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Inhibitory effect of triptolide on production of IL - 1β from PBMC is associated with IL - 1β gene polymorphism*

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[**ABSTRACT**]　**AIM**: To explore whether the inhibitory effect of triptolide on IL - 1β production by PBMC is associated with IL - 1β gene polymorphisms. **METHODS**: IL - 1β gene polymorphism was analyzed in 31 healthy volunteers. From genomic DNA, the C - T polymorphism at IL - 1β - 511 was typed by PCR - RFLP. Meanwhile the IL - 1β was also measured in the supernatants of the cultured and stimulated peripheral blood mononuclear cells (PBMC) by ELISA. **RESULTS**: After LPS stimulation in PBMC cultures of healthy subjects, the secretion levels of IL - 1β in 9 volunteers who carried IL - 1β - 511 T/T genotype were higher than in volunteers who are not T/T genotype ($P < 0.05$). Triptolide suppressed the production of IL - 1β significantly in LPS - treated human PBMC carried C/C and C/T genotype ($P < 0.05$), but this significant inhibitory effect of triptolide was not seen in T/T genotype ($P > 0.05$). **CONCLUSION**: The gene polymorphism at IL - 1β - 511 was related to the production of IL - 1β, and the inhibitory effect of triptolide on the production of IL - 1β was different in C/C, C/T, T/T genotype of IL - 1β - 511, which may be one of the reasons for the phenomenon that people respond differently to triptolide.

[**KEY WORDS**]　Interleukin - 1; Genes; Polymorphism(Genetics); Tripterygium; Arthritis, rheumatoid

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IL - 1β is the main proinflammatory cytokine in the pathogenesis of rheumatoid arthritis (RA). It plays a key role in the inflammation of joints, proliferation of synovium, erosion and destruction of cartilages and bones. IL - 1 receptor antagonist is one of the newly important agents to treating patients with RA that significantly improves the signs and symptoms of the disease, and reduces joint destruction[1]. As a disease modifying antirheumatic drug (DMARD), traditional Chinese herb Tripterygium wilfordii Hook F (TWHF) has been used to treat RA for hundreds of years and ameliorate many rheumatisms including RA. One of the mechanisms of its efficacy on RA is that TWHF can inhibit the expression of proinflammatory cytokines such as IL - 1β, TNFα, and recover the balance of cytokine network[2]. Many patients with RA, however, respond differently to TWHF. What is the genetic background of this clinical phenomenon? Whether does it correlate with the gene polymorphisms of IL - 1β? So, we conducted this experiment, and reported as follows.

MATERIALS AND METHODS

1　Experiment materials

1.1　Subjects　Thirty - one healthy adults of the Han nationality were selected in random, including 17 men and 14 female. The mean age was 33.10 years (the range between 21 and 50 years). These subjects did not have any chronic illness and were not affected by any acute medical problem at the time of the study.

1.2　Main reagents　PUREGENE® DNA purification kit for whole blood (Gentra systems), AVA Ⅰ restriction endonuclease (New England Biolabs), lymphocyte cell separation buffer (Tianjin Haoyang Biological manufacture Co. Ltd.), RPMI - 1640 culture medium (Hyclone), lipopolysaccharide (LPS) (Sigma), triptolide (TP) (Institute of Medical Science of Fujian

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Province, purity 99.99%), adenosine triphosphate (ATP) (Sigma), Endogen® human IL-1β ELISA kit (Pierce), PCR primers were synthesized by Shanghai Biotechnology Company Limited.

1.3 Main instruments UV1101 nucleic acid analysis instrument (Biometra, Germany), PCR amplification instrument (MJ Research, America), gel documentation and gel imaging systems (UVP, Britain), super clean bench (Suzhou Antai Air Technique Company Limited, China), CO_2 incubator (SHEL-LAB®, America), automated microplate reader (Bio-Tek Instruments, America), inverted microscope, supercentrifuge and so on.

2 Experimental methods

2.1 Preparation of genomic DNA Genomic DNA was extracted from 1 mL heparinized venous blood by DNA purification kit for whole blood according to the information sheet. The concentration and purity of DNA were detected by nucleic acid analysis instrument.

