Zen M, Regola F, Fredi M, Andreoli L, Tincani A, Urban ML, Emmi G, Ceccarelli F, Conti F, Bortoluzzi A, Govoni M, Tani C, Mosca M, Ubiali T, Gerosa M, Bozzolo E, Canti V, Cardinaletti P, Gabrielli A, Tanti G, Gremese E, De Marchi G, De Vita S, Fasano S, Ciccia F, Pazzola G, Salvarani C, Negrini S, Puppo F, Di Matteo A, De Angelis R, Orsolini G, Rossini M, Faggioli P, Laria A, Piga M, Mathieu A, Scarpato S, Rossi FW, de Paulis A, Brunetta E, Ceribelli A, Selmi C, Prete M, Racanelli V, Vacca A, Bartoloni E, Gerli R, Larosa M, Iaccarino L, Doria A. Early Disease and Low Baseline Damage as Predictors of Response to Belimumab in Patients With Systemic Lupus Erythematosus in a Real-Life Setting. Arthritis Rheumatol. 2020 Aug;72(8):1314-1324. DOI: 10.1002/art.41253. Epub 2020 Jun 12. PMID: 32275125.
- Guimarães PM, Scavuzzi BM, Stadtlober NP, Franchi Santos LFDR, Lozovoy MAB, Iriyoda TMV, Costa NT, Reiche EMV, Maes M, Dichi I, Simão ANC. Cytokines in systemic lupus erythematosus: far beyond Th1/Th2 dualism lupus: cytokine profiles. Immunol Cell Biol. 2017 Oct;95(9):824-831. DOI: 10.1038/icb.2017.53. Epub 2017 Jun 26. PMID: 28649995.
- Han JW, Zheng HF, Cui Y, Sun LD, Ye DQ, Hu Z, Xu JH, Cai ZM, Huang W, Zhao GP, Xie HF, Fang H, Lu QJ, Xu JH, Li XP, Pan YF, Deng DQ, Zeng FQ, Ye ZZ, Zhang XY, Wang QW, Hao F, Ma L, Zuo XB, Zhou FS, Du WH, Cheng YL, Yang JQ, Shen SK, Li J, Sheng YJ, Zuo XX, Zhu WF, Gao F, Zhang PL, Guo Q, Li B, Gao M, Xiao FL, Quan C, Zhang C, Zhang Z, Zhu KJ, Li Y, Hu DY, Lu WS, Huang JL, Liu SX, Li H, Ren YQ, Wang ZX, Yang CJ, Wang PG, Zhou WM, Lv YM, Zhang AP, Zhang SQ, Lin D, Li Y, Low HQ, Shen M, Zhai ZF, Wang Y, Zhang FY, Yang S, Liu JJ, Zhang XJ. Genome-wide association study in a Chinese Han population identifies nine new susceptibility loci for systemic lupus erythematosus. Nat Genet. 2009 Nov;41(11):1234-7. DOI: 10.1038/ng.472. Epub 2009 Oct 18. PMID: 19838193.
- Hartman EAR, van Royen-Kerkhof A, Jacobs JWG, Welsing PMJ, Fritsch-Stork RDE. Performance of the 2012 Systemic Lupus International Collaborating Clinics classification criteria versus the 1997 American

College of Rheumatology classification criteria in adult and juvenile systemic lupus erythematosus. A systematic review and meta-analysis. Autoimmun Rev. 2018 Mar;17(3):316-322. DOI: 10.1016/j.autrev.2018.01.007. Epub 2018 Jan 31. PMID: 29366725.

- Hayball JD, Lake RA. Altered superantigenic ligands demonstrate the quantitative nature of T-cell activation. Immunol Cell Biol. 2000 Dec;78(6):623-32. DOI: 10.1046/j.1440-1711.2000.00971.x. PMID: 11114973.
- Jandl C, King C. Cytokines in the Germinal Center Niche. Antibodies (Basel). 2016 Feb 5;5(1):5. DOI: 10.3390/antib5010005. Erratum in: Antibodies (Basel). 2016 Apr 27;5(2): PMID: 31557986; PMCID: PMC6698856.
