NINO KIKODZE [1,2], MANANA IOBADZE [2], NINO TSISKARISHVILI [3], IA PANTSULAIA [1,2], NONA JANIKASHVILI [1], TINATIN CHIKOVANI[1]

TH SUBSETS AND SERUM CYTOKINES IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

[1]Immunology department, Tbilisi State Medical University; [2]V.Bakhutashvili Institute of medical Biotechnology, Tbilisi State Medical University; [3]Todua Medical Center

ნინო ქიქოძე [1,2], მანანა იობაძე [2], ნინო ცისკარიშვილი [3], ია ფანცულაია [1,2], ნონა ჯანიკაშვილი [1], თინათინ ჩიქოვანი [1]

T ჰელპერების სუბპოპულაციები და შრატის ციტოკინები რევმატოიდული ართრიტის პათოგენეზში

[1]იმუნოლოგიის დეპარტამენტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; [2]ვ.ბახუტაშვილის სახ. სამედიცინო ბიოტექნოლოგიის ინსტიტუტი, თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი; [3]ფ. თოდუას სამედიცინო ცენტრი

რეზიუმე

რევმატოიდული ართრიტი სისტემური ანთებითი დაავადებაა. ის ხასიათდება ხრტილის და ძვლის დაზიანებით, რაც სახსრის ფუნქციის დარღვევას იწვევს. დროთა განმავლობაში, შესაძლოა მულტიორგანული: ფილტვის, გულის და თირკმლის დაზიანებები განვითარდეს. დაავადების დაწყებაში, სისტემური აქტიური ფაზის დროს, ასევე მკურნალობის შემდეგ უფრო ლოკალურ არააქტიურ ფაზაში გადასვლის დროს, იმუნური და სტრომის უჯრედების, ამ უჯრედების მიერ წარმოქმნილი ციტოკინებისა და ქემოკინების რთული ქსელი მონაწილეობს. რევმატოიდული არტრიტის მქონე პაციენტების პერიფერიული სისხლის Th1, Th17, Tregs და CD4⁺CD39⁺ უჯრედების და პრო- და ანტიანთებითი ციტოკინების პროფილის შეფასება მოხდა 47 პაციენტსა და 20 ჯანმრთელ ინდივიდში.

რევმატოიდული ართრიტის მქონე პაციენტები დაიყო ორ ჯგუფად: აქტიური და არააქტიური ართრიტი. მოცირკულირე Th1, Th17, Tregs და $CD4^+CD39^+$ უჯრედების სიხშირის განისაზღვრა ციტოფლუორომეტრიის საშუალებით. შრატში ციტოკინების IL-6, IL-10, IL-4, IL-17, TNF-α და TGF-β1 დონე განისაზღვრა იმუნოფერმენტული მეთოდით. აქტიური რევმატოიდული ართრიტის ჯგუფში Th1 და Th17 უჯრედების სიხშირე იზრდებოდა, მაშინ როცა Tregs სიხშირე, უცვლელი იყო ართრიტის ჯგუფებში. CD39 მარკერი სარწმუნოდ დაქვეითდა აქტიურ ჯგუფში, არააქტიურ და კონტროლის ჯგუფებთან შედარებით. თანდაყოლილი იმუნური სისტემის ციტოკინები: IL-6 და TNF-α სარწმუნოდ გაზრდილია აქტიური ართრიტის ჯგუფში, მაშინ როცა შრატში IL-17/IL-21 ციტოკინების კონცენტრაციის მიხედვით ეს ჯგუფი ერთგვაროვანი არ არის. ანტიანთებითი ციტოკინები: TGF-β და IL-4 დაქვეითებულია აქტიური ართრიტის ჯგუფში კონტროლთან შედარებით.

კვლევა აჩვენებს, რომ რევმატოიდული ართრიტის განვითარება ასოცირებულია შრატის პრო- და ანტიანთებითი ციტოკინების ცვლილებებთან, რაც გავლენას ახდენს T ჰელპერების სუბპოპულაციების ბალანსზე და ახალი თერაპიული შესაძლებლობების ფანჯარას ხსნის.

Introduction. Rheumatoid arthritis (RA) is a systemic inflammatory disease. It is characterized by damage of cartilages and bones which results in destruction of joint function. Multi-organic disorders of lung, heart and kidney can be developed in time. Complex network of immune and stromal cells, cytokines and chemokines produced by these cells participate in onset of the disease, in the systemic active phase and during the transition to more localized inactive disease after the treatment. Recent studies have shown that the balance between T cell subsets plays a crucial role in the development of RA [1,2]. Cytokines produced by dendritic cells and macrophages: IL-1, IL-6, IL-12, IL-15, IL-18, TNF-α determine early onset and development of rheumatoid arthritis. They form pro-inflammatory cytokine milieu which drives differentiation Th1 cells in the synovial tissues of RA patients but not in osteoarthritis patients [3,4,5]. Around 40% of citrulline-reactive $CD4^+$ T cells were found to be CXCR3 (surface marker for Th1 cells) positive [6] in the blood of RA patients [7]. $CD4^+$ T cells, triggered simultaneously by IL-6 and TGF-β differentiated into Th17 cells, which have been reported to play crucial role in the pathogenesis of RA [8,9]: its cytokine IL-17 induces osteoclastogenesis [10], stimulates synovial fibroblasts to produce IL-6 [11] and macrophages to produce TNF-α [12]. Th17 cells produce IL-21, which plays a central role in the amplification of inflammatory processes, the activation and proliferation of various immune cells including

