Relationship Between Cartilage Oligomeric Matrix Protein Level and Helper T Cell 17/regulatory T Cell Balance in Patients With Rheumatoid Arthritis

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Research

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Abstract

Objective To explore the correlation among the level of cartilage oligomeric matrix protein(COMP) as well as the balancing relationship of helper T cell 17(Th17) /regulatory T cells(Treg) of serum in patients with Rheumatoid Arthritis (RA).

Methods 40 patients with RA in the Second Hospital of Shanxi Medical University from May 2019 to May 2020 were selected as RA group, while 40 healthy subjects were collected as a control group. After collecting their serum, the figure of Th17, as well as Treg cells, were encountered by flow cytometry(FC), and the proportion of Th17/Treg was counted. Cytometric was also used to detect serum interleukin(IL)-17, IL-10 and IL-6 concentration. Enzyme-linked immunosorbent (ELISA)was also applied to track down the concentrations of COMP as well as tumor necrosis factor-α(TNF-α). All indicators above were considered among groups, and the interrelationship between COMP and the expression of various cells as well as related cytokines was analyzed.

Results The concentration of COMP, Th17 and Th17/Treg in RA group were higher than those in the control group, while Treg was lower than that in the control group, and the variation was statistically weighty($P<0.05$). The levels of IL-17, IL-6 and TNF-α in RA group were higher than those in the control group, while the concentration of IL-2 was smaller than that of the control group, the differences were statistically weighty($P<0.01$). Interrelationship analysis displayed that COMP was undeniably correlated with Th17, Th17/Treg, IL-17, IL-6 as well as TNF-α levels($r =0.687$, 0.478, 0.759, 0.903, 0.813, all $P<0.01$). There was a negative correlation between the levels of COMP and Treg, IL-2($r =-0.356$, -0.455, all $P<0.01$).

Conclusion Compared with healthy people, COMP in peripheral blood of patients with RA is abnormally high, and there is obvious T cell immune abnormality, suggesting that COMP may participate in the development of RA by regulating Th17/Treg balance.

Background

Rheumatoid arthritis (RA) is the most common type of autoimmune disease. It mainly occurs in small joints such as hand, wrist and foot, and some occur in large joints such as hip, knee and ankle[1]. It is an autoimmune disease with complex etiology. There are many causes leading to the disease, including pathogenic microorganisms, physical and chemical factors, immune damage, allergy and even drugs, but the exact pathogenesis is still being explored. Some experiments about COMP have shown that COMP plays an important role in the occurrence, development and prognosis of RA. Some other studies have shown that abnormal T cells play an important role in the pathogenesis and progression of RA. The concentration of helper T cell 17 (Th17) and regulatory T cell (Treg) in abnormal $CD4^+$T cell subsets and their imbalance ratio participate in the occurrence and subsequent development of RA[2], and Th17 / Treg tends to Th17, playing a pro-inflammatory role and aggravating the development of the disease [2]. However, there is no clinical study on the correlation between serum COMP and Th17 / Treg balance in RA patients. In this study, we aimed to explore the relationship between COMP and Th17 / Treg balance in RA patients.

1. Methods And Materials

1.1 General materials

RA patients who were treated in the second hospital of Shanxi Medical University from July 2019 to March 2020 were randomly selected. The inclusion criteria were according to the diagnosis standards made by ACR in 1987 as follows: at least 3 joints had inflammatory arthritis; RF and / or anti citrullinated peptide / protein antibody (such as anti CCP

antibody) were positive; CRP or ESR were elevated; diseases with similar clinical characteristics, especially psoriatic joints, were excluded The symptoms lasted for more than 6 weeks. They were all confirmed by the laboratory rheumatoid factor determination and joint X-ray diagnosis. Patients with long-term or severe cardiogenic shock, burn, postoperative and trauma were excluded. Finally, 40 RA patients were selected in the RA group, with an average age of (36.28 ± 3.25) years. 40 healthy people were randomly selected as the control group, with an average age of (36.39 ± 3.21) years. Informed consent has been obtained from all patients and their relatives. There was no significant difference in the age among two groups ($P > 0.05$).

