·Original Article in English·

Effect of mesangial cell-derived interleukin-13 on expression of cytokines synthesized by human mesangial cells

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Abstract: Objective This study aims to investigate the interleukin-13 (IL-13) expression in the human mesangial cells (HMC) and its effect on expressions of cytokines synthesized by HMC so as to study the role of IL-13 in the inflammatory process of glomerulonephritis. **Methods** The HMC were cultured and treated with LPS and/or recombinant human IL-13. The IL-13 mRNA expression and the IL-13 protein level in the cultured HMC were detected by semiquantitative reverse transcription polymerase chain reaction (RT-PCR) and enzyme-linked immunosorbent assay (ELISA) respectively. The effects of IL-13 on the expressions of proinflammatory cytokines, chemokines and profibrogenic cytokine in the HMC were determined by ribonuclease protection assay (RPA). The cultured HMC without LPS or recombinant human IL-13 stimulation were used as the controls. **Results** The IL-13 mRNA expression and IL-13 protein were undetected in the controls without stimulation. The IL-13 mRNA expression in the HMC with LPS stimulation was induced as early as 12 hrs after LPS stimulation, reached a peak at 48 hrs and remained high level until 72 hrs. The activation of HMC with LPS resulted in the induction of IL-13 mRNA expression in a dose and time dependent way. The IL-13 protein was induced 24 hrs after LPS stimulation and was further increased with stimulation time. The recombinant IL-13 inhibited TNF-α, IL-1α, IL-1β, MCP-1, IL-8 and TGF-β1 mRNA expressions were induced by LPS in a dose-dependent way. The expressions of TNF-α, IL-1α, IL-1β, MCP-1, IL-8, and TGF-β1 mRNA were increased after endogenously produced IL-13 was neutralized with anti-IL-13 mAb. **Conclusions** LPS can induce the IL-13 expression in HMC. The mesangial cell-derived IL-13 can inhibit the production of proinflammatory cytokines, chemokines, and profibrogenic cytokine synthesized by HMC. **[Chin J Contemp Pediatr, 2004, 6(2): 85-88]**

Key words: Mesangial cell; Interleukin-13; Inflammation

系膜细胞来源的白细胞介素-13 对系膜细胞细胞因子基因表达的研究

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[摘 要] **目的** 白细胞介素-13(IL-13)是新近发现的一种抗炎性细胞因子,其在肾小球肾炎中的作用尚不清楚,该研究探讨脂多糖(LPS)对体外培养的人肾小球系膜细胞(HMC)表达 IL-13 作用以及 IL-13 对 HMC 促炎性细胞因子、趋化因子和促纤维化因子基因表达的影响。**方法** 体外培养 HMC,加入不同浓度的 LPS 和(或) IL-13 后,用逆转录-聚合酶链反应和 ELISA 检测 HMC IL-13 mRNA 表达和细胞培养上清液中 IL-13 蛋白含量;应用核酸酶保护法检测 HMC 肿瘤坏死因子-α(TNF-α)、白介素-1α(IL-1α)、白介素-1β(IL-1β)、单核细胞趋化蛋白-1(MCP-1)、白介素-8(IL-8)、转化生长因子-β1(TGF-β1) mRNA 的表达。**结果** 未予 LPS 刺激的 HMC 不表达 IL-13 mRNA 和蛋白;LPS 呈剂量依赖性和时间依赖性诱导 HMC 表达 IL-13 mRNA 和分泌 IL-13 蛋白。HMC 受 LPS 刺激后 12 h 即可表达 IL-13 mRNA,48 h 达高峰,72 h 仍维持在较高的水平。HMC 受 LPS 刺激后 24 h,其培养上清液中检测到 IL-13 蛋白,48 h 和 72 h 进一步增加。外源性 IL-13 呈剂量依赖性地抑制 LPS 诱导的系膜细胞 TNF-α,IL-1α,IL-1β,MCP-1,IL-8,TGF-β1 mRNA 的表达。应用抗 IL-13 抗体中和内源性 IL-13 后,上述炎症因子

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表达增强。**结论** IL-13 是 HMC 自分泌因子。IL-13 可抑制 LPS 诱导的系膜细胞促炎性细胞因子、趋化因子和促纤维化因子的表达，提示自分泌和旁分泌的 IL-13 对于肾小球疾病状态下肾脏系膜细胞的炎症反应具有抑制作用。

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[**关 键 词**] 系膜细胞；白细胞介素-13；炎症

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It has been demonstrated that mesangial cells with LPS or immune complexes stimulation can secrete newly synthesized IL-1, IL-6, IL-8, TNF-α and colony-stimulating factors. These cytokines are important in amplifying the glomerulus inflammatory reaction[1]. IL-13, a novel lymphokine, is isolated from activated human T lymphocytes and is expressed in a variety of cells. It exhibits both anti-inflammatory and immuno-regulatory properties. It can inhibit production of macrophage inflammatory proteins such as IL-1, IL-6, IL-8, macrophage inflammatory protein (MIP)-1, TNF-α and enhance the production of IL-1 receptor antagonist[1]. This study aims to investigate whether LPS can induce human mesangial cells (HMC) to produce IL-13 and to explore the effect of IL-13 on the expression of cytokines synthesized by HMC.

