Correlation of nuclear factor-κB, regulatory T cell and transforming growth factor β with rheumatoid arthritis

You Sun a,1,*, De-Li Zhao b,1, Zi-Xuan Liua, Xue-Hui Suna, Yang Lia

a Department of Rheumatology, The 2nd Affiliated Hospital, Harbin Medical University, Harbin 150000, China
b Department of Radiology, The 2nd Affiliated Hospital, Harbin Medical University, Harbin 150000, China

Original article

Objective: Investigated the correlation of nuclear factor-κB, regulatory cells and transforming growth factor-β with rheumatoid arthritis. Methods: Included 65 cases of RA patients admitted in our hospital from June 2015 to December 2016 into case group, and included 50 healthy people into control group during the same period. Collected the peripheral detection of nuclear factor-κB, regulatory cells and transforming growth factor beta levels, and compared them between two groups. Results: The percentage of CD4+, CD25+ T cells in the case group was significantly lower than that in the control group \((P < 0.05)\); There was no significant difference in the percentage of CD4+, CD25+ CD127low T cells between groups \((P > 0.05)\); The levels of TGF-β and NF-kappa B in the case group were higher than those in the control group, and the difference between the two groups was statistically significant \((P < 0.05)\); The levels of ESR, CRP and RF in the case group were higher than those in the control group \((P < 0.05)\). There was a negative correlation between the expression of nuclear factor-κB, transforming growth factor-β and RF level in RA patients by pearson correlation analysis, \(r = -0.652, P < 0.05\). Conclusion: The expression levels of CD4+, CD25+ T cells in patients with RA are significantly decrease, which has a negative correlation with RA activity index RF, and showed that the pathogenesis of RA is related to the regulation of immune system.

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A B S T R A C T

Rheumatoid arthritis (RA) is a kind of systemic autoimmune disease and is mainly characterized in chronic joint disease. It is mainly manifested as joint symmetric multiple arthritis of hands, knee, wrist, ankle and foot. If it is not treated in time, RA protracts course of disease, even causing joint deformity. This disease is also one of major diseases that lead to joint deformity and loss of labor (Li et al., 2011). References (Limin et al., 2015) report that RA morbidity is about 0.34%. RA characteristic pathologic change is that, antigen polypeptide activates T cells via antigen presenting cells, leads to activation of other immune cells, increase of immune globulin, proinflammatory cytokines and other media, and causes vasculitis, synovial hyperplasia and cartilage destruction. Pathogenesis mechanism relevant to the disease is still not precise at present, but it initially think that it may be relevant to body immune modulating dysfunction. This study specially analyzes expression of nuclear factor κB, regulatory T cell and transforming growth factor β of patients with rheumatoid arthritis, analyzes correlation of them and clinical indexes of RA, and then analyzes relevant effect of them on RA morbidity.

1. Data and method

1.1. General data

Select 65 cases with rheumatoid arthritis received and cured in hospital in June, 2015–December, 2016, i.e. case group: male/female = 23/42, age is 39–78 years old, average age is 58.69 ± 8.23 years old, course of disease is three months ~ 29 years, average course of disease is 8.26 ± 3.16 years; and select 50 healthy cases in the same period in the hospital in control group, male/female = 16/34, age is 38–75 years old, average age is 57.96 ± 8.15 years old. There is no statistical difference \((P > 0.05)\) in comparison of sex, age and other general data of two groups, which has comparability.

Diagnostic criteria (Jia and Cibo, 2010): morning stiffness time is at least one hour and lasts six weeks and over; there are three
or more joint galls that lasts at least six weeks; there are gall symptoms in wrist, palm and finger or proximal interphalangeal joint that lasts at least six weeks; there are symmetric joint gall on both sides lasts at least six weeks; subcutaneous nodules; osteoporosis or joint space narrowing can be found at least during X-ray examination; examination of rheumatoid factor is positive.

Inclusion criteria: observation group: personnel that is in accordance with RA diagnostic criteria; personnel who volunteers to participate in this study, whose family is informed and consents and signs informed consent; personnel checked and approved by relevant medical ethics committee in the hospital. Control group: no RA disease; volunteer to participate in this study.