2.2 Analysis of IL-1β gene polymorphism There is a single base pair polymorphism at position -511 in the promoter region of IL-1β gene[3]. The genotype was analyzed by PCR-RFLP. A 304 bp PCR fragment of the IL-1β promoter region was amplified using the following primers: 5'-TGG CAT TGA TCT GGT TCA TC-3' and 5'-GTT TAG GAA TCT TCC CAC TT-3'. The amplification reaction system was 50 μL totally, containing genomic DNA 1 μg, dNTP 200 μmol/L, each primer 0.5 μmol/L, respectively, Taq DNA polymerase 2.5 U, $MgCl_2$ 2 μmol/L, 10× PCR buffer. PCR conditions were as follows: denaturation at 95 ℃ for 7 min, then 35 cycles at 94 ℃ for 30 s, at 55 ℃ for 30 s, at 72 ℃ for 1 min, and final extention at 72 ℃ for 5 min. 10-15 μL of PCR products was digested with 5 U of AVA Ⅰ at 37 ℃ for 3 h and ran on a 20 g/L agarose gel stained with ethidium bromide. AVA Ⅰ digestion of the PCR products of IL-1β resulted in three genotypes, consisting of T/T (intact, 304 bp), C/T (three fragments of 304, 190 and 114 bp) and C/C (two fragments of 190 and 114 bp) (Fig 1).

2.3 Separation and culture of PBMC Venous blood (6 mL) from healthy volunteers was collected in sterile heparinized tubes. In super clean bench, PBMC were purified within 2 hours of collection by density gradient centrifugation, washed and suspended in RPMI-1640 supplemented with 10% heat-inactived fetal bovine serum, 1×10^5 U/L penicillin and streptomycin, and adjusted to 2×10^9 cells/L. Then they were distributed in 96-well culture plates equivalently, and incubated at 37 ℃ with 5% CO_2 for 24 h. Subsequently LPS was added at a final concentration of 1 mg/L, meanwhile half of the wells were treated by triptolide at a final concentration of 5.4 μg/L, and RPMI-1640 was added to each well and the final volume was 200 μL per well. The cells were maintained in incubator for another 46 h. Finally ATP was added at a final concentration of 6 mmol/L. After 2 h, the samples were centrifuged at 2 000 r/min for 20 min. Supernatants were collected and frozen at -70 ℃.

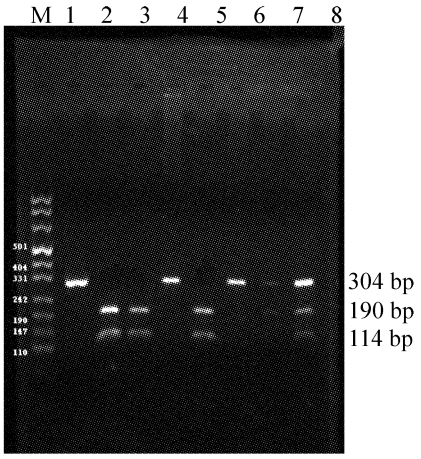

Fig 1 Results of IL-1β-511 gene typing. Lane M: DNA molecular mass marker; Lane 1, 4, 6: T/T; Lane7, 8: C/T; Lane 2, 3, 5: C/C.

2.4 Detection of IL-1β IL-1β in supernatants were detected by human IL-1β ELISA kit according to the manufacturer's instructions.

2.5 Statistical analysis The data were expressed as the mean ± standard deviation ($\bar{x} \pm s$). According to the characteristics of data, analysis of variance and nonparametric test were used to compare the discrepancies of different groups.

RESULTS

1 The distribution of genotype Among 31 subjects, there were ten homozygotes for C/C genotype, twelve heterozygotes for C/T genotype, and nine homozygotes for T/T genotype. These subjects were divided

into three groups according to their genotype of IL – 1β –511.

2 The influence of different genotype on IL – 1β production from PBMC stimulated by LPS After LPS stimulation, the secretion levels of IL – 1β in PBMC of 9 volunteers who carried IL – 1β – 511 T/T genotype were higher than volunteers who are not T/T genotype (Tab 1).

