·BASIC RESEARCH·

Relationship of the occurrence and development of rheumatism arthritis with T lymphocyte subsets and CD_4^+ T_{H1}/T_{H2} cell functional subsets★

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Abstract

BACKGROUND: Rheumatoid arthritis (RA) is an autoallergic disease, but its immunological pathogenesis has not been completely known. T lymphocytes, especially CD_4^+ T_{H1}/T_{H2} cells, may have an important effect in the occurrence and development of RA.

OBJECTIVE: To investigate the action of CD_4^+ T_{H1} and T_{H2} cells which separately mediate cellular immunity and humoral immunologic response (respectively) function in the occurrence and development of RA.

DESIGN: Case-control, comparative observation.

SETTING: Department of Immunology, Medical College of Chinese People's Armed Police Forces.

PARTICIPANTS: Totally 15 patients with RA hospitalized in the Department of Internal Medicine of General Hospital of Tianjin Medical University between March 1999 and March 2000 were selected for a RA patient group, consisting of 2 males and 13 females. Of them, 12 patients whose serumal rheumatoid factor (RF)was positive and the three others' was negative. At the same time, 30 healthy individuals from persons receiving health examination in the hospital or from our department were selected for a healthy control group, consisting of 4 males and 26 females. Informed consents were obtained from the participants.

METHODS: ①CD_3^+T cells (total T cells), CD_4^+ T cells and CD_8^+ T cells in peripheral blood mononuclear cells(PBMC) from the two groups of subjects were detected by enzyme-linked immunospot assay (ELISPOT). 200-500 lymphocytes were counted under a normal light microscope, and the percentages of their positive cells were calculated. ②Activated TH cells which secreted cytokines were detected by ELISPOT, and those which had red spots in their plasma after staining were positive cells. Among them, the cells secreting γ-interferon (IFN-γ) were T_{H1} cells while those secreting interleukin-4 (IL-4) were T_{H2} cells. 200-500 lymphocytes were counted under a normal light microscope and the percentages of T_{H1} and T_{H2} cells and the ratio of T_{H1} cells to T_{H2} cells were calculated. ③ *t*-test and chi-square test were used for comparing statistical differences of data between the two groups.

MAIN OUTCOME MEASURES: Quantitative analysis of T lymphocyte subsets (including CD_3^+ T cells, CD_4^+ T cells and CD_8^+ T cells) and CD_4^+ T_{H1}/T_{H2} cells in peripheral blood from the two groups of subjects.

RESULTS: Analysis of the results was from the study on all of subjects including 15 patients suffering from RA and 30 healthy controls. ①The percentages of total T cells (CD_3^+ T cells), CD_4^+ T cells and CD_8^+T cells in peripheral blood were not significantly different between the two groups ($P > 0.05$). ②The percentage of T_{H1} cells in peripheral blood was significantly higher in RA patient group than that in healthy control group [(24.44±5.25)%, (14.93±3.82)%, $P < 0.05$],while in RA patient group the percentage of T_{H2} cells and ratio of T_{H1} cells to T_{H2} cells from peripheral blood were not significantly different as compared with those of control group($P > 0.05$).

CONCLUSION: Cellular immunologic function mediated by T_{H1} cells may be associated with the occurrence and development of RA.

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INTRODUCTION

Rheumatoid arthritis (RA)has been generally accepted as an autoallergic disease, but its immunological pathogenesis has not been completely known up to now. Previous studies identified the existence of autoantibodies targeting destructure IgG, that is, rheumatoid factor(RF), *in vivo* in RA patients and therefore the onset of RA was presumed to be associated with humoral immunologic response mediated by RF. In recent years, further studies on T lymphocyte subsets, especially on CD_4^+ T_{H1}/T_{H2} cell functional subsets, have been performed, and most results showed that the number of TH1 cells which mainly induced cellular immunity significantly increased *in vivo* in RA patients, which implicated that the occurrence and development of RA was associated with inflammatory injure induced by autoreactive TH1 cells [1] and also strongly suggested the complexity of pathogenesis of RA. In our experiment, T lymphocyte subsets and CD_4^+ T_{H1}/T_{H2} cell functional subsets in peripheral blood from RA patients were detected by enzyme-linked immunospot assay (ELISPOT) so as to investigate the effect of T lymphocyte subsets ,especially CD_4^+ T_{H1}/T_{H2} cell functional subsets, on the onset and development of RA.