Exclusion criteria: personnel that is not in accordance with the above inclusion criteria; personnel that combines other chronic diseases, such as primary hypertension, diabetes, coronary heart disease, malignancy disease and other diseases; personnel that is accompanied with dysfunction of heart, liver, kidney and other organs; personnel that is accompanied with endocrine disease; personnel that is accompanied with hematological system diseases; personnel that combines various acute and chronic infection and is accompanied with other infectious disease; personnel that combines with serious complications; pregnant and lactant women; personnel without perfect case information.

1.2. Method

1.2.1. Required reagent

Mouse anti-human CD25 antibody labeled with phycoerythrin, mouse anti-human CD4 antibody labeled with fluorescein isothiocyanate, mouse anti-human CD127 antibody labeled with phycoerythrin-anthocyanin 5, mouse anti-human IgG-1 immune globulin, mouse anti-human IgG-1 immune globulin.

1.2.2. Detection method

Regulatory T cell detection: adopt the method of first labeling on monoclonal antibody on film surface and later dissolution of red blood cells. Firstly, separately add anti-human CD4 antibody labeled with phycoerythrin and 20 μL mouse anti-human CD4 antibody labeled with fluorescein isothiocyanate in 100 μL heparin anticoagulation, and add 5 μL mouse anti-human CD127 antibody labeled with phycoerythrin - anthocyanin 5. Then separately add mouse anti-human IgG-1 immune globulin and 20 μL mouse anti-human CD4 antibody labeled with fluorescein isothiocyanate in 100 μL anticoagulation of control tube with the same type, and add 5 μL mouse anti-human IgG-1 immune globulin labeled with phycoerythrin - anthocyanin 5. After incubating for 10 min out of the sun at room temperature, wash once with PBS liquid, skin off supernatant after centrifugation, add 1 ml PBS liquid and incubate it again. Operate American COULTER EPICA XL flow cytometry of BECKMAN COULTER according to instruction. Detection results are expressed as percentage of CD4+ CD25+ T cell and CD4+ CD25+ CD127low/- T cells between groups. Details are shown in Table 1.

2. Result

2.1. Result analysis of regulatory T cells of two groups

For case group, percentage of CD4+ CD25+ T cell is lower than that of control group, there is significant statistical difference (P < .05) between groups; there is no significant difference (P > .05) in percentage of CD4+ CD25+ CD127low/- T cells between groups. Details are shown in Table 1.

2.2. Comparison of nuclear factor -κB, levels of transforming growth factor β of two groups

Transforming growth factor β, nuclear factor –κB level of patient in case group are higher than that in control group, and there is significant statistical difference (P < .05) between groups. Details are shown in Table 2.

2.3. Comparison of RA clinical activity indexes of patients in two groups

ESR, CRP and RF levels in case group are higher than that in control group, and there is significant difference (P < .05) between groups. Details are shown in Table 3.

2.4. Analysis of correlation of nuclear factor-κB, regulatory T cell and level of transforming growth factor β with RA activity index of RA patient

Through analysis of correlation of Pearson, there is negative correlation of expression of nuclear factor –κB, transforming growth factor β with RF level of RA patient, r = −0.652, P < .05. Details are shown in Table 4 and Fig. 1.

3. Discussion

Rheumatoid arthritis is a kind of chronic systemic autoimmune disease that strictly influences human health and one of major morbidity diseases (Ningning et al., 2017). Its main lesion presents as synovial hyperplasia, abnormal proliferation on surrounding tissue and progressive joint destruction. The courses of disease have not been clear. In recent years, molecular biology science continuously...

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**Table 1**

| Group       | Case number | CD4+ CD25+ T cell | CD4+ CD25+ CD127low/- T cell |
|------------|-------------|------------------|-----------------------------|
| Case group | 65          | 3.42 ± 0.24      | 3.29 ± 0.46                 |
| Control    | 50          | 4.35 ± 0.34      | 3.42 ± 0.39                 |
| t          |             | 17.187           | 1.603                       |
| P          | <0.01       | 0.112            |                             |

**Table 2**

| Group       | Case number | Transforming growth factor β (pg/mL) | Nuclear factor-κB |
|------------|-------------|-------------------------------------|-------------------|
| Case group | 65          | 731.89 ± 245.15                    | 1.86 ± 0.45       |
| Control    | 50          | 468.35 ± 195.35                    | 0.67 ± 0.19       |
| t          |             | 6.187                              | 17.522            |
| P          | <0.01       | 0.112                              | 0.01              |
Comparison of RA clinical activity indexes of patients in two groups.