- Jin W, Dong C. IL-17 cytokines in immunity and inflammation. Emerg Microbes Infect. 2013 Sep;2(9):e60. DOI: 10.1038/emi.2013.58. Epub 2013 Sep 18. PMID: 26038490; PMCID: PMC3820987.
- Justiz Vaillant AA, Goyal A, Bansal P, Varacallo M. Systemic Lupus Erythematosus. 2020 Dec 30. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan–. PMID: 30571026.
- Kailashiya V, Singh U, Rana R, Singh NK, Dash D, Kailashiya J. Regulatory T Cells and Their Association with Serum Markers and Symptoms in Systemic Lupus Erythematosus and Rheumatoid Arthritis. Immunol Invest. 2019 Jan;48(1):64-78. DOI: 10.1080/08820139.2018.1527852. Epub 2018 Oct 16. PMID: 30325682.
- Kailashiya V, Singh U, Rana R, Singh NK, Dash D, Kailashiya J. Regulatory T Cells and Their Association with Serum Markers and Symptoms in Systemic Lupus Erythematosus and Rheumatoid Arthritis. Immunol Invest. 2019 Jan;48(1):64-78. DOI: 10.1080/08820139.2018.1527852. Epub 2018 Oct 16. PMID: 30325682.
- Karagiannidis C, Akdis M, Holopainen P, Woolley NJ, Hense G, Rückert B, Mantel PY, Menz G, Akdis CA, Blaser K, Schmidt-Weber CB. Glucocorticoids upregulate FOXP3 expression and regulatory T cells in asthma. J Allergy Clin Immunol. 2004 Dec;114(6):1425-33. DOI: 10.1016/j.jaci.2004.07.014. PMID: 15577848.
- Knochelmann HM, Dwyer CJ, Bailey SR, Amaya SM, Elston DM, Mazza-McCrann JM, Paulos CM. When worlds collide: Th17 and Treg cells in cancer and autoimmunity. Cell Mol Immunol. 2018 May;15(5):458-469. DOI: 10.1038/s41423-018-0004-4. Epub 2018 Mar 21. PMID: 29563615; PMCID: PMC6068176.
- Koga T, Ichinose K, Kawakami A, Tsokos GC. The role of IL-17 in systemic lupus erythematosus and its potential as a therapeutic target. Expert Rev Clin Immunol. 2019 Jun;15(6):629-637. DOI: 10.1080/1744666X.2019.1593141. Epub 2019 Mar 25. PMID: 30874446.
- Koga T, Ichinose K, Tsokos GC. T cells and IL-17 in lupus nephritis. Clin Immunol. 2017 Dec;185:95-99. DOI: 10.1016/j.clim.2016.04.010. Epub 2016 Apr 21. PMID: 27109641; PMCID: PMC5074925.
- Kuwabara T, Ishikawa F, Kondo M, Kakiuchi T. The Role of IL-17 and Related Cytokines in Inflammatory Autoimmune Diseases. Mediators Inflamm. 2017;2017:3908061. DOI: 10.1155/2017/3908061. Epub 2017 Feb 20. PMID: 28316374; PMCID: PMC5337858.
- Li D, Guo B, Wu H, Tan L, Chang C, Lu Q. Interleukin-17 in systemic lupus erythematosus: a comprehensive review. Autoimmunity. 2015;48(6):353-361.
- Lourenço EV, La Cava A. Cytokines in systemic lupus erythematosus. Curr Mol Med. 2009 Apr;9(3):242-54. DOI:

10.2174/156652409787847263. PMID: 19355907; PMCID: PMC3589140.

- Manjili MH, Payne KK. Immune Regulatory Function of Tregs. Immunol Invest. 2016 Nov;45(8):708-711. DOI: 10.1080/08820139.2016.1235394. PMID: 27775448.
- Mathias LM, Stohl W. Systemic lupus erythematosus (SLE): emerging therapeutic targets. Expert Opin Ther Targets. 2020 Dec;24(12):1283-1302. DOI: 10.1080/14728222.2020.1832464. Epub 2020 Dec 1. PMID: 33034541.
