Int J Clin Exp Med 2022;15(4):136-146
www.ijcem.com /ISSN:1940-5901/IJCEM0140813

Original Article

Analysis of immune indices in patients with rheumatoid arthritis and variations among TCM syndromes

Mifeng Liu*, Yangli Zhang*, Wen Gu, Bo Wen, Qin Zhang, Jing Wang, Bei Wang

*Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, Beijing 100010, China. *Equal contributors.*

Received November 27, 2021; Accepted March 11, 2022; Epub April 15, 2022; Published April 30, 2022

Abstract: Objective: Inflammatory response and autoimmune abnormalities play an important role in the etiology and development of rheumatoid arthritis (RA). This study sought to explore changes in immune indices among patients with RA and healthy people, and to elucidate variations among immune indices of different traditional Chinese medicine (TCM) syndromes of RA, focusing on the differences of autoimmunity and inflammatory response indicators in RA patients with damp-heat obstruction syndrome (DHOS) and cold-heat complex syndrome (CHCS). Methods: According to the diagnostic criteria of TCM syndrome differentiation, 68 patients with RA were identified, including 19 patients with DHOS and 49 patients with CHCS; these and 20 healthy individuals were included in the study. Biomarkers related to clinical immunity and autoimmunity, including myeloid-derived suppressor cells (MDSCs) and each phenotypes, Th17 and regulatory T (Treg)-cells, and inflammatory cytokines were examined in the peripheral blood of all subjects. Results: Autoimmune indicators such as MDSCs, polymorphonuclear MDSCs (PMN-MDSCs), monocytic MDSCs (MO-MDSCs), Th17 cells, and serum pro-/anti-inflammatory factors were also significantly increased in RA patients relative to healthy controls ($P < 0.01$), but the proportion of Treg cells was decreased ($P < 0.05$). The proportion of MO-MDSCs in the peripheral blood of RA patients was positively correlated with the results of ESR, CRP, and complement 3, and negatively correlated with the proportion of Treg cells ($P < 0.05$). Furthermore, the proportion of MDSCs and PMN-MDSCs were positively correlated with interleukin-6 content and negatively correlated with Treg cell count ($P < 0.05$). Separately, ESR, CRP, and MO-MDSCs findings in CHCS-RA patients were lower relative to those in DHOS-RA patients ($P < 0.01$). Conclusion: Clinical immune and autoimmune indices of RA patients were significantly abnormal relative to those of healthy people. DHOS-RA and CHCS-RA exerted different immunological profiles. It is uncertain whether ESR, CRP and MO-MDSCs can be used as important biomarkers to distinguish DHOS-RA and CHCS-RA, and further research is needed.

Keywords: Rheumatoid arthritis (RA), autoimmune diseases, myeloid-derived suppressor cells (MDSCs), TCM syndrome differentiation

Introduction

Rheumatoid arthritis (RA) is a multisystem inflammatory autoimmune disease characterized by chronic inflammation and the progressive destruction of multiple peripheral joints. The global prevalence of RA is 0.5%-1.0% [1]. Females tend to be more susceptible to RA as the morbidity of this disease among women is about three- to five-fold higher than that among men [2]. Extraarticular manifestations (EAM) are common in patients with RA. The 10-year and 15-year cumulative incidence of EAM can reach 40.6% and 53% [3-5]. Modern medicine believes that RA is caused by complex interactions among genes, environmental factors, and the immune system, but the specific pathogenesis of RA remains unclear [6]. Numerous studies have suggested that the inflammatory response and autoimmune abnormalities are involved throughout the course of RA, with the latter playing an important role in the occurrence and development of RA. The immunological processes can occur many years before symptoms of joint inflammation, the so-called pre-RA phase [7]. The etiology of RA involves an interaction among components of innate and adaptive immune responses [8]. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of cells mainly

composed of immature myeloid cells (IMCs) and myeloid cell progenitors. Under physiological conditions, MDSCs rapidly differentiate into mature granulocytes, macrophages, or dendritic cells (DCs) and have a remarkable ability to suppress T-cell responses. However, under pathological conditions such as cancer, various infectious diseases, or inflammation, the differentiation of IMCs into mature myeloid cells is partially blocked, leading to their massive proliferation. MDSCs are generated in the bone marrow from the myeloid progenitor cells and then travel through the circulatory system or migrate into the tissues, accumulating in inflammatory sites during some autoimmune disorders, and can be an important factor in the pathogenesis of these conditions [9]. Various studies have suggested a pro-/anti-inflammatory role of MDSCs in autoimmune diseases, but the exact function of MDSCs in this context remains controversial. MDSCs can accelerate the progress of arthritis [10] or alleviate disease advancement [11, 12] due to varying inhibition abilities and functional mechanisms of MDSCs [13]. The specific role of MDSCs and their subsets in the pathological processes of immune diseases related to inflammatory autoimmune abnormalities is still unknown [11, 13].

RA belongs to the category of arthromyodynia among traditional Chinese medicines (TCMs). Arthralgia has been described in detail in ancient TCM literature. For instance, *Su-Wen • Bi-Lun* pointed out that pathogenic factors of RA include wind, cold, heat, and dampness. *Zheng-Zhi Zhun-Sheng* recorded that "wind, damp, cold, and heat are superficial causes of arthralgia, and deficiency of the kidney plays the fundamental role in causing RA". Therefore, the TCM etiology of RA is mainly attributed to deficiencies in the amounts of Qi and blood, external invasions by pathogenic factors of wind, cold, dampness, and heat. Furthermore, congenital weakness, insufficient healthy Qi and weakened defense are considered as inherent reasons. In the case of internal injury, the body is more vulnerable to attack by external wind, cold, damp, heat, and other pathogenic factors, resulting in blockage of meridians and collaterals, Qi and blood circulation, and eventually lead to diseases. Cold is the most important causative factor of RA, and it is also a Yin pathogen able to compromise the body's Yang Qi. In the context of deficient amounts of Qi and blood, cold carries wind to invade the body and directly attack the sinews and bones. In addition, damp produced in the body is combined with external damp, which together lead to joint obstruction and form cold-damp obstruction syndrome. The accumulation of cold-dampness converts into heat, which may result in the syndrome of cold-heat complex or damp-heat obstruction. Cold or heat coagulates into phlegm as the disease persists, which leads to a complex situation of phlegm stasis obstruction accompanied by the simultaneous appearance of excess and deficiency syndrome. Based on this theory, many famous TCM doctors have applied it to the diagnosis and classification of RA in combination with clinical TCM syndrome. Syndrome differentiation-based diagnosis and treatment are unique characteristics of TCM and are the specific manifestations of individualized treatment of TCM. As the core of syndrome differentiation-based treatment, the standardization and objectification of TCM syndromes are key foci in TCM research. The purpose of this study was to explore the inherent relationship between macro- and micro-disease information of RA and TCM syndromes. RA patients were classified according to the criteria of TCM syndrome differentiation, and immune-related indicators in peripheral blood were tested to elucidate immunologic differences among RA patients with damp-heat obstruction syndrome (DHOS) and patients with cold-heat complex syndrome (CHCS).

