Cell. Mol. Biol. (ISSN: 1165-158X)

Journal Homepage: www.cellmolbiol.org

Cellular and Molecular Biology

Original Article

Effect of ADSCs on Th17/Treg and T-bet/GATA-3 in model mice with primary immune thrombocytopenia

Haijiao Dong#, Zhaoqi Ren#, Wen Shao, Guanghua Duan, Aicui Du, Bin Du*

Department of Transfusion Medicine, PLA Rocket Force Characteristic Medical Center, Beijing, China

Article Info

Article history:

Received: January 15, 2024
Accepted: April 10, 2024
Published: May 31, 2024

Use your device to scan and read the article online

Abstract

We aimed to observe the effects of adipose-derived mesenchymal stem cells (ADSCs) on T helper 17 (Th17)/regulatory T cells (Treg) and T-box transcription factor (T-bet)/GATA-binding protein 3 (GATA-3) in model mice with primary immune thrombocytopenia (ITP). 32 BALB/C mice were selected. ADSCs were isolated from 2 mice and cultured. The other 30 mice were randomly divided into the normal control group, the ITP model control group, and the ITP experimental group. Platelet count (PLT), Th17/Treg cells, related serum cytokines [interleukin-6 (IL-6), IL-17A, IL-10, and transforming growth factor β1 (TGF-β1)], T-bet and GATA-3 mRNA levels in peripheral blood mononuclear cells (PBMCs) in the 3 groups were detected. PLT and Treg in the ITP experimental group were significantly lower than those in the normal control group (P<0.05), but significantly higher than those in the ITP model control group (P<0.05). Th17 and Th17/Treg in the ITP experimental group were significantly higher than those in the normal control group (P<0.05), but significantly lower than those in the ITP model control group (P<0.05). Serum IL-6 and IL-17A levels, and T-bet mRNA level in the ITP experimental group were significantly higher than those in the normal control group (P<0.05), but significantly lower than those in the ITP model control group (P<0.05). Serum IL-10 and TGF-β levels, and GATA-3 mRNA level in the ITP experimental group were significantly lower than those in the normal control group (P<0.05), but significantly higher than those in the ITP model control group (P<0.05). ADSCs can effectively regulate Th17/Treg balance and improve T-bet/GATA-3 mRNA expression levels in ITP model mice.

Keywords: Primary immune thrombocytopenia; Adipose-derived mesenchymal stem cells; Th17/Treg cells; T-box transcription factor; GATA-binding protein 3

1. Introduction

Primary immune thrombocytopenia (ITP) is a blood disease caused by an autoimmune system disorder, resulting in a decreased number of platelets in the blood of patients. Platelets are key components of the blood, responsible for helping clotting and stopping bleeding. In ITP, the patient's immune system mistakenly recognizes and attacks its own platelets, resulting in a decrease in their number, which increases the risk of bleeding. ITP is a heterogeneous disease, meaning that its presentation and severity in different patients can vary considerably. Some patients may experience only mild symptoms, while others may suffer more severe bleeding problems. The diversity of symptoms also means that clinical treatment will have different effects, and treatment plans need to be tailored to the specific circumstances of each patient. Among all hemorrhagic diseases, ITP accounted for a relatively high proportion, about 30%. This suggests that it is a relatively common hemorrhagic disorder that requires attention from both medical professionals and the public. One of the most serious complications of ITP is visceral bleeding, which can occur in any visceral organ, such as the gastrointestinal tract or the brain. Visceral hemorrhage is a serious medical emergency that may endanger the life of the patient and requires immediate medical intervention [1]. At present, the conventional treatment of ITP mainly includes splenectomy, hormone drugs and immunosuppressants, etc. However, the long-term efficacy is not ideal and there are many adverse reactions. Moreover, there is no ideal method to prevent and treat refractory ITP (ITP with no obvious effect of splenectomy and hormone drugs) [2,3]. It is very important to find new prevention and treatment methods based on the pathogenesis of ITP. In terms of clinical treatment concept of immune diseases, mesenchymal stem cells have been developed rapidly and are now considered as a new treatment method. As for the source and type of stem cells, compared with other types of mesenchymal stem cells such as bone marrow, adipose-derived mesenchymal stem cells (ADSCs) show more advantages, such as sufficient donor, low immunogenicity, easy acquisition and culture, etc., showing great application prospects and value [4]. Whether ADSCs can be used in the clinical treatment of ITP and achieve good results needs to be supported by more experimental evidence. At