- Mok MY, Wu HJ, Lo Y, Lau CS. The relation of interleukin 17 (IL-17) and IL-23 to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus. J Rheumatol. 2010 Oct;37(10):2046-52. DOI: 10.3899/jrheum.100293. Epub 2010 Aug 3. PMID: 20682672.
- Moore E, Putterman C. Are lupus animal models useful for understanding and developing new therapies for human SLE? J Autoimmun. 2020 Aug;112:102490. DOI: 10.1016/j.jaut.2020.102490. Epub 2020 Jun 11. PMID: 32535128; PMCID: PMC7384952.
- Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, Cua DJ. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med. 2003 Dec 15;198(12):1951-7. DOI: 10.1084/jem.20030896. Epub 2003 Dec 8. PMID: 14662908; PMCID: PMC2194162.
- Murphy CA, Langrish CL, Chen Y, Blumenschein W, McClanahan T, Kastelein RA, Sedgwick JD, Cua DJ. Divergent pro- and antiinflammatory roles for IL-23 and IL-12 in joint autoimmune inflammation. J Exp Med. 2003 Dec 15;198(12):1951-7. DOI: 10.1084/jem.20030896. Epub 2003 Dec 8. PMID: 14662908; PMCID: PMC2194162.
- Okamoto A, Fujio K, Okamura T, Yamamoto K. Regulatory T-cell-associated cytokines in systemic lupus erythematosus. J Biomed Biotechnol. 2011;2011:463412. DOI: 10.1155/2011/463412. Epub 2011 Dec 18. PMID: 22219657; PMCID: PMC3247013.
- Pan L, Lu MP, Wang JH, Xu M, Yang SR. Immunological pathogenesis and treatment of systemic lupus erythematosus. World J Pediatr. 2020 Feb;16(1):19-30. DOI: 10.1007/s12519-019-00229-3. Epub 2019 Feb 22. PMID: 30796732; PMCID: PMC7040062.
- Puliaeva I, Puliaev R, Via CS. Therapeutic potential of CD8+ cytotoxic T lymphocytes in SLE. Autoimmun Rev. 2009 Jan;8(3):219-23. DOI: 10.1016/j.autrev.2008.07.045. Epub 2008 Aug 24. PMID: 18725326; PMCID: PMC3215296.
- Shah K, Lee WW, Lee SH, Kim SH, Kang SW, Craft J, Kang I. Dysregulated balance of Th17 and Th1 cells in systemic lupus erythematosus. Arthritis Res Ther. 2010;12(2): R53. DOI: 10.1186/ar2964. Epub 2010 Mar 24. Erratum in: Arthritis Res Ther. 2010;12(3):402. PMID: 20334681; PMCID: PMC2888202.
- Singla S, Wenderfer SE, Muscal E, Sagcal-Gironella ACP, Orange JS, Makedonas G. Changes in Frequency and Activation Status of Major CD4+ T-Cell Subsets after Initiation of Immunosuppressive Therapy in a Patient with New Diagnosis Childhood-Onset Systemic Lupus Erythematosus. Front Pediatr. 2017 May 15;5:104. DOI: 10.3389/fped.2017.00104. PMID: 28555177; PMCID: PMC5430328.
- Stritesky GL, Yeh N, Kaplan MH. IL-23 promotes maintenance but not commitment to the Th17 lineage. J Immunol. 2008 Nov 1;181(9):5948-55. DOI: 10.4049/jimmunol. 181.9.5948. PMID: 18941183; PMCID: PMC2678905.
- Strzępa A, Szczepanik M. IL-17-expressing cells as a potential therapeutic target for treatment of

immunological disorders. Pharmacol Rep. 2011;63(1):30-44. DOI: 10.1016/s1734-1140(11)70396-6. PMID: 21441609.

- Su DL, Lu ZM, Shen MN, Li X, Sun LY. Roles of pro-and anti-inflammatory cytokines in the pathogenesis of SLE. J Biomed Biotechnol. 2012;2012:347141. DOI: 10.1155/2012/347141. Epub 2012 Feb 15. PMID: 22500087; PMCID: PMC3303597.