Methods

Inclusion and exclusion criteria of RA

The experimental protocol was organized in accordance with the guidelines of the Declaration of Helsinki and was approved by the Medical Ethical Review Board of Beijing Hospital of TCM affiliated with Capital Medical University (CMU) (ethical approval No. 2016BL-083-02). The diagnostic criteria of RA used were based on the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria [14]. Sixty-eight patients with RA were included in this study, clinically diagnosed with RA at the Beijing Hospital of TCM affiliated with CMU. No patients received any immunosuppressive ag-

ents such as glucocorticoids and cytotoxic drugs within 24 h before blood sampling. Patients with other rheumatic diseases such as severe osteoarthritis, spondyloarthritis, or systemic lupus erythematosus; those with serious illness of the heart, brain, liver, kidney, or hematopoietic system; those taking cortisol hormones within one month of the study period; pregnant or lactating women; and patients with psychosis, were excluded. Twenty healthy subjects from the Health Examination Center of Beijing Hospital of TCM affiliated with CMU were also involved in the study as healthy controls (HCs).

Diagnostic criteria of TCM syndrome differentiation

The syndrome differentiation of DHOS [15] used referred to the diagnostic standard of DHOS in the *Guideline of Clinical Research of TCM New Drugs (Trial)* promulgated by the National Medical Products Administration in 2002. The syndrome differentiation of CHCS [16] used referred to the diagnostic standard of CHCS in *Chinese Rheumatology*.

Experimental methods

Analysis of clinical indicators: A total of 4 mL of peripheral venous blood anticoagulated with heparin sodium was collected from subjects in a fasting state and centrifuged to obtain supernatant. Levels of C-reactive protein (CRP), rheumatoid factor (RF); anti-cyclic citrullinated peptide (anti-CCP) antibody; immunoglobulin (Ig) G, A, M, and E; and complement (C) 3, 4, and 1q were analyzed by immunoturbidimetry using the Beckman AU5821 automatic biochemical analyzer (Beckman Coulter, Brea, CA, USA). ESR levels were detected using the Monitor-JPLUS dynamic automatic ESR analyzer (Vital Diagnostics, Italy). Morning stiffness time, swollen joint count (SJC), and tender joint count (TJC) were recorded, while the DAS28 score, clinical disease activity index (CDAI), and simplified disease activity index (SDAI) were calculated.

Frequency and phenotype of MDSCs detected by flow cytometry (FCM): A total of 4 mL of peripheral venous blood anticoagulated with heparin sodium was collected from subjects in a fasting state. FCM identified the frequency and phenotype of MDSCs in peripheral blood mononuclear cells (PBMCs) within 24 h, using the following antibodies: mouse anti-human fluorescent markers CD14-FITC (BD Biosciences, San Diego, CA, USA), HLA-DR-PerCP (BD Biosciences), CD11b-APC (Beckman Coulter), CD33-PE (Beckman Coulter). FCM showed the distribution of MDSCs ($CD33^+CD11b^+$ $HLA\text{-}DR^{-/low}$), PMN-MDSCs ($CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^-$), and MO-MDSCs ($CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^+$). The acquisition and analysis of FACS data were performed using a BD FACS Calibur flow cytometer with the Cell Quest Pro software (BD Biosciences).

Analysis of Th17 and Treg cells detected by FCM: A total of 4 mL of peripheral venous blood anticoagulated with heparin sodium was collected from subjects in a fasting state and incubated with Cell Activation Cocktail (with Brefeldin A) for five hours, the cells were collected into the flow tube after incubation. For Th17 cells analysis, stimulated peripheral blood cells were collected and stained with fluorescent markers CD4-FITC and PE-IL-17A antibody (BD Biosciences). For the analysis of Treg cells, fresh peripheral blood cells were stained with FITC-CD4 and APC-CD25 antibodies, fixed and permeabilized with a Foxp3 staining buffer kit (BD Biosciences) according to the manufacturer's instructions, and intracellularly stained with PE-Foxp3 antibodies. $CD4^+IL\text{-}17^+$ and $CD4^+CD25^+Foxp3^+$ were the representatives of Th17 cell and Treg cells, respectively. The acquisitions and analyses of FACS data were performed using a BD FACS Calibur flow cytometer with the Cell Quest Pro software (BD Biosciences).