3 The influence of triptolide on IL – 1β production by PBMC induced with LPS In general, IL – 1β production from PBMC treated by LPS and triptolide decreased obviously than that from PBMC treated by LPS only ($P < 0.05$). The variation of IL – 1β production from T/T homozygote genotype PBMC treated with triptolide and without triptolide was not significantly different ($P > 0.05$), but that in C/C and C/T genotypes changed greatly ($P < 0.05$) (Tab 1). Triptolide could lower the levels of IL – 1β by 31.05%. In three groups, the values were 63.19% in C/C genotype, 47.23% in C/T genotype, but only 8.10% in T/T genotype. These statistical data suggested that the inhibitory effect of triptolide on the production of IL – 1β by PBMC was obviously weak in T/T genotype.

Tab 1 The influence of triptolide on IL – 1β production by PBMC induced with LPS ($\bar{x} \pm s$)

Group	n	IL – 1β (ng/L)	
		LPS	LPS + TP
C/C genotype	10	69.92 ± 66.45$^{\triangle}$	25.74 ± 19.59*
C/T genotype	12	71.75 ± 73.54$^{\triangle}$	37.80 ± 43.25*
T/T genotype	9	176.61 ± 127.00	162.31 ± 161.17
Total	31	101.60 ± 99.94	70.06 ± 106.55*

$^{\triangle}P < 0.05$ *vs* T/T genotype; $^{*}P < 0.05$ *vs* corresponding LPS treated class.

DISCUSSION

Genetic factors play a key role in the course of RA. HLA – DR gene polymorphisms is studied extensively in RA, but it dose not explain the total genetic background of this disease. More and more evidences indicate that the gene polymorphisms of some cytokines that play key roles in RA may be more important in determining the course of RA. Among these cytokines, IL – 1β is the main proinflammatory cytokine in the pathogenesis of RA. It can lead to the inflammation of synovium, the erosion and destruction of cartilages and bones[1]. There is a C – T gene polymorphism at position – 511 in the promoter region of IL – 1β gene[3], which is associated with several diseases. Pan et al[4] reported that the patients with IL – 1β – 511 allele T/T had a higher swollen joint index, higher tender joint index and erythrocyte sedimentation rate than those without IL – 1β allele T/T. And the levels of IL – 1β in supernatants of stimulated PBMC from patients with IL – 1β allele T/T were higher than those from patients without allele T/T. Further studies show that the presence of IL – 1β allele T/T contributes to the expression of IL – 1β mRNA[5], and it may predict the severity and prognosis of RA. For TWHF can inhibit the gene expression and production of IL – 1β[2], the gene polymorphisms of IL – 1β may influence the therapeutic effect of TWHF. The aim of this study is to demonstrate our hypothesis by observing the effect of triptolide (a main active component of TWHF) on the IL – 1β production from PBMC stimulated by LPS *in vitro*.

Unlike most secreted proteins, the release of IL – 1β is an accurately regulated process that requires induced synthesis of the precursor pro – IL – 1β and a second stimulus that initiates cleavage and secretion of mature IL – 1β. Cultured monocytes and macrophages stimulated with LPS produce large quantities of 34 kD proIL – 1β, but release little mature 17 kD cytokine to the medium. Moreover, monocytes that were cultured overnight prior to LPS treatment released no 17 kD cytokine. Through activation of the P2X7 receptor, extracellular ATP can cause monocytes to release mature 17 kD cytokine[6,7]. So we used LPS /ATP two – step treating method to promote the secretion of IL – 1β, which helped us observe the effects of triptolide on the synthesis of IL – 1β.