Th17 cells, T follicular helpers, B cells and macrophages facilitating pathogenic role of this cytokine in the development of RA [7,13]. Throughout the course of the disease high quantity of IL-21 is produced by Tfh cells in secondary follicles in joints and lymph nodes, resulting in B cell activation and production of auto-antibodies such as rheumatoid factor, anti-CCP and anti-MCP antibodies [14]. Immune complexes, which deposit in synovium induce activation of neutrophils. Lysosomal enzymes released from neutrophils lead to tissues damage. Pro-inflammatory cytokines activate synovial fibroblasts, cause expansion and differentiation of osteoclasts via RANKL, that lead to bone resorption [15]. Degradation of the collagen matrix is caused by IL-1-induced matrix metalloproteinases (MMPs), membrane-type 1 MMP (MT1-MMP) from synovial cells, that enhance joint injury [16]. Activated autoreactive T helpers can be suppressed by T regulatory cells (Tregs) which are key players in maintenance of immunologic homeostasis and prevention autoimmunity [17]. Presumably microenvironment of chronic inflammation established in RA patients leads to aberrations in Tregs.

It is shown that proportion of Tregs ($CD4^+CD25^+FoxP3^+$) in $CD4^+$ T cells correlates with clinical response in RA patients [18,19]. Kikuchi et al. reported that expression of transcriptional factor Foxp3 positively correlates with expression of CD39 molecules on $CD4^+$ cells [20,21]. The ectonucleotidase CD39 has recently been reported as being responsible for hydrolysis of proinflammatory extracellular ATP, generated adenosine expresses immunosuppressive effects through adenosine A2A receptor (A2AR). This leads to the inhibition of effector T cell activation [22]. Deprivation of ATP, that inhibits differentiation of Th17 cells is additional mechanism of suppression of autoimmune processes [23,24,25,26].

Thus, imbalance between Th17 and Treg cells is an important mechanism which may lead to RA [2]. Influence of cytokines on Th17/Tregs balance is under the intense investigation.

Despite the fact that investigators are mostly focused on the pro-inflammatory cytokines which trigger RA, anti-inflammatory cytokines responsible for the suppression and regulation of the disease are not less important in the pathophysiology of RA [27]. Many authors report lower plasma TGF-β concentration in RA patients. TGF-β leads to Tregs polarization in vitro by increasing FoxP3 and CD39 expression on differentiating and differentiated Tregs and enhances their suppressive activity [28,29].

C.H. QU et al. showed that RA patients had remarkably lower serum IL-10 level [30], main source of which is synovium macrophages. It is worth to note, that data are highly controversial about IL-10 concentration in RA patients. It is reported significant increase in the level of IL-10 in patients having DAS > 3.2 [31]. In animal models IL-10 reduces severity of arthritis [32]. Hui Shen et al. reported, that plasma level of other anti-inflammatory cytokine IL-4 was elevated only in RA patients with interstitial lung disease (ILD), progressive complication of RA compare to RA patients without ILD [33].

The aim was to study Th subsets and pro- and anti-inflammatory cytokines in patients with rheumatoid arthritis.

Methods and materials. Study Design. 47 patients included in the study were recruited at Todua Medical Center and Caraps Medline. They fulfilled the American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) 2010 classification criteria for RA [34].

Disease activity were assessed by calculating DAS-28 score using an online application. The patients were divided into two groups: active rheumatoid arthritis (active RA group) and low disease activity (inactive RA group). There were 18 females and 9 males in the active group (n = 27) (mean age: 49.6$\pm$9.3). The mean disease duration was 5.2$\pm$4.2 years. In the inactive group (n = 20) 14 patients were females and 6 - males (mean age: 52.7$\pm$6.7). The mean disease duration was 6.1$\pm$5.2 years. 15 females and 5 males healthy individuals (mean age: 50$\pm$4.5) form control group (n = 20).

The patients with active disease: DAS-28$\geq$3.2 ESR>32mm/hour, CRP>9.8mg/l. The patients with inactive disease DAS-28<3.2 ESR<24 mm/hour, CRP<5.6 mg/l. None of the patients had been treated with any corticosteroid during the last three months. All patients were receiving nonsteroidal anti-inflammatory drugs at the time of sampling.

Cellular Subsets. Peripheral blood samples were collected from each patient in an EDTA anticoagulant-treated tube on day 0 (before starting treatment). The immunophenotypic analysis was accomplished within 24 h of the sample collections. Treg cells - $CD4^+CD25^+FoxP3^+$ and $CD4^+CD39^+$, Th1 cells - $CD4^+$T-bet^+, Th17 cells - $CD4^+$RoR-γt^+ were evaluated in samples.