Detection of serum cytokines by flow immunofluorescence microspheres: Composite microspheres labeled with monoclonal antibodies corresponding to different fluoresceins and serum or standard solution were mixed in a ratio of 1:1 to establish the total volume of 90 μL in a reaction pore, which was then shaken in the dark. The sheep anti-human immunoglobulin G labeled with fluorescein was added and incubated by vibration. Microsphere suspensions were detected by a Luminex 200 Multifunctional Flow Lattice Instrument according to the Median Fluorescence Intensity (MFI) value. Serum levels of interleukin (IL)-2, 4, 6, 10, 17A, and 23; interferon (IFN)-γ; and tumor

Table 1. The results of clinical immune indicators of RA Patients with DHOS and CHCS

Group	ESR (g/L)	CRP (/L)	RF (IU/mL)	anti-CCP (U/mL)	IgG (g/L)	IgA (g/L)
HCs (n = 20)	12.75 ± 2.10	2.68 ± 2.02	11.28 ± 4.20	10.38 ± 3.20	11.65 ± 2.27	2.25 ± 0.74
RA patients (n = 68)	48.01 ± 24.01**	38.19 ± 22.62**	208.10 ± 198.79**	125.30 ± 172.75**	12.60 ± 3.56	3.10 ± 1.39*
DHOS (n = 19)	55.47 ± 28.78**	51.04 ± 25.80**	181.11 ± 267.41*	131.62 ± 207.60	12.42 ± 4.42	3.29 ± 1.08*
CHCS (n = 49)	45.12 ± 21.53**,▲	33.21 ± 20.74**,▲	218.56 ± 225.94**	122.23 ± 156.38**	12.66 ± 3.21	3.02 ± 1.50
Group	**IgM (g/L)**	**IgE (IU/mL)**	**C3 (g/L)**	**C4 (g/L)**	**C1q (mg/L)**	
HCs (n = 20)	1.07 ± 0.39	34.72 ± 31.08	0.94 ± 0.12	0.19 ± 0.04	184.68 ± 20.94	
RA patients (n = 68)	1.62 ± 1.29**	179.25 ± 315.05**	1.19 ± 0.23**	0.24 ± 0.09**	221.06 ± 47.62**	
DHOS (n = 19)	1.56 ± 0.84	155.71 ± 305.35	1.26 ± 0.20**	0.22 ± 0.07	237.55 ± 61.48**	
CHCS (n = 49)	1.64 ± 1.43*	189.07 ± 321.35**	1.16 ± 0.24**	0.24 ± 0.09**	214.67 ± 39.98**	

Note: Compared with HCs, *P < 0.05; **P < 0.01; Compared with DHOS, ▲P < 0.05. HCs, Healthy Control Group; DHOS, Damp-Heat Obstruction Syndrome; CHCS, Cold And Heat Complex Syndrome (CHCS); ESR, Erythrocyte Sedimentation Rate; CRP, C-Reactive Protein Level; RF, Rheumatoid Factor; anti-CCP, anti-Cyclic Citrullinated Peptide; IgG, Immunoglobulin G; IgA, Immunoglobulin A; IgM, Immunoglobulin M; IgE, Immunoglobulin E; C3, Complement 3; C4, Complement 4; C1q, Complement 1q.

necrosis factor (TNF)-α were analyzed by flow immunofluorescence microspheres.

Statistical method

Data analyses were performed using the SPSS version 17.0 software program (IBM Corp., Armonk, NY, USA). Data are expressed as means ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) with the Bonferroni test or Tamhane's test. Data of non-normal distribution were analyzed by independent multi-sample nonparametric Kruskal-Wallis rank-sum tests. Differences between groups were identified as statistically significant at two levels: P < 0.05 and P < 0.01.

Results

Clinical evaluation

Based on the criteria of RA diagnosis and TCM syndrome differentiation, 68 RA patients were included in the present study, including 19 diagnosed with DHOS and 49 diagnosed with CHCS. There was no age difference between RA patients and HCs in this study (P > 0.05). There were no significant differences in the course of disease, SJC, TJC, DAS28-ESR, DAS28-CRP, CDAI, or SDAI among RA patients with DHOS and CHCS. Serum ESR, CRP, RF, and anti-CCP antibody values of RA patients were significantly increased relative to those of the HCs (P < 0.01). Except for the increase in RF value, the ESR, CRP level, and anti-CCP antibody count of RA patients with CHCS were lower than those of RA patients with DHOS, though only the ESR and CRP level results showed significant differences (P < 0.05). Serum IgG, IgA, IgM, and IgE and C3, C4, and C1q values of RA patients were significantly higher as compared with among the HCs, but there was no significant difference in the abovementioned indicators between RA patients with DHOS and CHCS (**Table 1**).

Phenotype and frequency analysis of MDSCs

The results of FCM showed the distribution of MDSCs ($CD33^{+}CD11b^{+}HLA\text{-}DR^{-/low}$), PMN-MDSCs ($CD33^{+}CD11b^{+}HLA\text{-}DR^{-/low}$ $CD14^{-}$) and MO-MDSCs ($CD33^{+}CD11b^{+}HLA\text{-}DR^{-/low}$ $CD14^{+}$) in PBMCs from RA patients and HCs (**Figure 1A**, **1B**). According to cell phenotype and morphological characteristics, MDSCs were divided into two main subsets including MO-MDSCs and PMN-MDSCs. As a group of cells expressing $CD33^{+}CD11b^{+}HLA\text{-}DR^{-/low}$, MDSCs were further divided into PMN-MDSCs ($CD14^{-}$) or MO-MDSCs ($CD14^{+}$) according to the expression of CD14. MDSCs and PMN/MO-MDSCs subsets of RA patients were significantly higher as compared with among the HCs (28.67% ± 6.36% vs. 20.38% ± 5.51%; 28.82% ± 8.15% vs. 20.17% ± 5.86%; 0.215% ± 0.190% vs. 0.077% ± 0.057%) (P < 0.01, **Figure 1C**, **1D**). Compared with DHOS-RA patients, the decrease of MO-MDSCs in CHCS-RA patients was more significant (0.305% ± 0.176% vs. 0.180% ± 0.185%) (P < 0.05, **Figure 1E**), while MDSCs and PMN-MDSCs increased slightly, but there was no significant difference (P > 0.05).