| Group      | Case number | ESR (mm/h) | CRP (µg/mL) | RF (IU/L) |
|------------|-------------|------------|-------------|-----------|
| Case group | 65          | 61.24 ± 19.62 | 36.85 ± 18.98 | 80.49 ± 35.48 |
| Control group | 50       | 12.32 ± 3.59     | 4.26 ± 1.62     | 12.05 ± 3.05   |

Analysis of correlation of nuclear factor-κB expression with RA activity index of RA patient.

| Indexes                        | ESR          | CRP          | RF           |
|-------------------------------|--------------|--------------|--------------|
|                               | r | P     | r | P     | r | P     |
| CD4+CD25+ T cell              | -0.201       | 0.791        | -0.224       | 0.197 | -0.652 | 0.023 |
| Nuclear factor-κB             | 0.205        | 0.625        | 0.198        | 0.795 | 0.204 | 0.632 |
| Transforming growth factor β  | 0.168        | 0.796        | 0.124        | 0.798 | 0.110 | 0.809 |

Fig. 1. Analysis of correlation of CD4+CD25+ T cell expression with RF level of RA patient.

Developed provides conditions for the pathogenesis studied from gene level (Hongbin et al., 2012). Previous studies (Xinwen et al., 2017) have pointed that regulatory T cell plays an important role in generation and development of RA disease, but there is a few studies of correlation of nuclear factor-κB regulatory T cell and transforming growth factor β of patients with rheumatoid arthritis. For this, this study analyzes expression of nuclear factor-κB regulatory T cell and transforming growth factor β of patients with rheumatoid arthritis, analyzes correlation of them and clinical indexes of RA, and then analyzes relevant effect of them on RA morbidity.

CD4+CD25+ regulatory T cell is a kind of T cell subset of the body, which can actively control T cell proliferation and regeneration of auto-reactive T cells, inhibit autoimmune response, then stop occurrence of immune response and maintain immunologic balance action of the body. Lower immune response and immunological suppression are two characteristics of CD4+CD25+ regulatory T cell (Xiang et al., 2010). Reduction or dysfunction of CD4+CD25+ regulatory T cell may damage stability of autoimmune environment and cause autoimmune diseases. Previous animal experiments (Jian et al., 2011) find that changes on regulatory T cell may be related to arthropathy degree of patients with rheumatoid arthritis. Combining this study, percentage of CD4+CD25+ regulatory T cell of patients in RA group is significantly lower than that in control group. However, there is no significant difference in comparison of percentage of CD4+CD25+ CD127low+ T cell. The result is the same to that of ______. Through analysis of correlation of Pearson, there is negative correlation of expression of nuclear factor-κB, transforming growth factor β with RF level of RA patient, r = -0.652, P < .05, i.e. expression level of CD4+CD25+ regulatory T cell of RA patient drops, level of activity RF index may increases. It can be found that regulatory T cell may play an important role in generation and development of RA disease.

NF-κB (NF-κB) is a transcription factor protein family, mainly including five sigmasubunits: Rel (cRel), p65 (RelA, NF-κB3), RelB, p50 (NF-κB1) and p52 (NF-κB2). It mainly involves body defense reaction, tissue damage, stress and cell differentiation and apoptosis, growth and inhibition of tumor cell and other information transmission processes. Different selection of sequence incorporation of NF-κB dimer may have certain difference, which is a fine control method of different gene expressions by combining different dimers (Wenxia et al., 2016). NF-κB and IκB in resting cells form compound (IκB kinase, IKK), existing in cytoplasm in inactive form. When external factors stimulate cells, IKK phosphorylates IκB, which exposes nucleus locating point of NF-κB. Free NF-κB rapidly transfers to cell nucleus. Combine specificity κB, and then induce transcription of relevant genes (Lei et al., 2013). As shown in this study, peripheral blood NF-κB level of RA patient is significantly higher than that in control group. But through analysis of correlation of Pearson, there is no correlation of NF-κB expression with clinical activity index of RA, so it can be initially determined that NF-κB expression may be not related to morbidity of RA patients.