* Corresponding author.
E-mail address: 18910883346@189.cn (B. Du).
# These authors contributed equally
Doi: http://dx.doi.org/10.14715/cmb/2024.70.5.21

present, the detailed pathogenesis of ITP has not been fully clarified. Recent studies have shown that the occurrence of ITP is closely related to the immune state of T cells in the body, especially the immune balance between helper T cells 17 (Th17) and regulatory T cells (Treg) plays an important role, and is closely related to the severity of ITP and the prognosis of patients [5]. Many previous studies have found that Th1/Th2 is unbalanced in ITP patients and shows Th1 bias advantage [6,7]. However, the mRNA levels of GATA-3 and T-box transcription factor (T-bet) can sensitively reflect the imbalance of Th1/Th2 [8]. At present, there are few animal experiments on the effect of ADSCs on Th17/Treg in ITP. In this paper, we explored the effects of Th17/Treg and T-bet/GATA-3 on ADSCs in ITP model mice, hoping to provide a basis for ADSCs for the treatment of ITP.

2. Materials and methods

2.1. Materials

Experimental animals: Thirty-two BALB/C mice were selected, all female specific pathogen free (SPF), 6 weeks old, body weight range 18-26 g, animal license number is SCXK (Beijing) > 2009-0004, the control feeding environment is: The air circulation was maintained, the light was in accordance with the circadian rhythm, and the temperature was about 21 ~ 25℃. After regular disinfection, the experiment began after 1 week of feeding. The study was approved by the ethics committee.

Main drugs, reagents and instruments: Phosphate buffer solution (PBS), MWReg30 monoclonal antibody, TRIzol reagent, Alexa Fluor® 647 interleukin 17 (IL-17), PE-Foxp3 reagent, polymerase chain reaction (PCR) kit, enzyme-linked immunosorbent assay (ELISA) kit, constant temperature shaker, centrifuge, automatic blood cell analyzer, flow cytometry.

2.2. Isolation and culture of ADSCs

Two mice were sacrificed, and 75% ethanol was used for disinfection. About 1g of inguinal adipose tissue was selected, rinsed through PBS, and then cut into pieces to control the size of tissue about 1 mm^2. The supernatant was discarded and placed in DMEM medium (containing 15% fetal bovine serum), and the cell suspension concentration was controlled to 1×10^6 /mL, and put into the culture dish. After 24 hours, the culture medium needed to be changed, and the adherent cells were subcultured until the growth showed 80% confluence. Finally, the third generation ADSCs were used for the experiment.

2.3. Grouping, modeling, and processing

Thirty BALB/C mice were randomly divided into normal control group, ITP model control group and ITP experimental group, with 10 mice in each group. The latter model was established according to ITP model establishment method. MWReg30 monoclonal antibody was diluted with PBS 200 μL and injected intraperitoneally every day. The dose of D1 and D2 was controlled at 68 μg/kg, D3 was controlled at 102 μg/kg, and D4-D8 was controlled at 136 μg/kg. Not restricting their activities and drinking water; The success of modeling was determined as following: on D2, ecchymosis began to occur, and the subcutaneous bleeding spots were displayed, most of which were near the injection point. Venous blood samples were collected for platelet count (PLT) determination, and it was found that the degree of reduction was more than 50%. The normal control group was not given any treatment. The ITP model control group was infused with PBS (200 μL) through the tail vein. In ITP group, ADSCs (1×10^6 /mL) were infused via tail vein (200 μL). The indexes were detected on the 7th day after treatment.

2.4. Detection of PLT and Th17/Treg cell

About 100 μL of tail vein blood was collected, mixed with anticoagulant, and PLT was detected by automatic blood cell analyzer.

The suspension of peripheral blood mononuclear cells (PBMC) was controlled to 1×10^6 /mL, and then 100 μL suspension was treated with phorbol ester and ionomycin for 4 h, followed by protein transport inhibitor (PPI). The final concentrations of these three agents were 100 ng/mL, 500 ng/mL and 1 μL/mL, respectively. The cell surface was stained with 10 μL CD4 and CD25 antibodies (labeled with fluorescein isothiocyanate), then incubated in the dark for 0.5 h, washed, incubated in 2 mL of fixative solution for 20 min, and then incubated in 2 mL of membrane disruption solution for 0.5 h. After washing, the cells were incubated with 10 μL Alexa Fluor® 647 IL-17 and PE-Foxp3 reagent for 20 min, followed by washing, and the Th17/Treg ratio was determined by flow cytometry.