Flow cytometric immunophenotyping was performed on peripheral venous blood in EDTA-anticoagulated vacutainers. The samples were maintained at room temperature and processed within 24 h of collection. Mononuclear cells were separated on ficol-paque gradient. The cells were added anti-CD3-FITC, anti-CD4-PE-Cy7, anti-CD39-PE, anti-CD25-PE, anti-FoxP3-APC, anti-T-bet-PE, anti-RoR-γt-PE monoclonal antibodies (eBioscience, USA) and incubated in the dark at C4^{0}C for 30 minutes. The flow cytometric analysis was done on a Facs calibur (BD Bioscience, USA). For the evaluation of the data, the events were gated on the forward side scatter to exclude remaining red blood cell and cellular debris. For each tube, at 10000 gated events were acquired. Data were processed using the Flow Jo v7 software (USA).

Cytokine assay. Human ELISA kits were used to detect the serum levels of TNF-α, TGF-β1, IL-6, IL-21, IL-17A, IL-10, IL-4 (ebioscience, USA) by following the manufacturer's protocol.

Statistical analysis. Continuous variables were analyzed with a Student's *t*-test, differences between groups were performed using the Mann–Whitney U test. P-values of <0.05 were regarded as significant.

Ethical Approval. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Results. Immunophenotyping of T cell subsets demonstrates that the frequency of Th1cells was significantly higher (P <0.005) in active group of patients (19.94±7.24) compare to healthy subjects (8.63±2.02) and inactive group (10.04±0.55) (Figure 1). Remarkable difference (P<0.05) was found between frequencies of Th17cell subset in active RA (10.86±4.18) and healthy subjects (2.00±0.9). The frequency of $CD4^{+}RoR\text{-}\gamma t^{+}$ cells was significantly (P<0.05) lower in inactive group (2.06±0.51) in comparison with active group. However, there was not statistically significant difference in the frequencies of Th17 cells between inactive and control groups (Figure 1).

The frequencies of circulating Tregs ($Foxp3^{+}CD4^{+}CD25^{+}$) were found unchanged in groups of the patients in comparison with healthy controls (P<0.05) (Figure 1).

On the contrary, significant changes of circulating $CD39^{+}$ cells in CD4 compartment in any studied groups were revealed. We observed significantly lower frequency in active RA group (1.07±0.80) compare to inactive group of patients (6.19±3.41) and healthy controls (14.60±5.62) (P<0.05) (Figure 1).