SUBJECTS AND METHORDS

Subjects

Totally 15 patients with RA hospitalized in the Department of Internal Medicine in General Hospital of Tianjin Medical University between March 1999 and March 2000, were selected for a RA patient group, consisting of 2 males and 13 females, with average age of (44±16)years, average disease course of(18.4±12.9)years; and average blood sedimentation rate of (51.03±14.87)mm/h. In the 15 RA patients, 12 patients' serumal rheumatoid factor(RF) was positive and the other threes' was negative(Furthermore, after two weeks the three negative patients were detected again and their serum RF was still negative).Inclusion criteria: ①Conforming to the criteria of American College of Rheumatology for RA(revised in 1987). ② Not receiving any treatment of immunosuppressive drugs and hormone or hormonelike drugs for three months at least up to blood sampling. At the same time, 30 healthy controls who were from persons receiving health examination in the hospital or from our department were included in the control group, consisting of 4 males and 26 females with average age of(39±14) years. Informed consents were obtained from the participants.

Methods

Reagents: ELISPOT kits, monensiin, ionomycin and PMA were supplied by Institute of Clinical Examination, China-Japan Friendship Hospital. Ficoll-Hypaque was supplied by Institute of Hematic Disease, China Academy of Medical Sciences.

Detection of T lymphocyte subsets in peripheral blood: CD_3^+ T cells (total T cells), CD_4^+ T cells and CD_8^+ T cells in PBMC were detected by ELISPOT. The test was performed strictly according to the specification of ELISPOT kit. The cells with their members stained red were positive. 200-500 lymphocytes were counted under a nor-

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mal light microscope and the percentages of positive cells of them were calculated.

Detection of T_{H1} and T_{H2} cells in peripheral blood: PBMC were suspended in RPMI-1640 complete medium containing 100 g/L fetal calf serum and regulated to $1\times10^9L^{-1}$. The suspension was added at 1 mL/well into 24-well culture plate and 21μL monensiin, 10 μL ionomycin and 10 μL PMA were also added into every well. The cells were cultured at 37 ℃ in a CO_2 incubator to be activated. Six hours later, after centrifugation, the supernatants were threw away. Activated TH cells secreting cytokines in the incubated PBMC were detected by ELISPOT. The operation was performed strictly according to the specification of ELISPOT kit. The cells in which there were red spots in staining were positive. Among them, the cells expressingγ-IFN were T_{H1} cells and those expressing IL-4 were T_{H2} cells. 200–500 lymphocytes were counted under a normal light microscope and the percentages of positive T_{H1} cells and T_{H2} cells and the ratio of T_{H1} cells to T_{H2} cells were calculated.

Statistical analysis: Experimental data were collected and analyzed with SPSS 7.5 statistic software by the first author. Chi-square test was used for comparing statistical differences of data between the two groups.

RESULTS

Quantitative analysis of the participants

All of participants including 15 patients with RA and 30 healthy controls were collected for result analysis. None of them were left out

Statistical inference

The results of T lymphocyte subsets (Table 1)

Table 1 Comparison of percentages of T lymphocyte subsets in peripheral blood from RA patients and healthy individuals ($\bar{x}\pm s$,%)

Group	n	CD_3^+ T cell	CD_4^+ Tcell	CD_8^+ T cell
Rheumatoid arthritis patients	15	75.23±14.81	49.41±8.60	28.46±6.17
Health controls	30	68.45±11.02	45.31±10.34	24.60±5.45

The percentages of total T cells (CD_3^+ T cells), CD_4^+ T cells and CD_8^+T cells in peripheral blood were not significantly different on statistics between the two groups($P > 0.05$).