TGF-β is an important inflammatory factor with complex functions. It not only inhibits inflammation, but also promotes inflammation. Relevant foreign references (Lei et al., 2013) report that, TGF-β expression quantity of serum of RA patient is obviously higher than that of healthy people, which can inhibit inflammation and immune response to achieve positive regulation. However, in light of strong balance among cell factors, stimulation of inflammatory factors caused promotes inadequate protective action of TGF-β, pushing development of RA. Recently, there is a few studies of correlation of TGF-β with morbidity of RA. It is not clear at present. As shown in this study, TGF-β level of RA patient significantly increases and Zhiguo et al. (2015) and other persons prove this.

ESR, VRP and RF are common activity observation indexes of RA patients, so specially analyzes expression and correlation of nuclear factor κB, regulatory T cell and transforming growth factor β of patients with rheumatoid arthritis. Results show that, there is negative correlation of expression level of CD4+CD25+ T of RA with RF index. It can be seen that regulatory T cell may be related to generation and development of rheumatoid arthritis which is to be further studied.
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References

Li, Jiang, Jingguo, Zhou, Yufeng, Qing, et al., 2011. Study on effect of toll-like receptor 2 and toll-like receptor 4 and signal channel on primary pathogenesis of gouty arthritis. Chin. J. Rheumatology 15 (5), 300–304.

Limin, Ren, Dan, Ma, Liyun, Zhang, et al., 2015. Effect of intraperitoneal injection arsenic trioxide on osteoprotegerin system of receptor activation factor ligand of serum nuclear factor j of rats with collagen induced arthritis. Chin. J. Rheumatology 19 (10), 682–685. Cover 3.

Jia, Huang, Cibo, Huang, 2010. Progress on diagnosis and treatment of rheumatoid arthritis. Clin. Med. J. 8 (1), 1–5.

Ningning, Liu, Gailian, Zhang, Liyun, Zhang, et al., 2017. Influence of articular injection with ozone on intervention curative effect and osteoprotegerin system of receptor activation factor ligand of serum nuclear factor kB of rats with collagen induced arthritis. Chin. J. Rheumatology 21 (7), 466–470.

Hongbin, Li, Ning, Tie, Yongfeng, Jia, et al., 2012. Analysis on correlation of expression of synovial anti-cyclic citrullinated peptide of patients with rheumatoid arthritis with unbalance and synovial inflammation of Th17 regulatory T cell. Chin. J. Rheumatology 16 (4), 224–228. Cover 3.

Xinwen, Ma., Zemin, Dong, Yong, Wang, et al., 2017. Influence of serum MK and Th17/regulatory T cell on generation and development of condition of patients with rheumatoid arthritis. Hainan Med. J. 28 (1), 2128–2130.

Xiang, Lu, Xiaochao, Wang, Yumin, Lu, et al., 2010. Study on expression of peripheral blood CD4+CD25 regulatory T cell and interleukin 17 and transforming growth factor β of patients with rheumatoid arthritis. Chin. Gen. Pract. 13 (30), 3355–3357.

Jian, Liu, Wei, Wan, Changjian, Sheng, et al., 2011. Study on correlation of pulmonary function change on rat with adjuvant arthritis with Th1/Th2 cell, regulatory T cell. Chin. J. Cell. Mol. Immunol. 27 (1), 56–60.

Wenxia, Hu., Jin, Yang, Xinxing, Yang, et al., 2016. Expression of helper T cell 17/regulatory T cell balance correlation factor and correlation with metaphase factor of serum. Chin. J. Rheumatology 20 (4), 224–228.

Lei, Wan, Jian, Liu, Chuanbing, Huang, et al., 2013. Correlation of pulmonary function reduction of rat with adjuvant arthritis with reduction of regulatory T cell and Foxp3 expression. Chin. J. Cell. Mol. Immunology 29 (3), 251–255.

Lei, Wan, Jian, Liu, Chuanbing, Huang, et al., 2013. Effect of xinfeng capsule on pulmonary function of rat with adjuvant arthritis and Treg, Foxp3, TGF-β1/Smads. J. Zhejiang Univ. (Med.) 42 (4), 418–425.

Zhiguo, Yin, Zhongjian, Cong, Yang, Cheng, et al., 2015. Comparative study on effect on mesenchyme stem cells on cartilage generation of serum PRG4 and TGF-β1 patients with infratemporal arthritis. Prog. Mod. Biomed. 15 (36), 7117–7120.