1.2 Methods

Taking 15 ml of venous blood of every patient to the gel tube of the serum separator and centrifuge them at room temperature for 3000r/min in 15 minutes. The separated serum was distributed into a 1 ml cryotube, and a portion of the isolated serum from each patient was immediately used for Th17 analysis at all sampling times, while the rest was stored at − 80 °C until performing COMP analysis. In order to avoid repeated freeze-thaw cycles, a single determination of the sample was carried out.

1.2.1 ELISA:

It is used to detect COMP and TNF-α. The instrument was Beckman AU5808 analyzer, and the reagents were purchased from Beckman, USA.

1.2.2 Flowcytometry:

It is used to detect Th17, Treg, IL-17, IL-6 and IL-2. The instrument was Beckman Coulter, and the reagents were purchased from Beckman, USA.

1.3 Statistical methods

The data were processed with IBM SPSS 23.0 statistical software. The homogeneity variance was used to test the normality. The quantitative data of normal distribution was given in the form of mean ± standard deviation (x ± SD). The t-test and Pearson correlation analysis were performed.

2. Results

2.1 Comparison of the levels of COMP, Th17, Treg, Th17/Treg between RA group and the control group

The concentrations of comp, Th17 and Th17 / Treg in RA group were higher than those in control group, while Treg concentration was lower in RA group than that in control group ($P < 0.05$). See Table 1

Table 1 Comparison of the levels of COMP, Th17, Treg, Th17/Treg between two groups (x±s)

group	Sample size	age (year)	COMP (U/L)	Th17 (Number/UL)	Treg (Number/UL)	Th17/Treg (%)
Control group	40	36.39±3.21	9.11±1.05	4.35±2.15	32.99±12.35	0.15±0.09
RA group	40	36.28±3.25	14.01±1.98	6.87±4.86	22.99±13.95	0.39±0.19
t		0.102	14.381	2.566	2.555	4.596
P		0.919	0.000	0.005	0.010	0.000

2.2 Comparison of levels of IL-17, IL-6, IL-2 and TNF-α between the RA groups and the control group

The concentrations of IL-17, IL-6 and TNF -α in RA group were higher than those in control group, while the concentration of IL-2 in RA group was lower than that in control group (P < 0.05). See Table 2

Table 2 Comparison of levels of IL-17, IL-6, IL-2 and TNF-α between the two groups (x±s)

group	Sample size	age (year)	IL-2(μg/L)	IL-6(ng/L)	IL-17(μg/L)	TNF-α(U/ml)	IL-32(pg/ml)
Control group	40	36.39±3.21	7.79±1.19	14.60±2.96	84.29±34.11	42.65±11.46	28.60±7.06
RA group	40	36.28±3.25	3.35±0.84	196.91±45.81	145.89±59.56	134.19±32.56	87.00±15.13
t		0.102	16.286	12.362	5.86	11.566	22.845
P		0.919	0.000	0.000	0.000	0.000	0.000

2.3 Correlation analysis results of COMP and other index

Comp was positively correlated with Th17, Th17 / Treg, IL-17, IL-6 and TNF-α (r = 0.687, 0.478, 0.759, 0.903, 0.813, all P < 0.01), but negatively correlated with Treg and IL-2 (r = - 0.356, - 0.455, all P < 0.01).

3. Conclusion

Compared with healthy people, COMP in peripheral blood of patients with RA is abnormally high, and there is obvious T cell immune abnormality, suggesting that COMP may participate in the development of RA by regulating Th17/Treg balance.

4. Discussion

There are a lot of thorough studies on comp, but most of them exist in sepsis and infectious diseases [4]. A few studies have shown that it plays an important role in the occurrence, development and prognosis of RA [5]. Under the stimulation of related factors, it can be released outside the nucleus and extracellular, participate in inflammatory reaction and express specific biological effects [5]. Comp B box is the domain of proinflammatory factors. The recombinant B box can activate monocyte macrophages and release tumor necrosis factor (TNF), interferon (INF),

interleukin-1 (IL-1), intercellular adhesion molecule-1 (cam-1), vascular cell adhesion molecule-1 (VCAM-1) and other inflammatory mediators [6]. In the inflammatory effect caused by comp, cytokines such as TNF, inf and IL-1 synthesized and released can react with monocyte macrophages to release more comp [7], and then aggravate the inflammatory reaction.