2.5. Detection of Serum related cytokines

The venous blood was centrifuged (at a rate of 1200× g) for 10min to obtain the upper serum. Interleukin-6 (IL-6), interleukin-17A (IL-17A), interleukin-10 (IL-10) and transforming growth factor-β1 (TGF-β1) were measured by enzyme-linked immunosorbent assay (ELISA) kit. T-bet and GATA-3 mRNA levels were measured by real-time fluorescent quantitative PCR (RT-PCR) for mRNA determination, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal reference, and the primer sequences are detailed in Table 1. Samples were mixed in TRIzol solution, total RNA was obtained according to the instructions of the kit, dissolved in diethyl pyrocarbonate (DEPC) water, and then 4.0 μl RNA template was used for reverse transcription reaction. The related system was as follows: RNA template volume 4.0

Table 1. Primer sequences.

Variables		Primer sequences
T-bet	Upstream	5'-GCCAGGGAACCGCTTATATG-3'
	Downstream	5'-GACGATCATCTGGGTCACATTGT-3'
GATA-3	Upstream	5'-GAGGTGGACGTACTTTTTAACATCG-3'
	Downstream	5'-GGCATACCTGGCTCCCGT-3'
GAPDH	Upstream	5'-ACCACAGTCCATGCCATCAC-3'
	Downstream	5'-TCCACCACCCTGTTGCTGTA-3'

μL, 4.0 μL reverse transcription buffer, DEPC water volume 10.5 μL, 10 mmol/L deoxyribonucleoside triphosphate (dNTPs), 10 pmol/μL downstream primers, 200 U/μL reverse transcriptase (MMLV) 0.5 μL, respectively, a total of 20.0 μl. RT-PCR related reaction system is as follows: 10 μL of 5×SYBR Green I buffer, 3 U/μL of Taq enzyme, 10 pmol/μL of upstream primers, 10 mmol/L of dNTPs, 10 pmol/μL of downstream primers, 31.0 μL of deionized water (ddH2O), and 31.0 μL of DDH2O. 5.0 μL complementary DNA (cDNA) for a total of 50.0 μL. The PCR reaction conditions were as follows: treatment at 93°C for 3 min, 55°C for 15 s, and 72°C for 30 s, for a total of 40 cycles.

2.6. Statistical analysis

Data were entered into Statistic Package for Social Science (SPSS) 25.0 software for analysis (IBM, Armonk, NY, USA), measurement data were described in the form of (±s), multiple groups were tested by one-way analysis of variance, and multiple group comparison was tested by SNK-q method. The test level was 0.05 (two-sided).

3. Results

3.1. Comparison of PLT among the three groups

Refer to Table 2. The platelet count in ITP experimental group was significantly lower than that in normal control group ($P < 0.05$), and was significantly higher than that in ITP model control group ($P < 0.05$).

3.2. Comparison of Th17/Treg cells among the three groups

Refer to Table 3. The levels of Th17 and Th17/Treg in ITP experimental group were significantly higher than those in normal control group ($P < 0.05$), but significantly lower than those in ITP model control group ($P < 0.05$). Treg in ITP experimental group was significantly lower than that in normal control group ($P < 0.05$), but significantly higher than that in ITP model control group ($P < 0.05$).

3.3. Comparison of serum related cytokines among the three groups

Refer to Table 4. The serum levels of IL-6 and IL-17A in ITP experimental group were significantly higher than those in normal control group ($P < 0.05$), but significantly lower than those in ITP model control group ($P < 0.05$). The serum levels of IL-10 and TGF-β1 in ITP experimental group were significantly lower than those in normal control group ($P < 0.05$), and were significantly higher than those in ITP model control group ($P < 0.05$).

3.4. Comparison of T-bet and GATA-3 mRNA levels among the three groups

Refer to Table 5. The expression of T-bet mRNA in ITP experimental group was significantly higher than that in normal control group ($P < 0.05$), but significantly lower than that in ITP model control group ($P < 0.05$). The GATA-3 mRNA level in ITP group was significantly lower

Table 2. Comparison of PLT among the three groups ($\bar{x}$±s).

Group	n	PLT (×10⁹/L)
ITP experimental group	10	335.14±74.85*#
ITP model control group	10	158.97±26.38*
Normal control group	10	405.23±74.67
F		40.674
P		<0.001

Compared with normal control group, *P < 0.05; Compared with ITP model control group, #P < 0.05.