Expressions of Th17, Treg cells and cytokines

Analysis of FCM for antibody combinations of CD4, CD25, and Foxp3 was performed (**Figure**

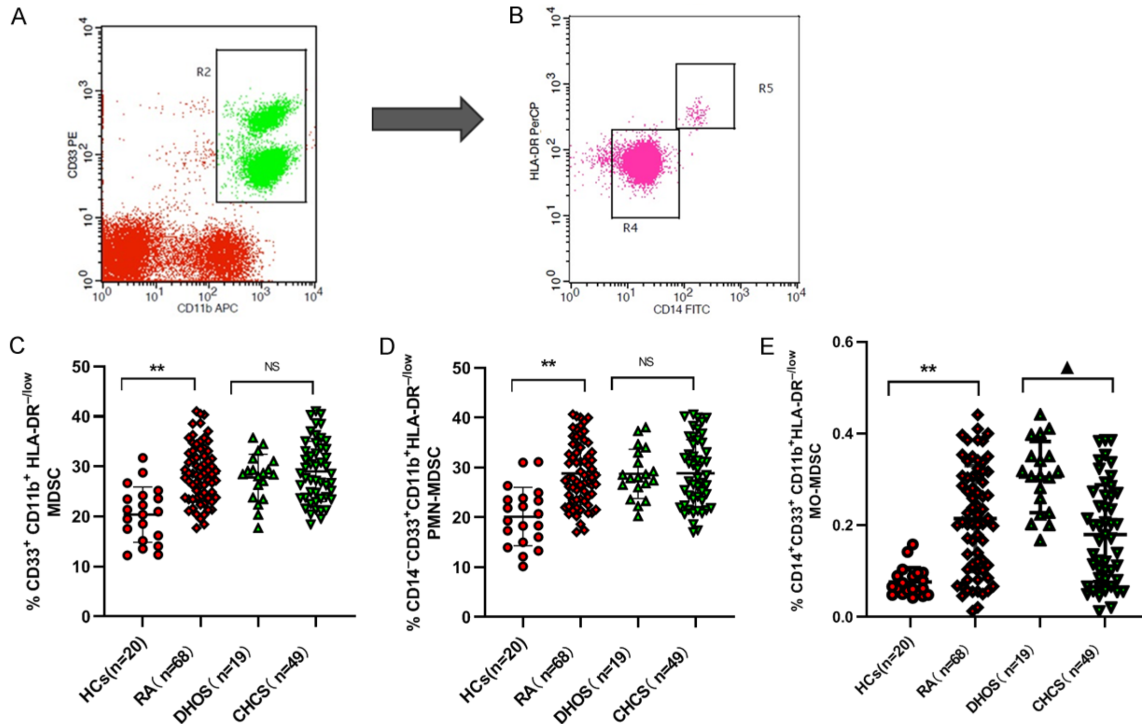

Figure 1. The results of MDSCs and each phenotype in PBMCs detected by FCM. The frequencies of MDSCs ($CD33^+CD11b^+HLA\text{-}DR^{-/low}$), PMN-MDSCs ($CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^-$), and MO-MDSCs ($CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^+$) between the HCs and RA patients as well as RA patients with DHOS and CHCS were compared. A. Representative gating of $CD33^+CD11b^+$ cells (R2). B. Representative gating of $CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^-$ cells (R4), of $CD33^+CD11b^+HLA\text{-}DR^{-/low}$ $CD14^+$ cells (R5). C. MDSCs frequency (% of all single cells). D. PMN-MDSCs frequency (% of all MDSCs). E. MO-MDSCs frequency (% of all MDSCs). The data shown are presented as means ± SD. *P < 0.05, **P < 0.01, versus HCs; ▲P < 0.05, versus DHOS.

2A-C). Th17 and Treg cells are important T-cell subsets that mediate the occurrence of autoimmune diseases, which emphasizes the balance between Treg and Th17 cells. Treg cells, as one of the core members of RA pathogenesis, regulate the inflammatory response of autoimmune diseases by inhibiting effector T-cells. Compared with in the HCs, the Th17 cells in peripheral blood cells of RA patients were significantly increased (0.87% ± 0.72% vs. 0.46% ± 0.41%) (P < 0.05), while the Treg cells in peripheral blood cells of RA patients expressed lower levels (0.58% ± 0.46% vs. 1.00% ± 0.776%; P < 0.05) (**Figure 2D**, **2E**). There were no significant differences in Th17 and Treg cells between RA patients with DHOS and CHCS (0.85% ± 0.50% vs. 0.87% ± 0.79%; 0.58% ± 0.36% vs. 0.64% ± 0.46%; (P > 0.05). The Th17/Treg ratio in RA patients was higher than that in the HCs (1.80 ± 1.39 vs. 0.83 ± 0.98; P < 0.01), but there was no significant difference between the two syndromes (1.85 ± 0.96 vs. 1.78 ± 1.54; P > 0.05) (**Figure 2F**).

Various proinflammatory and anti-inflammatory cytokines can promote or restrict each other to form a complex network of cytokines. The levels of proinflammatory factors TNF-α, IL-6, IL-17A, IL-23, and IFN-γ and anti-inflammatory factors IL-2, IL-4, and IL-10 in serum between the HCs and RA patients were detected by flow immunofluorescence microspheres and comparatively analyzed (**Figure 3A-H**). Levels of serum pro-/anti-inflammatory factors in RA patients were significantly higher than those of the HCs, and the increase of proinflammatory factor IL-23 was the most obvious (60.16% ± 23.88% vs. 4.14% ± 3.34%; P < 0.01). There was no significant difference between the above cytokines in RA patients with DHOS and CHCS (P > 0.05).