Abnormal T cells play an important role in the pathogenesis and progression of RA [8]. After activation, T cells can regulate the functions of macrophages, dendritic cells, B cells, synovial cells and osteoclasts through high expression of CD69, CD25, cd3hla-dr and release various cytokines, antibodies and enzymes, which can lead to synovitis and hyperplasia, and eventually lead to pathological joint damage [9]. Th17 and Treg are newly discovered CD4 + T cell subsets in recent years. It is a hot spot in recent years to detect the expression of surface marker molecules of abnormally activated T cells and explore the correlation between them and RA disease activity indicators [10].

Th17, Treg and their balance play an important role in the process of human autoimmune stabilization and anti-tumor immunity. The concentration of helper T cell 17 (Th17) and regulatory T cell (Treg) in abnormal CD4 + T cell subsets and their imbalance ratio participate in the occurrence and subsequent development of RA, and Th17 / Treg tends to Th17, playing a pro-inflammatory role and aggravating the development of the disease [11]. Some studies have emphasized the role and mechanism of miR-181a in regulating Th17 / Treg cell immunity in RA [12]. Other studies have shown that peripheral blood comp regulates Th17 / Treg via rage-il-6 pathway. The high expression of comp in peripheral blood of patients with pre RA may regulate the shift of Th17 / Treg balance to Th17 through rage-il-6 signal pathway, aggravate inflammatory reaction and participate in the pathogenesis of pre RA [13]. Some studies have analyzed the relationship between comp and Th17, Treg cells and cytokines levels in peripheral blood of patients with pre RA, and found that comp affects the balance of Th17 / Treg through rage-il-6 axis [14]. The interaction of rage with ages, S100B and comp ligands can lead to persistent inflammatory state, and then lead to a variety of chronic diseases including RA. Therefore, Th17 / Treg imbalance plays an important role in the pathogenesis of rheumatoid arthritis.

The expression of serum COMP induced by inflammation may be related to the pathological process of RA (systemic synovial inflammation) [14]. Relevant data showed that the expression of comp was synergistically correlated with the expression of TNF il-1in and IL-6 in synovium [15]. Therefore, the expression of comp and related inflammatory factors in proliferative synovial tissue may be one of the important reasons for chronic synovial inflammation of RA.

Of course, there are also limitations in this study, such as the interference of sample size and single determination (in order to avoid repeated freeze-thaw cycles, we only conduct a single determination on samples. Although we have carried out the quality control of the inspection instrument before the determination, the results show that it is normal, but a single measurement may still have a slight impact on some data).

Comp and T cells are two important indexes in RA detection. Monitoring comp, Th17 / Treg balance is conducive to the diagnosis and monitoring of the disease [16]. Their rise and fall is closely related to the severity of RA, which is helpful for monitoring, beneficial to observe the curative effect of drugs, guiding personalized treatment, so as to optimize the drug treatment of RA, and has important significance for improving the level of differential diagnosis of rheumatoid arthritis [17].

This study confirmed the abnormal expression of comp in serum of RA patients, and compared with the reference interval of healthy people, the abnormal T-cell immunity was obviously existed, suggesting that comp may participate in the occurrence and subsequent development of RA by regulating Th17 / Treg balance.

5. Declaration

- Ethical Approval and Consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of shanxi medical university. Written informed consent was obtained from individual or guardian participants.
- Consent for publication

All authors agree for publication
- Availability of data and materials

Data extracted from the second affiliated hospital of shanxi medical university
- Competing interests

There was not competing interests between authors.
- Funding

Not applicable.
- Authors' contributions

HAN Rong analyzed and interpreted the patient data regarding the hematological disease and the transplant. All authors read and approved the final manuscript.
- Acknowledgements

Not applicable.
- Authors' information

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