Table 3. Comparison of Th17/Treg cells among the three groups ($\bar{x}$±s).

Group	n	Th17 (%)	Treg (%)	Th17/Treg
ITP experimental group	10	2.23±0.40*#	5.03±0.09*#	0.44±0.07*#
ITP model control group	10	4.23±0.81*	3.72±0.59*	1.14±0.20*
Normal control group	10	1.55±0.32	6.08±0.12	0.25±0.05
F		63.390	113.171	139.051
P		<0.001	<0.001	<0.001

Compared with normal control group, *P < 0.05; Compared with ITP model control group, #P < 0.05.

Table 4. Comparison of serum related cytokines among the three groups (ng/L, $\bar{x}$±s).

Group	n	IL-6	IL-17A	IL-10	TGF-β1
ITP experimental group	10	25.62±4.37*#	24.73±4.18*#	32.65±6.01*#	83.67±15.39*#
ITP model control group	10	39.64±5.92*	62.35±11.74*	20.13±3.82*	40.05±7.63*
Normal control group	10	18.87±3.46	11.05±1.98	41.37±7.92	99.18±17.65
F		50.935	132.963	30.145	46.486
P		<0.001	<0.001	<0.001	<0.001

Compared with normal control group, *P < 0.05; Compared with ITP model control group, #P < 0.05.

Table 5. Comparison of T-bet and GATA-3 mRNA levels among the three groups ($\bar{x}$±s).

Group	n	T-bet mRNA	GATA-3 mRNA
ITP experimental group	10	2.87±0.54*#	0.76±0.14*#
ITP model control group	10	4.65±0.73*	0.54±0.08*
Normal control group	10	1.13±0.21	0.92±0.17
F		106.991	19.891
P		<0.001	<0.001

Compared with normal control group, *P < 0.05; Compared with ITP model control group, #P < 0.05.

than that in normal control group (P < 0.05), but significantly higher than that in ITP model control group (P < 0.05).

4. Discussion

ITP is an immune comprehensive disease, which is also a hemorrhagic disease. It is common in clinical practice, mainly characterized by the block of new platelet formation and the continuous reduction of the number of existing platelets, which can occur in people of any age [9]. The pathogenesis of ITP is still not completely clear in clinical practice, so there are few effective treatment methods for the etiology, which seriously affects the quality of life of patients. At present, in the clinical treatment of autoimmune diseases, the use of stem cells for biological immunotherapy is a new method, especially in the improvement of Th1/Th2 immune imbalance, and mesenchymal stem cells have made some progress in the treatment of autoimmune diseases [10]. With the emergence of this biological treatment idea, it effectively solves the problems of drug side effects and application safety in basic treatment (such as hormone drugs and immunosuppressants), and has good application prospects and value. ADSCs are mainly isolated from adipose tissue and have multi-directional differentiation potential. They show low immunogenicity among biological individuals and can inhibit excessive cellular immunity, improve immune disorders, induce immune tolerance, and reduce damage caused by inflammation [11,12].