The results of T_{H1}/T_{H2} cells (Table 2)

Table 2 Comparison of percentages of T_{H1} and T_{H2} cells and ratio of T_{H1} to T_{H2} cells in peripheral blood between two groups ($\bar{x}\pm s$)

Group	n	T_{H1} cells(%)	T_{H2} cells(%)	T_{H1}/T_{H2} cells
Rheumatoid arthritis patients	15	24.44±5.25[a]	21.18±6.31	1.15
Health controls	30	14.93±3.82	18.22±4.09	0.82

[a]$P < 0.05$, *vs* control group

The percentage of T_{H1} cells in peripheral blood from RA patient group was significantly higher than that of control group [(24.44±5.25)%, (14.93±3.82)%, $P < 0.05$], while in RA patient group its percentage of T_{H2} cells and ratio of T_{H1} cells to T_{H2} cells in peripheral blood were not significantly different, as compared with those of control group($P > 0.05$).

DISCUSSION

Our experimental results showed that the percentages of total T cells (CD_3^+ T cells), CD_4^+ T cell and CD_8^+ T cells in peripheral blood from RA patient group were not significantly different on statistics, as compared with healthy control ($P > 0.05$). Such results may be associated with the physical constitution of patients included in RA group. As RA is a chronic systemic disease, most sufferers in this study had suffered from this disease for quite a longer time and their physical condition was worse. On the other hand, a part of them had accepted drug treatment of adrenal cortical hormone or immunodepressant. Although they had not used these drugs for three months at least before our study began, these factors may still probably resulted in some depressive effect on their immunologic function.

Our observation on T_{H1}/T_{H2} cells in the peripheral blood from RA patients showed that the percentage of T_{H1} cells in RA patient group was higher than that of the healthy control group ($P < 0.05$), but in RA patient group its percentage of T_{H2} cells and ratio of T_{H1} cells to T_{H2} cells were not significantly different, as compared with those of control group ($P > 0.05$). These results indicated that cellular immunity mediated by T_{H1} cells were associated with the pathogenesis of rheumatoid arthritis. Autoreactive T_{H1} cells can induce and maintain synovitis by immunologic response to autoantigens and finally result in damage of arthrodial cartilage. Furthermore, T cells existing in the tissue of synovial membrane can also secrete cytokines to affect further the activities of other immunocytes, for example, they can activate macrophages, *etc* [1,2]. T_{H2} cells can help with activation and proliferation of B cells, and enhance the production of antibodies , leading to up-regulation of humoral immunologic response mediated by antibodies. Many studies demonstrated that in the majority of RA patients there existed in vivo autoantibodies targeting degenerative IgG, namely rheumatoid factor (RF). Our study showed that of the 15 RA patients there were 12 patients whose rheumatoid factor (RF) was positive. In addition, histological studies also evidenced that in RA patients, besides many T lymphocytes and macrophages, a great quantity of plasma cells infiltrated into the inflammatory tissue of joint synovial membrane [3]. The above implicated that pathogenesis of RA was also related to humoral immunity, and at the same time strongly suggested the complexity of RA etiology. Our investigation indicated that the percentage of T_{H2} cells and the ratio of T_{H1} cells to T_{H2} cells from RA patients were a close approximation to those from healthy control. The cause of such results might be that in RA patients their concentrations of T_{H1}/T_{H2} cells in the peripheral blood could not exactly reflect the actual levels of T_{H1}/T_{H2} cells existing in inflammatory tissue of joint synovial membrane. Therefore, it can not be simply concluded that humoral immunity mediated by T_{H2} cells are not associated with the pathogenesis of RA.