Correlation of MDSCs and each phenotype with other variables in the peripheral blood of RA patients

In order to clarify the correlation between the proportion of MDSCs with various phenotypes

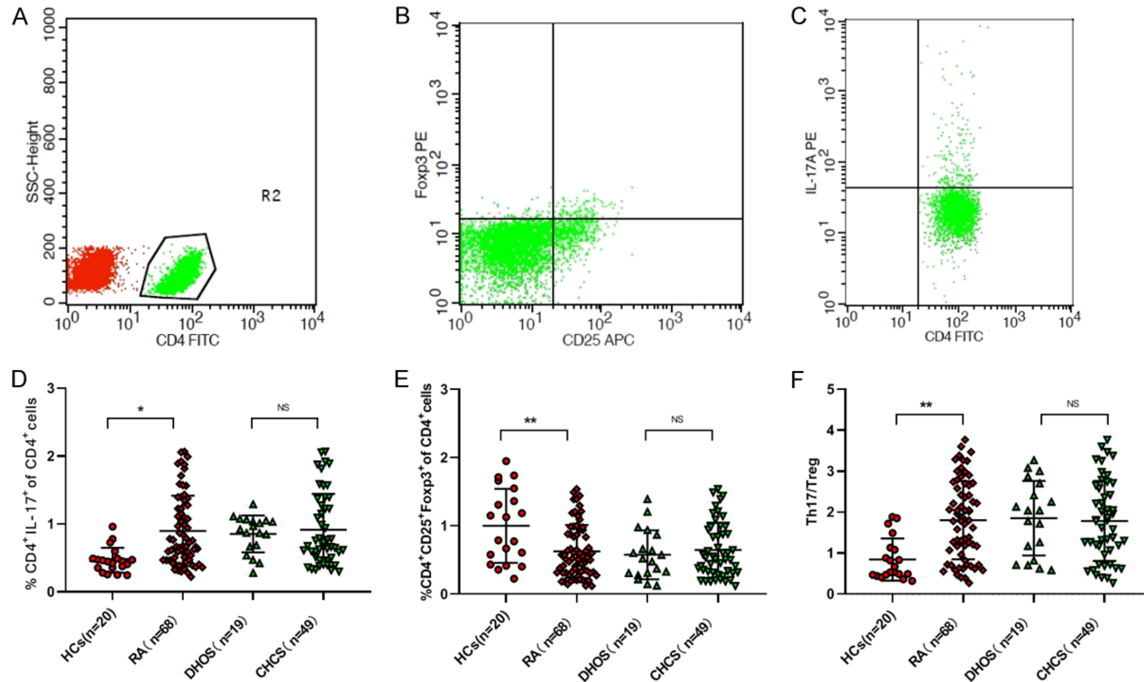

Figure 2. The results of Th17 and Treg cells in peripheral blood cells of RA patients and HCs detected by FCM. The results of FCM showed the distribution of Th17 cells ($CD4^+IL\text{-}17^+$) and Treg cells ($CD4^+CD25^+Foxp3^+$). The frequencies of Th17 and Treg cells and the ratio of Th17/Treg between the HCs and RA patients, and between the RA patients with DHOS and CHCS were compared. A. Representative gating of $CD4^+$ cells. B. Representative gating of $CD25^+FOXP3^+$ of $CD4^+$ cells. C. Representative gating of $IL\text{-}17A^+$ of $CD4^+$ cells. D. Th17 cells frequency (% of $CD4^+$ cells). E. Treg cells frequency (% of $CD4^+$ cells). F. The Th17/Treg ratio. The data shown are presented as means ± SD. *$P < 0.05$, **$P < 0.01$, versus HCs.

and clinical, immune and other autoimmune indexes of RA patients, a correlation analysis was performed. There was a positive correlation between the ratio of MO-MDSCs and ESR, CRP, C3 ($r = 0.293$, 0.249, and 0.277; $P < 0.05$); furthermore, the proportion of MDSCs and PMN-MDSCs were positively correlated with the level of IL-6 in RA patients ($r = 0.253$ and 0.257; $P < 0.05$), while the proportions of MDSCs and PMN/MO-MDSCs were negatively correlated with the proportion of Treg cells ($r = -0.310$, -0.315, and -0.239; $P < 0.05$). Nevertheless, no significant correlation was found between MDSCs and each phenotypes and age; course of disease; SJC; TJC; DAS28; CDAI; SDAI; serum IgG, IgA, IgM, and IgE; C4 and C1q values; and various proinflammatory and anti-inflammatory cytokines except IL-6 in RA patients ($P > 0.05$).

Discussion

In this study, DHOS and CHCS, the two most common clinical syndromes of RA in TCM, were analyzed to explore differences in immunologic indicators in peripheral blood collected from RA patients with different TCM syndromes. The phenotypic and functional characteristics of MDSCs help to vary the microenvironment under different pathological conditions [17, 18]. In this study, the proportions of MDSCs and each phenotype in PBMCs of RA patients with different TCM syndromes were detected and compared. Our results showed that the proportions of MDSCs and each phenotype of RA patients were higher than those of the HCs. In addition, the local environment plays an important role in functional alterations of MDSCs and phenotypes. The role of MDSCs at the periphery and center of the lesions is different due to functional changes among MDSCs [19] and the maturation or composition of immune cells in different parts. Different phenotypes of MDSCs maintain the immune homeostasis of autoimmune arthritis through different effector molecules and pathways [20]. It is suggested that the proportions of MDSCs with different phenotypes are closely related to clinical outcomes.