In the present study, it showed that the levels of IL – 1β in supernatants of PBMC from volunteers with IL – 1β – 511 T/T genotype were higher than those from volunteers without allele T/T, which was in line with a previous study by Pan Yunfeng, et al. Triptolide suppressed the production of IL – 1β significantly in LPS – treated human PBMC carried C/C and C/T genotype, but this significantly inhibitory effect of triptolide was not seen in T/T genotype, suggesting that the inhibitory

effect of triptolide on IL－1β production by PBMC from healthy subjects was weak obviously in T/T genotype. Recent studies showed that IL－1β－511 C－T polymorphism was located within the promoter region of IL－1β gene and resulted in the loss of a putative AP－2 binding site. In addition, there was significant linkage disequilibrium between the IL－1β－511 C－T polymorphism and－31 T－C polymorphism which resulted in a loss of first T of the TATA box, and influences the formation of transcriptional initiation complex[8,9]. We can infer that the reasons why the inhibitory effect of triptolide on IL－1 β production from cultured PBMC was weak obviously in T/T genotype are associated with the triptolide's effects on AP－2 and the formation of transcriptional initiation complex. With the further study on TWHF, the specific way of this effect may be clarified.

Traditional Chinese herb TWHF has reliable therapeutic effect on RA in clinical practice, and plays an important role in the treatment of RA. But its clinical utilization is seriously limited because of the individual difference, its toxicity and side effects[10]. How to use TWHF correctly and how to utilize its virtue, avoid its defect, and exert its unique therapeutic effects is the most important thing for us. The level of IL－1β in serum is closely associated with the activity and the morphological feature of RA[11]. And we found that the inhibitory effect of triptolid on IL－1β production was different in C/C, C/T, T/T genotype of IL－1β－511. Therefore, it is reasonable to think that the therapeutic effects of TWHF on RA may be different in people with various genotype of IL－1β. Our experiment supported that people who carried T/T genotype at IL－1β－511 might achieve unsatisfactory therapeutic result of TWHF in comparison with other genotypes.

From the point of pharmacogenomics, our study revealed firstly that the IL－1β－511 C－T polymorphism might be one of the reasons why the therapeutic effects of triptolide on RA were different in different people. But this conclusion was found *in vitro*, and the experiment conditions couldn't simulate the real states of patients with RA completely. Our conclusion needs to be demonstrated by large clinical trials in future.

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雷公藤甲素抑制 PBMC 分泌 IL－1β 蛋白量与 IL－1β 基因多态性有关*

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［摘　要］ **目的**：探讨雷公藤甲素对外周血单个核细胞（PBMC）分泌白细胞介素－1β（IL－1β）的抑制作用与 IL－1β 基因多态性的关系。**方法**：采用 PCR－RFLP 法对 31 名健康志愿者 IL－1β 基因启动子区－511 位点 C－T 多态性进行基因型检测，同时进行 PBMC 培养，用脂多糖（LPS）刺激培养细胞，并予以雷公藤甲素处理 PBMC，收集培养上清液，ELISA 法检测上清液中 IL－1β 的含量。**结果**：携带 IL－1β－511T/T 纯合子基因型 PBMC 经 LPS 刺激后 IL－1β 的分泌量明显较非 T/T 纯合子为高（$P<0.05$）；雷公藤甲素能显著抑制 LPS 诱导的 C/C 和 C/T 基因型 PBMC 分泌 IL－1β（$P<0.05$），但对 T/T 基因型的抑制作用不明显（$P>0.05$）。**结论**：IL－1β 基因－511C－T 多态性与 IL－1β 的分泌量相关，雷公藤甲素对不同基因型的 IL－1β 抑制作用有差异，这可能是导致雷公藤药理作用出现个体差异的原因之一。

［关键词］ 白细胞介素 1；基因；多态现象（遗传学）；雷公藤属；关节炎，类风湿
［中图分类号］ R363　　［文献标识码］ A

2007 年全国老年周围动脉硬化疾病防治专题研讨会征文通知

周围动脉硬化疾病（PAD）是近年来发展迅速的疾病之一，其发生、发展及病变涉及多学科多领域。由中华医学会老年医学分会与《中华老年医学杂志》编辑委员会主办，山东大学齐鲁医院承办的老年周围动脉硬化疾病专题研讨会将于 2007 年 4 月 20～23 日在济南市举行。大会将邀请多位权威专家到会作报告。参会者可获国家级继续医学教育Ⅰ类学分 8 分，优秀论文将发表在《中华老年医学杂志》2007 年第 5 期。竭诚欢迎内科、介入科、血管外科、检验科、影像科及基础研究等相关领域研究的同道们参加并踊跃投稿。

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