Previous studies have shown that T lymphocyte subsets play an important role in inducing immune responses in ITP. Induction promotes the activation of T lymphocytes, which can damage normal immune cells [13]. Th17 cells belong to the CD4+T cell subtype. The synthesis of IL-17 is a pro-inflammatory factor, which can exert effects on neutrophils, increase the levels of various inflammatory factors, and participate in the formation of autoimmune diseases and the process of collective defense response. Treg cells are mainly produced by natural thymic cells, which can inhibit the proliferation of Th1 and Th17, and prevent autoimmune damage by protecting peripheral immune tolerance. Relevant reports indicate that there is an antagonistic relationship between Th17 and Treg in terms of differentiation and function, and the balance of Th17/Treg is conducive to protecting the normal immune response process and reducing the risk of autoimmune diseases [14]. This study showed that compared with the normal control group, Th17 and Th17/Treg in the ITP experimental group were significantly increased, while Treg was significantly decreased. After ADSCs treatment, Th17 and Th17/Treg in the ITP experimental group were significantly decreased compared with the ITP model control group, and Treg was significantly increased. ADSCs play an important role in improving the balance of Th17/Treg in ITP model mice. Th17 cells can synthesize specific inflammatory factors, including IL-6 and IL-17, which are the initiating factors of inflammatory response and play an inflammatory condition. Treg cells can synthesize inhibitory factors, including IL-10 and TGF-β1, which can inhibit autoreactive T cells, thus participating in the process of immune tolerance, maintaining immune homeostasis and establishing tolerance, and avoiding immune inflammation [15]. In this study, the levels of IL-6 and IL-17A in ITP experimental group were significantly lower than those in ITP model control group, and the levels of IL-10 and TGF-β1 were significantly higher than those in ITP model control group, indicating that ADSCs could effectively regulate Th17/Treg related cytokines in ITP mice, which further confirmed that ADSCs could regulate Th17/Treg. It can reduce immune inflammatory injury and maintain immune stability. T-bet, an important member of the T-box gene family, is also a Th1 transcription factor (with specificity), which can be specifically synthesized and expressed in Th1 cells [16-18]. GATA-3, a zinc finger protein, is also a Th2 transcription factor, which can be specifically synthesized by Th2 and has the function of regulating all important Th2 cytokines (such as IL-4, L-5 and IL-13) [19]. This study found that compared with the ITP model control group, the ITP experimental group had a significant decrease in T-bet mRNA level and a significant increase in GATA-3 mRNA level, which was consistent with the previous experiment [20], suggesting that ADSCs treatment could correct Th1 shift in ITP mice and promote the recovery of Th1/Th2 balance. This study showed that PLT in ITP experimental group was significantly higher than that in ITP model control group, but significantly lower than that in normal control group, indicating that ADSCs could effectively improve the condition of ITP model mice. The mechanism may be related to the regulation of Th17/Treg and Th1/Th2 balance by ADSCs, down-regulating the expression of T-bet mRNA and up-regulating the expression of GATA-3 mRNA.

In conclusion, ADSCs may regulate Th1/Th2 balance by improving the balance of Th17/Treg cells and the expression of related cytokines, up-regulating the expression of GATA-3 mRNA and down-regulating the expression of T-bet mRNA, and thus alleviate the condition of ITP mice.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

This study was approved by the Animal Ethics Committee of PLA Rocket Force Characteristic Medical Center Animal Center.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Haijiao Dong, Zhaoqi Ren: Conceptualization, methodology, writing original draft preparation. Wen Shao, Guanghua Duan, Aicui Du: Investigation, software, statistical analysis. Bin Du: Reviewing and editing, funding acquisition, supervision. All authors read and approved the final manuscript.

Funding

None.