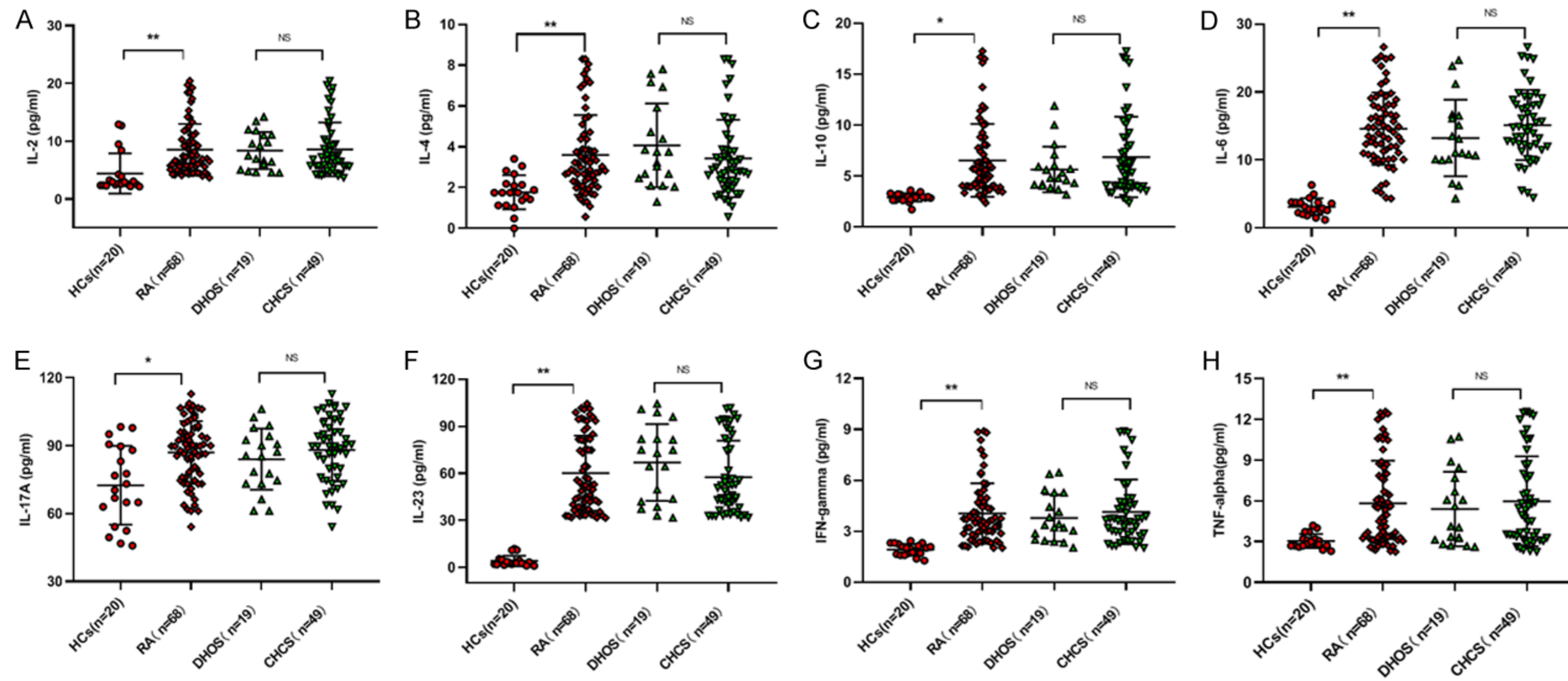

Figure 3. The results of serum cytokines between the HCs and RA patients were detected by flow immunofluorescence microspheres. The levels of serum IL-2 (A), IL-4 (B), IL-10 (C), IL-6 (D), IL-17A (E), IL-23 (F), IFN-γ (G), and TNF-α (H). The data shown are presented as means ± SD. *P < 0.05, **P < 0.01, versus HCs.

The frequency of MDSCs and of each phenotypes was increased in an RA mouse model, which was positively [10, 21-23] or negatively [11, 12] correlated with disease or progression. The proportion of PMN/MO among MDSCs subsets varied at different stages of the disease [20]. MO-MDSCs inhibited the function of T- and B-cells and the progression of disease in a collagen-induced arthritis model [24]. The alteration of the proportions of MDSCs and MO-MDSCs was one of the risk factors for RA. MDSCs and MO-MDSCs were positively correlated with DAS28 score and anti-citrullinated protein antibody (ACPA) [24]. In this study, MDSCs and each phenotype were not correlated with DAS28. The frequency of PMN- and MO-MDSCs in the spleen of experimental autoimmune arthritis model was decreased in the early stage of disease and but increased in a later stage, which was probably related to the formation and function of bone marrow cells in the process of immune regulation [25]. MDSCs in RA mice were stimulated by macrophage colony-stimulating factor and receptor activator of NF-kappa B ligand and differentiated into osteoclasts, leading to bone erosion in *vitro* [25]. The differentiation of MDSCs into osteoclasts depends on NF-kappa B and IL-1 signaling pathways [26, 27].

Antigen-presenting cells, including dendritic cells, macrophages, and activated B-cells, induce T-cells to differentiate into pathogenic T-cell subsets (Th1, Th17) under the inflammatory state [28, 29] and lead to the destruction of joints by secreting cytokines and other mediators. B-cells further strengthen the T-cell-induced inflammation by producing cytokines and autoantibodies such as RF and ACPA [30, 31]. Th17 cells play an important role in regulating the progress of autoimmune arthritis. The activation, expansion, and differentiation of Th17 cells are regulated by cytokines, such as the synergistic effect of TGF-β with IL-6 or IL-21 leads to the differentiation of Th17 cells [32]. Communication between Th17 and other cells is achieved through Th17 cells secreting cytokines including IL-17, IL-21, IL-22, and IL-23 [32], which can further induce the production of matrix metalloproteinase and osteoclasts, consequently promoting the infiltration of inflammatory cells in synovium as well as the destruction of bone and cartilage [33]. IL-17 is the main cytokine secreted by Th17 cells, which can promote an inflammatory response of synovium and play an important role in osteoclast formation and osteolysis [34, 35]. The balance between the inducible effect of IL-17 [36] and the inhibitory effect of IFN-γ [37] plays a key role in IL-23-induced osteoclast differentiation. As a multipotent proinflammatory cytokine, IL-6 can stimulate the number of Th17 cells and the secretion of IL-17, while increased IL-17 can also induce the production of IL-6, thus forming a positive feedback loop to accelerate bone destruction [38]. Suppressive cofactor cytotoxic T-lymphocyte-associated antigen 4 is highly expressed in Treg cells [36] and binds to CD80/CD86 in effector cells to inhibit the function of effector cells. Treg cells secrete inhibitory cytokines, such as IL-10, TGF-β, and IL-2, which are involved in immune regulation and the reduction of Treg cells, leading to autoimmune diseases [39]. The relationship and restriction between Th17 and Treg cells can be interchangeable with each other under specific cytokine microenvironments, and the imbalance in cell number and function between them is an important causative factor of RA [40, 41]. In this study, Th17 cell count and the Th17/Treg ratio in RA patients were significantly increased, while the Treg cell count was decreased. Inflammatory factors and proinflammatory factors in RA patients were significantly higher, but there was no significant difference among RA with DHOS and CHCS. The proportion of MDSCs and Th17 cells of RA patients was increased, but there was no significant correlation between them.