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类风湿性关节炎的发生发展与外周血T淋巴细胞亚群及CD4+ T_{H1}/T_{H2} 细胞功能亚型的关系*

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摘要
背景:类风湿性关节炎作为一种自身免疫病其免疫学发病机制目前尚未完全明确。T淋巴细胞、尤其是CD4+ T_{H1}/T_{H2} 细胞在类风湿性关节炎发生发展中可能有重要作用。
目的:探讨主要参与细胞免疫的CD4+ T_{H1} 细胞和辅助体液免疫应答的 T_{H2} 细胞在类风湿性关节炎发生发展过程中的作用。

设计 :病例-对照,对比观察。
单位 :武警医学院免疫学教研室。
对象 :选择 1999-03/2000-03 在天津医科大学总医院内科就诊的类风湿性关节炎患者 15 例为患者组,男 2 例,女 13 例,其中 12 例受检者类风湿因子阳性,3 例为阴性。同期选取健康体检者或本单位工作人员健康者 30 人为对照组,男 4 人,女 26 人。纳入对象均对实验目的知情同意。
方法 :①采用酶联免疫斑点法对两组对象的外周血单个核细胞中 $CD3^+$ T 细胞(总 T 细胞)、$CD4^+$ T 细胞和 $CD8^+$ T 细胞进行检测,普通光学显微镜油镜下计数 200~500 个淋巴细胞,计算出阳性细胞的百分率。②采用酶联免疫斑点法检测活化的分泌细胞因子的 TH 细胞 :细胞内有红色斑点的细胞为阳性细胞,分泌 γ-干扰素的细胞为 T_{H1} 细胞,分泌白细胞介素 4 的细胞为 T_{H2} 细胞 ;普通光学显微镜油镜下计数 200~500 个淋巴细胞,计算出 T_{H1} 细胞、T_{H2} 细胞的百分率及 T_{H1}/T_{H2} 细胞的比值。③采用 t 检验或 χ^2 检验比较数据间差异性。
主要观察指标 :两组对象外周血 T 淋巴细胞亚群 (包括 $CD3^+$T 细胞、$CD4^+$ T 细胞、$CD8^+$ T 细胞)及 $CD4^+$ T_{H1}/T_{H2} 细胞定量分析结果。
结果 :类风湿性关节炎患者 15 例和健康者 30 人均进入结果分析。①两组外周血的总 T 细胞(即 CD_3^+ T 细胞)、$CD4^+$ T 细胞及 $CD8^+$ T 细胞的百分率差异不明显($P > 0.05$)。②患者组外周血的 T_{H1} 细胞的百分率明显高于对照组[(24.44±5.25)%,(14.93±3.82)%, $P < 0.05$] ;而其外周血 T_{H2} 细胞的百分率及 T_{H1}/T_{H2} 细胞的比值与对照组相近($P > 0.05$)。
结论 :T_{H1} 细胞介导的细胞免疫可能与类风湿性关节炎的发生发展有关。
主题词 :关节炎, 类风湿 ;T 淋巴细胞亚群 ;免疫,细胞
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·TRADITIONAL CHINESE MEDICINE FOR REHABILITATION·

Observation of effects of *tiaozhi zengshou tang* on regulation of dyslipidemia★

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Abstract

BACKGROUND: Dyslipidemia is the important risk of cardiac cerebral vascular diseases, such as arthrosclerosis and coronary heart disease, *etc*.

OBJECTIVE: To observe the clinical therapeutic effects of *tiaozhi zengshou tang* (herbal decoction) on intervention of dyslipidemia.

DESIGN: Randomized, group controlled observation.

SETTING: Department of Oncology, Department of Chinese Medicine and Department of Cardiology of General Hospital of Chinese PLA.

PARTICIPANTS: Totally 120 cases from inpatients with dyslipidemia in Department of Chinese Medicine and Department of Cardiology of General Hospital of Chinese PLA and outpatients in Clinic of Blood lipid from February 2002 to January 2004.