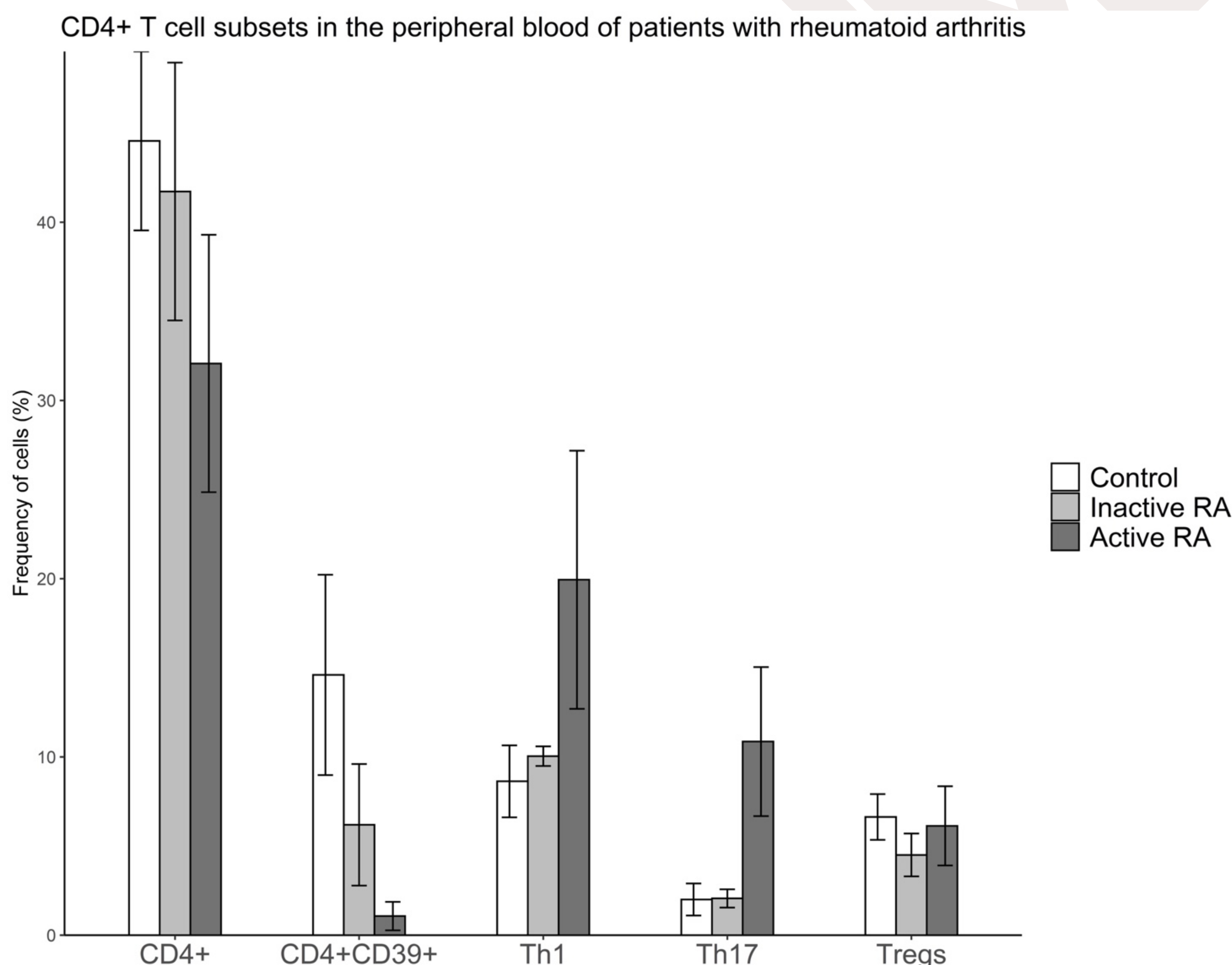

Figure 1. Th1, Th17, Treg cells and the CD4+CD39+/CD4+ cells changes in both study groups and control. A significant decrease was observed in the CD4+CD39+/CD4+ cells in active RA group. A significant increase was shown in Th1 and Th17 cells in active RA group.

Plasma concentrations of IL-17, IL-21, IL-6 and TNF-α in patients with RA are increased compare to healthy controls. Active RA [IL-17: 25.13±10.80 pg/ml; IL-21: 2.55±1.59 ng/ml; IL-6: 30.10±13.35 pg/ml; TNF-α: 120.87±20.02 pg/ml] and inactive RA [IL-17: 22.83±5.63 pg/ml; IL-21: 1.62±0.70 ng/ml; IL-6: 5.24±0.57 pg/ml; TNF-α: 12.56±1.06 pg/ml] in comparison with healthy subjects [IL-17: 13.95±4.28

pg/ml; IL-21: 0.88±0.06 ng/ml; IL-6: 3.25±1.98 pg/ml; TNF-α: 8.11±2.45 pg/ml] (P <0.001), while there was no obvious difference between two RA groups (P<0.05) in plasma concentrations of TGF-β1 and IL-10 in active RA [TGF-β1: 32.63±4.16 ng/ml; IL-10: 3.51±0.86 pg/ml] and inactive RA groups [TGF-β1: 32.76+3.94 ng/ml; IL-10: 4.29±1.20 pg/ml]. TGF-β1 significantly decreased when compared with healthy control group [78.48±6.34ng/ml] (P<0.001). Other anti-inflammatory cytokine IL-4 significantly decreased in RA patients (active RA: 3.27±2.13pg/ml; inactive RA: 19.05±4.71pg/ml) compare to healthy control group (34.30±8.92 pg/ml) (Figure 2).

In addition, frequencies of Th17 cells were positively correlated with plasma concentrations of IL-21 (r = 0.338, P<0.01), IL-17 (r = 0.398, P<0.05) and negatively correlated with plasma concentrations of TGF-β1 (r = -0.402, P<0.05).