Conclusion

Guided by the holistic concept of TCM and the basic theory of syndrome differentiation-based treatment, the macro- and micro-disease information of RA patients with DHOS and CHCS was analyzed in this study. Our results showed that the clinical immune and autoimmune indices of RA patients were significantly abnormal relative to those of healthy people. The immune indexes ESR, CRP, and MO-MDSCs were significantly different between RA patients with DHOS and CHCS. It is uncertain whether ESR, CRP and MO-MDSCs can be used as important biomarkers to distinguish DHOS-RA and CHCS-RA, which needs to be further studied. In future studies, more RA cases will be collected, and

multicenter studies should be conducted to validate the findings observed in this study. In addition, the role of MDSCs and each phenotype in RA patients with DHOS and CHCS and their related mechanisms must be further explored.

Acknowledgements

This study was supported by the Beijing Municipality Hospital Scientific Research and Cultivation Program Foundation (No. PZ2017-015).

Disclosure of conflict of interest

None.

Abbreviations

ACPA, Anti-Citrullinated Protein Antibody; Anti-CCP, Anti-Cyclic Citrullinated Peptide; CDAI, clinical disease activity index; CHCS, cold-heat complex syndrome; DAS28, Disease Activity Score 28; DCs, Dendritic Cells; DHOS, Damp-Heat Obstruction Syndrome; EAM, Extraarticular Manifestations; Foxp3, Forkhead Box P3; HCs, Healthy Controls; IMCs, Immature Myeloid Cells; MDSCs, Myeloid-Derived Suppressor Cells; MO-MDSCs, Monocytic MDSCs; PMN-MDSCs, Polymorphonuclear MDSCs; PBMCs, Peripheral Blood Mononuclear Cells; RA, Rheumatoid Arthritis; SDAI, Simplified Disease Activity Index; SJC, Swollen Joint Count; TCM, Traditional Chinese medicine; TJC, Tender Joint Count; Treg, Regulatory T.

Address correspondence to: Bei Wang, Beijing Hospital of Traditional Chinese Medicine, Capital Medical University, No. 23 Meishuguan Back Street, Dongcheng District, Beijing 100010, China. E-mail: wangbei@bjzhongyi.com

References

[1] Scott D, Wolfe F and Huizinga T. Rheumatoid arthritis. Lancet 2010; 376: 1094-1108.
[2] Radu A and Bungau SG. Management of rheumatoid arthritis: an overview. Cells-Basel 2021; 10: 2857.
[3] Zhu J, Zhou Y, Chen X and Li J. A metaanalysis of the increased risk of rheumatoid arthritis-related pulmonary disease as a result of serum anticitrullinated protein antibody positivity. J Rheumatol 2014; 41: 1282-1289.
[4] Norton S, Koduri G, Nikiphorou E, Dixey J, Williams P and Young A. A study of baseline prevalence and cumulative incidence of comorbidity and extra-articular manifestations in RA and their impact on outcome. Rheumatology 2012; 52: 99-110.
[5] Mckay N and Kelly CA. Extra-articular features of rheumatoid arthritis. Medicine 2006; 34: 383-386.
[6] Svendsen AJ, Kyvik KO, Houen G, Junker P, Christensen K, Christiansen L, Nielsen C, Skytthe A and Hjelmborg JV. On the origin of rheumatoid arthritis: the impact of environment and genes-a population based twin study. PLoS One 2013; 8: e57304.
[7] Firestein GS and Mcinnes IB. Immunopathogenesis of rheumatoid arthritis. Immunity 2017; 46: 183-196.
[8] Veale DJ, Orr C and Fearon U. Cellular and molecular perspectives in rheumatoid arthritis. Semin Immunopathol 2017; 4: 343-354.
[9] Rajabinejad M, Salari F, Gorgin Karaji A and Rezaiemanesh A. The role of myeloid-derived suppressor cells in the pathogenesis of rheumatoid arthritis; anti- or pro-inflammatory cells? Artif Cell Nanomed B 2019; 47: 4149-4158.
[10] Guo C, Hu F, Yi H, Feng Z, Li C, Shi L, Li Y, Liu H, Yu X, Wang H, Li J, Li Z and Wang X. Myeloid-derived suppressor cells have a proinflammatory role in the pathogenesis of autoimmune arthritis. Ann Rheum Dis 2015; 75: 278-285.
[11] Fujii W, Ashihara E, Hirai H, Nagahara H, Kajitani N, Fujioka K, Murakami K, Seno T, Yamamoto A, Ishino H, Kohno M, Maekawa T and Kawahito Y. Myeloid-Derived suppressor cells play crucial roles in the regulation of mouse Collagen-Induced arthritis. J Immunol 2013; 191: 1073-1081.
[12] Jiao Z, Hua S, Wang W, Wang H, Gao J and Wang X. Increased circulating myeloid-derived suppressor cells correlated negatively with Th17 cells in patients with rheumatoid arthritis. Scand J Rheumatol 2012; 42: 85-90.
[13] Brys L, Beschin A, Raes G, Ghassabeh GH, Noël W, Brandt J, Brombacher F and Baetselier PD. Reactive oxygen species and 12/15-Lipoxygenase contribute to the antiproliferative capacity of alternatively activated myeloid cells elicited during helminth infection. J Immunol 2005; 174: 6095-6104.
[14] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Ménard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Sym-