References

1. Rubenstein AI, Pierson SK, Shyamsundar S, Bustamante MS, Gonzalez MV, Millller ID et al (2024) Immune-mediated thrombocytopenia and IL-6-mediated thrombocytosis observed in idiopathic multicentric Castleman disease. Brit J Haematol doi: 10.1111/bjh.19279
2. Kuwana M, Ito T, Kowata S, Hatta Y, Fujimaki K, Naito K et al (2024) Long-term treatment with fostamatinib in Japanese patients with primary immune thrombocytopenia: An open-label extension study following a phase 3 placebo-controlled, double-blind, parallel-group study. Am J Hematol doi: 10.1002/ajh.27176
3. Cao Q, Zhu H, Xu W, Zhang R, Wang Y, Tian Z et al (2023) Predicting the efficacy of glucocorticoids in pediatric primary immune thrombocytopenia using plasma proteomics. Front Immunol 14:1301227. doi: 10.3389/fimmu.2023.1301227
4. Yassin MA, Al-Rasheed M, Al-Khaboori M, Marashi M, Osman H, Wali Y et al (2023) Thrombopoietin-receptor agonists for adult patients with immune thrombocytopenia: a narrative review and an approach for managing patients fasting intermittently. Front Cardiovasc Med 10:1260487. doi: 10.3389/fcvm.2023.1260487
5. Zhou H, Han S, Jin J, Huang R, Guo X, Shen X et al (2023) Efficacy and safety of QL0911 in adult patients with chronic primary immune thrombocytopenia: A multicenter, randomized, double-blind, placebo-controlled, phase III trial. J Transl Intern Med 11:423-432. doi: 10.2478/jtim-2023-0106
6. Chang L, Wang C, Peng J, Song Y, Zhang W, Chen Y et al (2024) Rattan Pepper Polysaccharide Regulates DSS-Induced Intestinal Inflammation and Depressive Behavior through Microbiota-Gut-Brain Axis. J Agr Food Chem doi: 10.1021/acs.jafc.3c08462
7. Zhang Y, Jiang X, Wang Q, Wu J, Zhou J (2023) Dexamethasone alleviates pulmonary sarcoidosis by regulating the TGF-beta/Smad3 signaling to promote Th17/Treg cell rebalance. Cell Immunol 395-396:104781. doi: 10.1016/j.cellimm.2023.104781
8. Tang D, Liu M, Gao S, Sun H, Peng Y, Li Y et al (2023) Thermally engineered MSC-derived extracellular vesicles ameliorate colitis in mice by restoring the imbalanced Th17/Treg cell ratio. Int Immunopharmacol 125:111077. doi: 10.1016/j.intimp.2023.111077
9. Wang T, Feng W, Ju M, Yu H, Guo Z, Sun X et al (2023) 27-hydroxycholesterol causes cognitive deficits by disturbing Th17/Treg balance and the related immune responses in mild cognitive impairment patients and C57BL/6J mice. J Neuroinflamm 20:305. doi: 10.1186/s12974-023-02986-5
10. Xu J, Zheng B, Xie C, Zhao Y, Wu H, Wang Y et al (2024) Inhibition of FABP5 attenuates inflammatory bowel disease by modulating macrophage alternative activation. Biochem Pharmacol 219:115974. doi: 10.1016/j.bcp.2023.115974
11. Pan Y, Wu Y, Liu Y, Wang P, Huang H, Jin J et al (2023) Long non-coding RNA ENSMUST00000197208 promotes a shift in the Th17/Treg ratio via the P2X7R-NLRP3 inflammasome axis in collagen-induced arthritis. Immunol Res doi: 10.1007/s12026-023-09439-4
12. Liu X, Wang S, Wu X, Zhao Z, Jian C, Li M et al (2023) Astragaloside IV Alleviates Depression in Rats by Modulating Intestinal Microbiota, T-Immune Balance, and Metabolome. J Agr Food Chem doi: 10.1021/acs.jafc.3c04063
13. Li Y, Yao G, Wang R, Zhu J, Li H, Yang D et al (2024) Maternal immune activation mediated prenatal chronic stress induces Th17/Treg cell imbalance may relate to the PI3K/Akt/NF-kappaB signaling pathway in offspring rats. Int Immunopharmacol 126:111308. doi: 10.1016/j.intimp.2023.111308
14. Zhang G, Wu S, Xia G (2023) MiR-326 sponges TET2 triggering imbalance of Th17/Treg differentiation to exacerbate pyroptosis of hepatocytes in concanavalin A-induced autoimmune hepatitis. Ann Hepatol 29:101183. doi: 10.1016/j.aohep.2023.101183
15. Mehmandar-Oskuie A, Jahankhani K, Rostamlou A, Mardafkan N, Karamali N, Razavi ZS et al (2024) Molecular mechanism of lncRNAs in pathogenesis and diagnosis of auto-immune diseases, with a special focus on lncRNA-based therapeutic approaches. Life Sci 336:122322. doi: 10.1016/j.lfs.2023.122322
16. Hosseini S, Mahmoudi M, Rezaieyazdi Z, Shapouri-Moghaddam A, Hosseinzadeh A, Arab FL et al (2024) Lupus mice derived mesenchymal stromal cells: Beneficial or detrimental on SLE disease outcome. Int Immunopharmacol 126:111306. doi: 10.1016/j.intimp.2023.111306
17. Cao M, Chen P, Peng B, Cheng Y, Xie J, Hou Z et al (2023) The transcription factor ELF4 alleviates inflammatory bowel disease by activating IL1RN transcription, suppressing inflammatory TH17 cell activity, and inducing macrophage M2 polarization. Front Immunol 14:1270411. doi: 10.3389/fimmu.2023.1270411
18. Berdiaki A, Vergadi E, Makrygiannakis F, Vrekoussis T, Makrigiannakis A (2023) Title: Repeated implantation failure is associated with increased Th17/Treg cell ratio, during the secretory phase of the human endometrium. J Reprod Immunol 161:104170. doi: 10.1016/j.jri.2023.104170
19. Wu D, Zhang Y, Zou B, Lu Y, Cao H (2023) Shaoyao decoction alleviates TNBS-induced ulcerative colitis by decreasing inflammation and balancing the homeostasis of Th17/Treg cells. Bmc Complement Med 23:424. doi: 10.1186/s12906-023-04237-9
20. Afsar B, Afsar RE (2023) Salt Behind the Scenes of Systemic Lupus Erythematosus and Rheumatoid Arthritis. Curr Nutr Rep 12:830-844. doi: 10.1007/s13668-023-00509-5