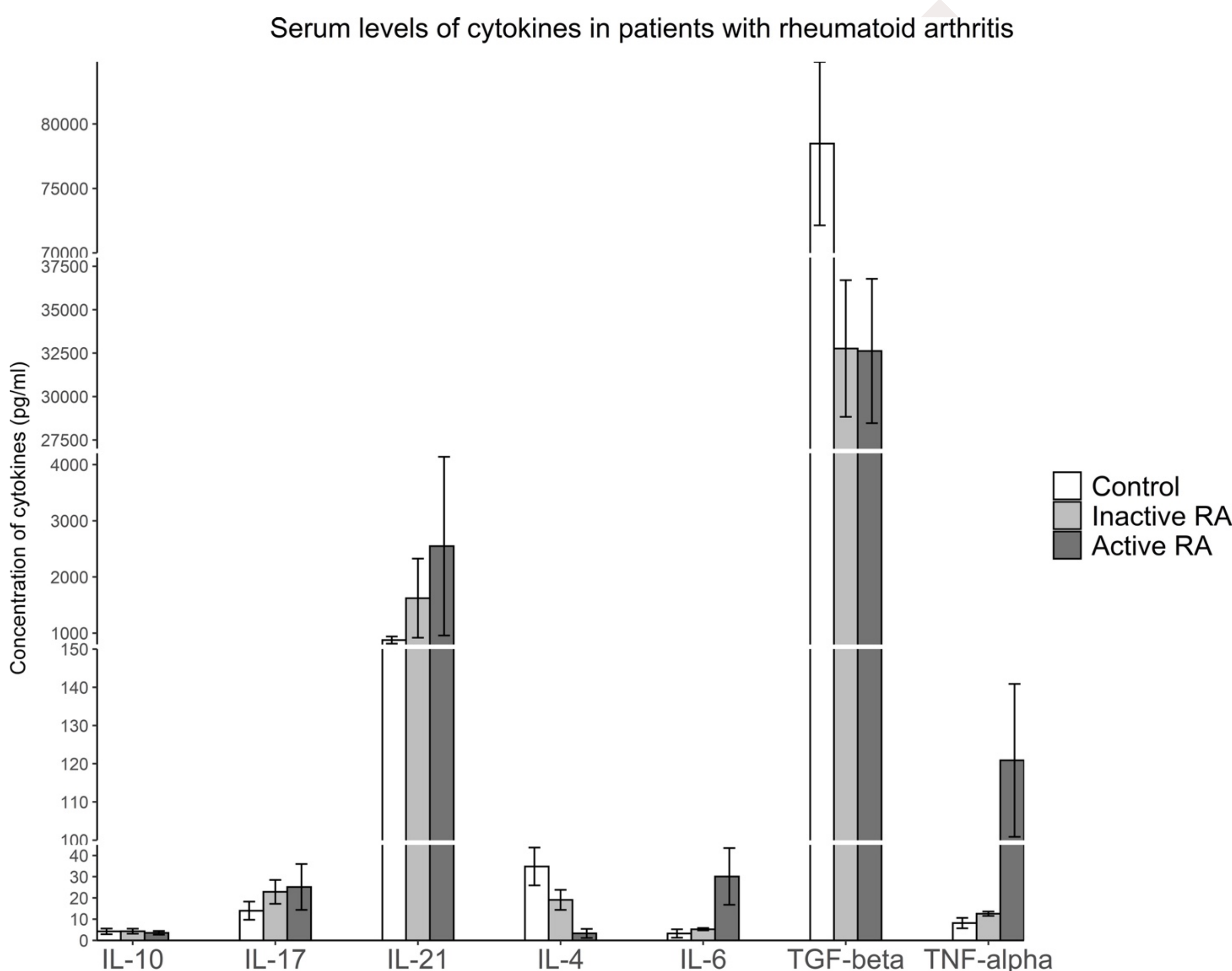

Figure 2. Serum IL-6, IL-10, IL-4, IL-17, IL-21, TNF-α and TGF-β1 concentration changes in both study groups and control. A significant increase was noted in TNF-α and IL-6 in active RA group. A significant decrease was noted in TGF-β and IL-4 in the both RA groups.

Discussion. Disturbed peripheral balances of Th1, Th17, Treg cells and serum pro- and anti-inflammatory cytokines were shown in the present study. Frequencies of Th1 and Th17 cells in the peripheral blood of active RA group are significantly increased in comparison with healthy controls and inactive RA group as it is reported by many authors [1,3]. That confirms suggestion about crucial role of these helper cells in the development of RA. Higher frequency of Th17 cells along with activated synovial fibroblasts and macrophages leads to the enhance production of cytokines: IL-17, TNF-α and IL-6 and sustains an inflammation of joint tissues in RA [35,36,37].

Hierarchical importance of IL-6 and TNF-α in the pathogenesis of RA is already well established [38]. Present study confirms significant difference between active and inactive RA groups and healthy controls according to the peripheral levels of these cytokines. Importance of identification of other cytokines (besides IL-6 and TNF-α) which could present additional therapeutic targets of treatment of RA is obvious. In active RA group of patients two subgroups are identified according to two correlated cytokines IL-17/IL-

21: with high and low level of serum IL-17 and IL-21. It is supposed that in patients with higher serum concentration of IL-17 and IL-21 remission is difficult to achieve. It is suggested that in this group of refractory disease IL-17, IL-21 can play role of therapeutic targets for biologics.

T regulatory cells (Tregs) express reciprocal developmental pathway of generation from pathogenic Th17 cells. Normal amount and function of Tregs prevent breach of self-tolerance at the periphery and avoid development of autoimmune diseases [38]. Expression of CD39 ectonucleotidase on Tregs intensifies their suppressive capacity on autoreactive T cells [39,40,41]. We revealed that frequency of Treg cells in the peripheral blood of RA patients did not significantly differ from that of in healthy individuals. Whereas, frequency of $CD39^{+}CD4^{+}$ cells in active RA group were significantly decreased than in inactive RA patients and healthy controls. We suppose, CD39 stabilizes suppressive activity of Tregs. Reduced expression of CD39 leads to the functional disorder of Tregs. Therefore, despite normal frequency of these cells in the peripheral blood breach of tolerance takes place. Enhance of CD39 expression on Tregs via activation of TGFβRII, TGFβRI and other molecules can be induced by TGF-β stimulation [29]. We suppose, that reduced serum TGF-β concentration in our RA patients explains decreased suppressive activity of Tregs in these patients. Reduced TGF-β and dramatically increased IL-6 at the same time form cytokine milieu beneficial for Th17 differentiation. Drastically increased frequency of Th1 and Th17 cells in active RA in combination with decreased suppressive activity of Tregs shifts balance to the side of autoreactive cells and triggers autoimmune process. Th17 cell cytokine - IL-17 along with IL-9 promotes the secretion of chemokine and cytokines which in turn accelerates the infiltration of neutrophils in the tissues thereby aggravates inflammation and tissue injury [42].