- Su DL, Lu ZM, Shen MN, Li X, Sun LY. Roles of pro-and anti-inflammatory cytokines in the pathogenesis of SLE. J Biomed Biotechnol. 2012;2012:347141. DOI: 10.1155/2012/347141. Epub 2012 Feb 15. PMID: 22500087; PMCID: PMC3303597.
- Suárez A, López P, Gómez J, Gutiérrez C. Enrichment of CD4+ CD25high T cell population in patients with systemic lupus erythematosus treated with glucocorticoids. Ann Rheum Dis. 2006 Nov;65(11):1512-7. DOI: 10.1136/ard.2005.049924. Epub 2006 Apr 10. PMID: 16606650; PMCID: PMC1798359.
- Suárez-Fueyo A, Bradley SJ, Tsokos GC. T cells in Systemic Lupus Erythematosus. Curr Opin Immunol. 2016 Dec;43:32-38. DOI: 10.1016/j.coi.2016.09.001. Epub 2016 Sep 13. PMID: 27636649; PMCID: PMC5125867.
- Talaat RM, Elmaghraby AM, Barakat SS, El-Shahat M. Alterations in immune cell subsets and their cytokine secretion profile in childhood idiopathic thrombocytopenic purpura (ITP). Clin Exp Immunol. 2014 May;176(2):291-300. DOI: 10.1111/cei.12279. PMID: 24460857; PMCID: PMC3992042.
- Talaat RM, Mohamed SF, Bassyouni IH, Raouf AA. Th1/Th2/Th17/Treg cytokine imbalance in systemic lupus erythematosus (SLE) patients: Correlation with disease activity. Cytokine. 2015 Apr;72(2):146-53. DOI: 10.1016/j.cyto.2014.12.027. Epub 2015 Jan 31. PMID: 25647269.
- Tang Y, Tao H, Gong Y, Chen F, Li C, Yang X. Changes of Serum IL-6, IL-17, and Complements in Systemic Lupus Erythematosus Patients. J Interferon Cytokine Res. 2019 Jul;39(7):410-415. DOI: 10.1089/jir.2018.0169. Epub 2019 Jun 7. PMID: 31173544.
- Wang D, Huang S, Yuan X, Liang J, Xu R, Yao G, Feng X, Sun L. The regulation of the Treg/Th17 balance by mesenchymal stem cells in human systemic lupus erythematosus. Cell Mol Immunol. 2017 May;14(5):423-431. DOI: 10.1038/cmi.2015.89. Epub 2015 Oct 5. PMID: 26435067; PMCID: PMC5423084.
- Wang X, Wei Y, Xiao H, Liu X, Zhang Y, Han G, Chen G, Hou C, Ma N, Shen B, Li Y, Egwuagu CE, Wang R. A novel IL-23p19/Ebi3 (IL-39) cytokine mediates inflammation in Lupus-like mice. Eur J Immunol. 2016 Jun;46(6):1343-50. DOI: 10.1002/eji.201546095. Epub 2016 Apr 13. PMID: 27019190.
- Yap DY, Lai KN. Cytokines and their roles in the pathogenesis of systemic lupus erythematosus: from basics to recent advances. J Biomed Biotechnol. 2010;2010:365083. DOI: 10.1155/2010/365083. Epub 2010 May 6. PMID: 20467470; PMCID: PMC2866250.
- Yazdani MR, Aflaki E, Gholijani N. Inflammatory and T Helper 17/ Regulatory T Cells Related Cytokines Balance in Cutaneous Lupus Erythematosus (CLE). Iran J Allergy Asthma Immunol. 2020 Feb 1;19(1):9-17. DOI: 10.18502/ijaai.v19i1.2411. PMID: 32245325.
- Zhang L, Yuan S, Cheng G, Guo B. Type I IFN promotes IL-10 production from T cells to suppress Th17 cells and Th17-associated autoimmune inflammation. PLoS One. 2011;6(12):e28432. DOI: 10.1371/journal.pone.0028432. Epub 2011 Dec 6. PMID: 22163016; PMCID: PMC3232207.