mons D, Tak PP, Upchurch KS, Vencovsky J, Wolfe F and Hawker G. 2010 rheumatoid arthritis classification criteria: an American College of Rheumatology/European League against Rheumatism collaborative initiative. Ann Rheum Dis 2010; 69: 1580-1588.
[15] Zheng XY. Clinical guiding principles of new Chinese medicine in the treatment of rheumatoid arthritis. In: Zheng XY, editor. Guiding principles for clinical research of new traditional Chinese medicine (Trial). Beijing: China Medical Science and Technology Publishing House; 2002. pp. 115-119.
[16] Lou YL. China rheumatology. Beijing: People's Health Publishing House; 2001. pp. 2103.
[17] Youn J and Gabrilovich DI. The biology of myeloid-derived suppressor cells: the blessing and the curse of morphological and functional heterogeneity. Eur J Immunol 2010; 40: 2969-2975.
[18] Pastuła A and Marcinkiewicz J. Myeloid-derived suppressor cells: a double-edged sword? Int J Exp Pathol 2011; 92: 73-78.
[19] Gabrilovich DI and Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009; 9: 162-174.
[20] Wang W, Jiao Z, Duan T, Liu M, Zhu B, Zhang Y, Xu Q, Wang R, Xiong Y, Xu H and Lu L. Functional characterization of myeloid-derived suppressor cell subpopulations during the development of experimental arthritis. Eur J Immunol 2015; 45: 464-473.
[21] Youn J, Nagaraj S, Collazo M and Gabrilovich D. Subsets of myeloid-derived suppressor cells in tumor-bearing mice. J Immunol 2008; 181: 5791-5802.
[22] Zhang H, Wang S, Huang Y, Wang H, Zhao J, Gaskin F, Yang N and Fu SM. Myeloid-derived suppressor cells are proinflammatory and regulate collagen-induced arthritis through manipulating Th17 cell differentiation. Clin Immunol 2015; 157: 175-186.
[23] Sun J, Xu P, Du X, Zhang Q and Zhu Y. Piperlongumine attenuates collagen-induced arthritis via expansion of myeloid-derived suppressor cells and inhibition of the activation of fibroblast-like synoviocytes. Mol Med Rep 2015; 11: 2689-2694.
[24] Corzo CA, Condamine T, Lu L, Cotter MJ, Youn JI, Cheng P, Cho HI, Celis E, Quiceno DG, Padhya T, Mccaffrey TV, Mccaffrey JC and Gabrilovich DI. HIF-1alpha regulates function and differentiation of myeloid-derived suppressor cells in the tumor microenvironment. J Exp Med 2010; 207: 2439-2453.
[25] Li M, Zhu D, Wang T, Xia X, Tian J and Wang S. Roles of Myeloid-Derived suppressor cell subpopulations in autoimmune arthritis. Front Immunol 2018; 9: 2849.
[26] Zhu J, Chen S, Wu L, Wang R, Zheng S, Zhao D, Wang X and Li J. The expansion of Myeloid-Derived suppressor cells is associated with joint inflammation in rheumatic patients with arthritis. Biomed Res Int 2018; 2018: 5474828.
[27] Zhang H, Huang Y, Wang S, Fu R, Guo C, Wang H, Zhao J, Gaskin F, Chen J, Yang N and Fu SM. Myeloid-derived suppressor cells contribute to bone erosion in collagen-induced arthritis by differentiating to osteoclasts. J Autoimmun 2015; 65: 82-89.
[28] Mucida D and Cheroutre H. The many face-lifts of CD4 T helper cells. Adv Immunol 2010; 107: 139-152.
[29] Bluestone JA, Mackay CR, O'Shea JJ and Stockinger B. The functional plasticity of T cell subsets. Nat Rev Immunol 2009; 9: 811-816.
[30] Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ and Xu J. Rheumatoid arthritis: pathological mechanisms and modern pharmacologic therapies. Bone Res 2018; 6: 15.
[31] Calabresi E, Petrelli F, Bonifacio AF, Puxeddu I and Alunno A. One year in review 2018: pathogenesis of rheumatoid arthritis. Clin Exp Rheumatol 2018; 36: 175-184.
[32] Bunte K and Beikler T. Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and Immune-Mediated inflammatory diseases. Int J Mol Sci 2019; 20: 3394.
[33] Kim K, Kim H, Kim B, Cho M and Lee S. Th17 cytokines regulate osteoclastogenesis in rheumatoid arthritis. Am J Pathol 2015; 185: 3011-3024.
[34] Kotake S, Udagawa N, Takahashi N, Matsuzaki K, Itoh K, Ishiyama S, Saito S, Inoue K, Kamatani N, Gillespie MT, Martin TJ and Suda T. IL-17 in synovial fluids from patients with rheumatoid arthritis is a potent stimulator of osteoclastogenesis. J Clin Invest 1999; 103: 1345-1352.
[35] Al-Ghamdi A and Attar SM. Extra-articular manifestations of rheumatoid arthritis: a hospital-based study. Ann Saudi Med 2009; 29: 189-193.
[36] Takahashi T, Tagami T, Yamazaki S, Uede T, Shimizu J, Sakaguchi N, Mak TW and Sakaguchi S. Immunologic self-tolerance maintained by CD25 (+) CD4 (+) regulatory T cells constitutively expressing cytotoxic T lymphocyte-associated antigen 4. J Exp Med 2000; 192: 303-310.
[37] Kotake S, Nanke Y, Mogi M, Kawamoto M, Furuya T, Yago T, Kobashigawa T, Togari A and Kamatani N. IFN-γ-producing human T cells directly induce osteoclastogenesis from human monocytes via the expression of RANKL. Eur J Immunol 2005; 35: 3353-3363.
[38] Schinnerling K, Aguillón JC, Catalán D and Soto L. The role of interleukin-6 signalling and its

therapeutic blockage in skewing the T cell balance in rheumatoid arthritis. Clin Exp Immunol 2017; 189: 12-20.
[39] Lawson CA, Brown AK, Bejarano V, Douglas SH, Burgoyne CH, Greenstein AS, Boylston AW, Emery P, Ponchel F and Isaacs JD. Early rheumatoid arthritis is associated with a deficit in the CD4+CD25high regulatory T cell population in peripheral blood. Rheumatology 2006; 45: 1210-1217.
[40] Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL and Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature 2006; 441: 235-238.
[41] Mackay K, Milicic A, Lee D, Tikly M, Laval S, Shatford J and Wordsworth P. Rheumatoid arthritis susceptibility and interleukin 10: a study of two ethnically diverse populations. Rheumatology 2003; 42: 149-153.