IL-10 is an anti-inflammatory and immunoregulatory cytokine which suppresses the formation of pro-inflammatory cytokines as well as down regulates the functioning of antigen-presenting cells [43]. It also inhibits the production of proteases and stimulates the formation of tissue inhibitor of metalloproteinases-1 (TIMP-1) by monocytes [32]. We did not find out significant changes in the serum level of IL-10 between groups unlike studies which detected higher level of IL-10 in response to higher inflammatory state of RA patients [31].

The role of Th2 cell cytokine - IL-4 is not clearly understood in the pathogenesis of RA. The study results indicate that anti-inflammatory and modulatory cytokine IL-4 seems to play an important role in the pathogenesis of disease. This cytokine is not detected in synovium and synovial fluid of patients and lack of IL-4 is likely to contribute to the uneven Th1/Th2 balance and to the chronic nature of RA. IL-4 gene therapy reduced IL-17 and RANKL expression in the synovium and prevents bone erosion [44]. Serum IL-4 concentration was significantly decreased in RA groups in comparison with healthy control. Therapeutic strategies that enhance local IL-4 production may protect against cartilage and bone destruction in RA.

Substantial plasticity between T cell subsets, different cytokine milieu during discrete phases of disease development provides various therapeutic opportunities to meet clinical needs of patients.

References:

1. Nistala K, Wedderburn LR. Th17 and regulatory T cells: rebalancing pro- and anti-inflammatory forces in autoimmune arthritis // Rheumatology, vol.48, pp.602–6, 2009.
2. Liu J, Hong X, Lin D, et al. Artesunate influences Th17/Treg lymphocyte balance by modulating Treg apoptosis and Th17 proliferation in a murine model of rheumatoid arthritis // J. Experimental and Therapeutic Medicine, vol.13, no.5, pp.2267-2273, 2017.
3. Gerli R, Bistoni O, Russano A, Fiorucci S, Borgato L, Cesarotti ME, Lunardi C. In vivo activated T cells in rheumatoid synovitis. Analysis of Th1- and Th2-type cytokine production at clonal level in different stages of disease // Clin Exp Immunol vol.29, pp.549-555, 2002.
4. Morita Y, Yamamura M, Nishida K, Harada S, Okamoto H, Inoue H, et al. Expression of interleukin-12 in synovial tissue from patients with rheumatoid arthritis // Arthritis Rheum., vol.41 pp.306–14, 1998, doi: 10.1002/1529- 0131(199802)41:2<306:AID-ART15>3.0.CO;2-4.
5. Gracie JA, Forsey RJ, Chan WL, Gilmour A, Leung BP, Greer MR, et al. A proinflammatory role for IL-18 in rheumatoid arthritis // J Clin Invest., Vol.104, pp.1393–401, 1999, doi: 10.1172/JCI7317.
6. Sallusto F, Lenig D, Mackay CR, Lanzavecchia A. Flexible programs of chemokine receptor expression on human polarized T helper 1 and 2 lymphocytes // J Exp Med., vol.187, pp.875–83, 1998.
7. DiFusco D., Izzo R., Figliuzzi M.M. Pallone F., Monteleone G. IL-21 as a therapeutic target in inflammatory disorders // Ex Opin. Ther. Targets, vol.18, pp.1329-1338, 2014.
8. Sato K., Suematsu A., Okamoto K., Yamaguchi A., Morishita Y., Kadono Y., Tanaka S., Kodama T., Akira S., Iwakura Y., et al. Th17 functions as an osteoclastogenic helper T cell subset that links T cell activation and bone destruction//J. Exp. Med., vol.203, pp.2673–2682, 2006, doi: 10.1084/jem.20061775.