METHODS: Totally 120 cases of dyslipidemia were randomized into 3 groups. In Chinese herb group (43 cases), *tiaozhi zengshou tang* (herbal decoction) was prescribed, one dose/d, taking separately before breakfast and dinner. In western drug group (40 cases), pravastatin sodium was prescribed, 10 mg/tablet, 1 tablet/d, taking orally before sleep at night. Integrative group (37 cases) both pravastatin sodium and herbal decoction were prescribed, the dosage, composition and administration were same as previous. The treatment lasted 8 weeks, and then, the therapeutic effects on changes of blood lipid and harmful effects were observed.

MAIN OUTCOME MEASURES: Comparisons of therapeutic effects and harmful effects among groups.

RESULTS: 120 cases were employed in the experiment, but 27 of those were dropped out due to loss of contact and absent re-visiting in time. Total ly, the rest 93 cases have all accomplished the datum collection. ① Comparison of therapeutic effects among groups: The effective rates in Chinese herb group and western drug group were similar (81%, 80%, $P > 0.05$), but all less than integrative group (97%, $P < 0.05$). ② Comparison of changes of blood lipid series before and after treatment in each group: In Chinese herb group, triglyceride (TG) (2.59±1.64) mmol/L was reduced to (1.56±0.72) mmol/L, serum total cholesterol (TC) (5.30±1.71) mmol/L was reduced to (4.35±0.85) mmol/L ($P < 0.01$) and HDL-Cholesterol (HDL-Ch) (1.32±0.37) mmol/L was increased to (1.50±0.22) mmol/L ($P < 0.05$). In western drug group, TG (2.84±1.50) mmol/L was reduced to (2.04±0.98) mmol/L, serum TC (5.50±1.22) mmol/L was reduced to (4.71±0.89) mmol/L ($P < 0.05$, $P < 0.01$) and $APoA_1$ (1.24±0.21) g/L was increased to (1.49±0.15) g/L ($P < 0.01$). In integrative group, the therapeutic effects were significant in reducing TG, TC, LDL-Ch and APoB and increasing $APoA_1$ and HDL-Ch ($P < 0.01$, $P < 0.05$). ③ Harmful accident and side effect: The cases with harmful effects in Chinese herb group were less than western drug group (1 case, 7 cases).

CONCLUSION: *Tiaozhi zengshou tang* provides definitely clinical therapeutic effects in dyslipidemia and presents low incident of harmful effects.

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INTRODUCTION

Dyslipidemia is the important risk of cardiac cerebral vascular diseases, such as arthrosclerosis (AS) and coronary heart disease, *etc*. To decrease blood lipid can retard the progression of AS [1,2], further reduce incidence and mortality of cardiac cerebral vascular diseases so as to prolong the life of patient. It is discovered in I-grade prevention of coronary heart disease that to reduce total cholesterol (TC) decreases the risk of coronary heart disease correspondently at 1:2 [3]. This paper was to observe the clinical therapeutic effects of *tiaozhi zengshou tang* (herbal decoction) on dyslipidemia.

SUBJECTS AND METHODS

Subjects

All of cases were collected from inpatients with dyslipidemia in Department of Chinese Medicine and Department of Cardiology of General Hospital of Chinese PLA and outpatients in Clinic of Blood lipid from February 2002 to January 2004. Inclusion criteria: to tally with the diagnostic criteria on the disease issued in *Principle for the Prevention of Dyslipidaemia* [4] and American Education Plan of Cholesterol in Adults [5]. Exclusion criteria: ① Severe cardiac cerebral vascular disease, disorders of liver and gallbladder, renal syndrome or functional damage of liver and kidney, diabetes, hypothyroidism, chronic pancreatitis, thrombocytopenia, cancer, deep vein thrombosis (DVT) and having taking drugs for blood lipid metabolism recently. ② To receive other medications for lowing lipid at same period. Totally 120 cases were tallied with the criteria, which was randomized into 3 groups, Chinese herb group (n=43), western drug group (n=40) and integrative group (n=37).