9. Komatsu N., Okamoto K., Sawa S., Nakashima T., Oh-hora M., Kodama T., Tanaka S., Bluestone J.A., Takayanagi H. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis // Nat. Med., vol.20, pp.62–68, 2014, doi: 10.1038/nm.3432.
10. Yago T., Nanke Y., Ichikawa N., Kobashigawa T., Mogi M., Kamatani N., Kotake S. IL-17 induces osteoclastogenesis from human monocytes alone in the absence of osteoblasts, which is potently inhibited by anti-TNF-alpha antibody: A novel mechanism of osteoclastogenesis by IL-17 // J. Cell. Biochem., vol.108, pp.947–955, 2009, doi: 10.1002/jcb.22326.
11. Yao Z., Painter S.L., Fanslow W.C., Ulrich D., Macduff B.M., Spriggs M.K., Armitage R.J. Human IL-17: A novel cytokine derived from T cells // J. Immunol., vol.155, pp.5483–5486, 1995.
12. Jovanovic D.V., DiBattista J.A., Martel-Pelletier J., et al. IL-17 stimulates the production and expression of proinflammatory cytokines, IL-1β and TNFα, by human macrophages // J. Immunol., vol.160, pp.3513–3521, 1998.
13. Palani Dinesh, Mahaboobkhan Rasool Multifaceted role of IL-21 in rheumatoid arthritis: Current understanding and future perspectives // J Cell Physiol., vol.233, pp.3918–3928, 2018.
14. Carbone, G., Wilson, A., Diehl, S. A., Bunn, J., Cooper, S. M., & Rincon, M. Interleukin-6 receptor blockade selectively reduces IL-21 production by CD4 T cells and IgG4 autoantibodies in rheumatoid arthritis // International Journal of Biological Sciences, vol.9, pp.279–288, 2013, https://doi.org./10.7150/ijbs.5996.
15. Piet Geusens The role of RANK ligand/osteoprotegerin in rheumatoid arthritis // Ther Adv Musculoskelet Dis., vol.4, no.4, pp.225-33, 2012, doi: 10.1177/1759720X12438080.
16. Mary-Clare Miller, Hugh B. Manning, Abhilash Jain, Linda Troeberg, Jayesh Dudhia, David Essex, Ann Sandison, Motoharu Seiki, Jagdeep Nanchahal, Hideaki Nagase, and Yoshifumi Itoh MT1-MMP is a crucial promotor of synovial invasion in human rheumatoid arthritis // Arthritis Rheum., Mar, vol.60, no.3, p.686, 2009, doi: 10.1002/art.24331.
17. Li Z, Li D, Tsun A, Li B. FOXP3+ regulatory T cells and their functional regulation // Cell Mol Immunol., vol.12, pp.558–565, 2015.
18. Nguyen D.X., Ehrenstein M.R. Anti-TNF drives regulatory T cell expansion by paradoxically promoting membrane TNF-TNF-RII binding in rheumatoid arthritis // J Exp Med., vol.213, pp.1241–1253, 2016.
19. Dao X. Nguyen, Alice Cotton, RN, Laura Attipoe, Coziana Ciurtin, Caroline J. Doré, and Michael R. Ehrenstein Regulatory T cells as a biomarker for response to adalimumab in rheumatoid arthritis // J Allergy Clin Immunol., Sep, vol.142, no.3, pp.978–980, 2018, doi: 10.1016/j.jaci.2018.04.026.
20. Kikuchi J., Hashizume M., Kaneko Y., Yoshimoto K., Nishina N. and Takeuchi Ts. Peripheral blood CD4+ CD25+ CD127low regulatory T cells are significantly increased by tocilizumab treatment in patients with rheumatoid arthritis: increase in regulatory T cells correlates with clinical response // Arthritis Research & Therapy, vol.17, p.10, 2015, doi: 10.1186/s13075-015-0526-4.
21. Gupta V., Katyar Sh., Singh A., Misra R. and Amita Aggarwal A. CD39 positive regulatory T cell frequency as a biomarker of treatment response to methotrexate in rheumatoid arthritis // International Journal of Rheumatic Diseases, vol.**21, pp.**1548–1556, 2018.
22. Deaglio S, Dwyer KM, Gao W, Friedman D, Usheva A, Erat A et al. Adenosine generation catalyzed by CD39 and CD73 expressed on regulatory T cells mediates immune suppression // J Exp Med., vol.204, pp.1257–1265, 2007.
23. Rissiek A, Baumann I, Cuapio A et al. The expression of CD39 on regulatory T cells is genetically driven and further upregulated at sites of inflammation // J Autoimmun., vol.**58**, pp.12–20, 2015.
24. Palmer TM, Trevethick MA Suppression of inflammatory and immune responses by the A(2A) adenosine receptor: an introduction // Br J Pharmacol vol.**153** (Suppl. 1); S27–34, 2008.
25. Purvis HA, Anderson AE, Young DA, Isaacs JD, Hilkens CMU A negative feedback loop mediated by STAT3 limits human Th17 responses // J Immunol., vol.193, pp.1142–50, 2014.
26. Pandolfi JB, Ferraro AA, Sananez I et al. ATP-induced inflammation drives tissue-resident Th17 cells in metabolically unhealthy obesity // J Immunol., vol.**196**, pp.3287–96, 2016.
27. Chen Zh., Bozec A., Ramming A. and Schett G. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis //Nature Reviews Rheumatology, vol. 15, pp.9–17, 2019.
28. Shevach E.M., Tran D.Q., Davidson T.S., Andersson J. The critical contribution of TGF-beta to the induction of Foxp3 expression and regulatory T cell function // Eur. J. Immunol., vol.38, pp.915-917, 2008.
29. Peres R.S., Donate P.B., Talbot Jh., et al. TGF- signalling defect is linked to low CD39 expression on regulatory T cells and methotrexate resistance in rheumatoid arthritis // Journal of Autoimmunity, pp.1-10, 2018.

30. Qu C.H., Hou Y., Bi Y.F., et al. Diagnostic values of serum IL-10 and IL-17 in rheumatoid arthritis and their correlation with serum 14-3-3η protein // European Review for Medical and Pharmacological Sciences, vol.23, pp.1899-1906, 2019.
31. IsomaÈ ki P., Punnonen J. Pro-and anti-inflammatory cytokines in rheumatoid arthritis // Annals of medicine, 29(6):499-507, 1997.
32. van Roon JA, Lafeber FP, Bijlsma J. Synergistic activity of interleukin-4 and interleukin-10 in suppression of inflammation and joint destruction in rheumatoid arthritis // Arthritis & Rheumatism, vol.44, no.1, pp.3-12, 2001.
33. Hui Shen, Liping Xia, and Jing Lu Interleukin-4 in rheumatoid arthritis patients with interstitial lung disease: a pilot study // Indian J Med Res., Dec, vol.138, no.6, pp.919–921, 2013.
34. Aletaha D, Neogi T, Silman AJ et al. Rheumatoid arthritis classification criteria: an American college of rheumatology/European league against rheumatism collaborative initiative // Ann Rheum Dis., vol.69, pp.1580–8, 2010.
35. Dulic S., Vásárhelyi Zs., Sava F., et al. T-Cell Subsets in Rheumatoid Arthritis Patients on Long-Term Anti-TNF or IL-6 Receptor Blocker // Therapy Mediators of Inflammation, Article ID 6894374, 19 pages, 2017.
36. Aravena O., Pescea B, Soto L. et al. Anti-TNF therapy in patients with rheumatoid arthritis decreases Th1 and Th17 cell populations and expands IFN-γ-producing NK cell and regulatory T cell subsets // Immunobiology, vol. 216, pp.1256–1263, 2011.
37. Huang Z., Yang B., Shi Y. et al. Anti-TNF-α therapy improves Treg and suppresses Teff in patients with rheumatoid arthritis // Cellular Immunology, vol. 279, no. 1, pp.25–29, 2012.
38. Iain B. McInnes I.B., Buckley Ch.D., John D., Isaacs J.D. Cytokines in rheumatoid arthritis — shaping the immunological landscape // Nature Reviews Rheumatology, Published online 2015, doi:10.1038/nrrheum.2015.171.
39. Hori S, Nomura T, Sakaguchi S Control of regulatory T cell development by the transcription factor Foxp3 // Science, vol.299, pp.1057–1061, 2003.
40. Borsellino G., Kleinewietfeld M., Di Mitri D., et al. Expression of ectonucleotidase CD39 by Foxp3+ Treg cells: hydrolysis of extracellular ATP and immune suppression // Blood, Aug, 15, vol.110, no.4, pp.1225-32, 2007, doi: 10.1182/blood-2006-12-064527.
41. Timperi E. and Barnaba V. CD39 Regulation and Functions in T Cells // Int. J. Mol. Sci., vol.22, p.8068, 2021, https://doi.org/10.3390/ijms22158068.
42. Al-Saadany HM, Hussein MS, Gaber RA, Zaytoun HA Th-17 cells and serum IL-17 in rheumatoid arthritis patients: Correlation with disease activity and severity // The Egyptian Rheumatologist, vol.38, no.1, pp.1-7, 2016.
43. Ding L, Linsley P, Huang L, Germain R, Shevach E. IL-10 inhibits macrophage costimulatory activity by selectively inhibiting the up-regulation of B7 expression // The Journal of Immunology, vol.151, no.3,pp.1224-34, 1993.
44. Lubberts E, Joosten L A B, Chabaud M. et al. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion // J Clin Invest., vol.105, pp.1697–1710, 2000.

NINO KIKODZE [1,2], MANANA IOBADZE [2], NINO TSISKARISHVILI [3], IA PANTSULAIA[1,2], NONA JANIKASHVILI [1], TINATIN CHIKOVANI [1]

TH SUBSETS AND SERUM CYTOKINES IN THE PATHOGENESIS OF RHEUMATOID ARTHRITIS

[1]Immunology department, Tbilisi State Medical University; [2]V.Bakhutashvili Institute of medical Biotechnology, Tbilisi State Medical University; [3]Todua Medical Center

SUMMARY

Rheumatoid arthritis (RA) is a systemic inflammatory disease. It is characterized by damage of cartilages and bones which results in destruction of joint function. Multi-organic disorders of lung, heart and kidney can be developed in time. Complex network of immune and stromal cells, cytokines and chemokines produced by these cells participate in onset of the disease, in the systemic active phase and during the transition to more localized inactive disease after the treatment. To evaluate the Th1, Th17, Tregs and $CD4^{+}CD39^{+}$ cells pattern and pro- and anti-inflammatory cytokines in peripheral blood of patients with RA, 47 RA patients and 20 healthy individuals were included in the study. RA patients were divided into active and inactive RA groups. Frequencies of circulating Th1, Th17, Tregs and $CD4^{+}CD39^{+}$ cells were analyzed by flow cytometry. Serum levels of cytokines: IL-6, IL-10, IL-4, IL-17, TNF-α and TGF-β1 were detected

by ELISA. The results demonstrated an increase of Th1, Th17 cell frequencies in active RA patients, whereas Tregs remain unchanged in RA groups. CD39 marker expression shows significant decline in active RA patients in comparison with inactive RA group and controls. Concentrations of innate cytokines IL-6, TNF-α in the peripheral blood was significantly increased in active RA while according to IL-17/IL-21 serum concentration this group was divided into two subgroups. Anti-inflammatory cytokines TGF-β, IL-4 shows decrease in active RA group compared to healthy controls.

Study demonstrated that development of RA is associated with changes of serum pro- and anti-inflammatory cytokines which influence balance of T helpers subsets and could provide therapeutic opportunities.

Keywords: Rheumatoid arthritis, Th1, Th1, Treg, $CD4^{+}CD39^{+}$ cells, IL-6, IL-10, IL-4, IL-17, IL-21, TNF-α and TGF-β1.