Materials and methods

Reagents

RPMI 1640, HEPES, fetal calf serum (FCS) and TRIzol reagent were provided by Gibco BRL (USA). LPS from Escherichis coli 026:B6, insulin, transferrin and the collagenase type IV were provided by Sigma (USA). Cell culture flasks were provided by Nunc (Denmark). The rat anti-IL-13 monoclonal antibodies and human multi-probe template set were provided by PharMingen (Canada). Oligo $(dT)_{15}$, dNTPs, AMV, and Taq polymerase were provided by Promega (USA).

Human mesangial cells culture

Human fetal kidneys with gestational ages from 4 to 6 months were obtained after therapeutic abortions. The mesangial cells were cultured in RPMI 1640 buffered with 10 mmol/L of HEPES to pH 7.4, and supplemented with 20 % FCS, 5 μg/ml of insulin and transferrin, 100 U/ml of penicillin, and 100 mg/ml of streptomycin at 37 ℃ in a humidified environment with 5 % CO_2. The third to fifth passage cells were used in this study.

The cells with a density of 1×10^6 cells/flask were cultured in 75 cm^2 flasks with 20 % FCS medium until approximately 80 % of these cells were confluent. These cells were then incubated with 0.5 % FCS medium for 48 hours to make them quiescent. The cultured cells were divided into the Control group and the Experiment group. The Experiment group was incubated with a 1640 medium containing 20 % FCS. Various concentrations of LPS (1, 10 and 100 μg/ml) were added to the medium for detecting the IL-13 mRNA expression induced by LPS in some of the Experiment group; some of the Experiment group were pretreated with recombinant human IL-13 for 30 minutes before LPS inducement in order to evaluate the effect of IL-13 on cytokines; and a combination of LPS and anti-IL-13 antibody were added to some of the Experiment group for detecting the effects of IL-13 endogenously produced by HMC on cytokins expressions. The Control group had no treatment.

RNA extraction

The total RNA of cultured HMC was extracted by an acid guanidinium thiocyanate-phenol-chloroform extraction procedure according to the instructions of the TRIzol reagent. The RNA extraction was dissolved in 20 - 30 μl of diethylpyrocarbonate-treated water.

Detection of IL-13 mRNA expression

The IL-13 mRNA expression was detected by a semiquantitative RT-PCR according to the protocol. PCR was performed on a thermocycler (PE Cetus, CT) under the following conditions: denaturation at 94 ℃ for 5 minutes, 92 ℃ for 30 seconds, 56 ℃ for 30 seconds, and 72 ℃ for 1 minutes, with 30 cycles. The downstream primer sequence of the internal control, β-actin, was 5′-CTACAATGAGCTGCGTGTTGGG-3′ and the upstream primer sequence was 5′-CAGGTCCAGACGCAGGATGGC-3′. The downstream primer sequence of IL-13 was 5′-CAGTTGAACCGTCCCTGCCG-3′ and the upstream

primer sequence was 5′-CCCA GAACCA-GAAA GGCTCCG-3′. The fragment size of β-actin and IL-13 was 206 bp and 279 bp respectively. The IL-13 and β-actin amplified products (20 μl repectively) were eletrophoresed on a 2 % agarose gel stained with ethidium bromide. The densitometric quantification of the PCR-amplified products was expressed in arbitrary units as the ratio of IL-13/β-actin levels.

Detection of IL-13 protein

The level of IL-13 protein in the culture supernatants of the HMC was detected using a quantitative sandwich enzyme immunoassay technique according to the instructions in the IL-13-specific ELISA kit. The lower limit of IL-13 protein was 7 pg/ml. All samples were measured in duplicate.

Riboprobe Synthesis

The multi-probe template set contained DNA templates, which can hybridize with target human mRNAs encoding TNF-α, IL-1α, IL-1β, MCP-1, IL-8, TGF-β1, and housekeeping gene products GAPDH, were transcribed by incubating with T7 polymerase and (α-α 32P) UTP to the cRNA probes with a mark of ^{32}P according to the protocol.

Detection of cytokins expressions

The expressions of the TNF-α, IL-1α, IL-1β, MCP-1, IL-8 and TGF-β1 were determined by ribonuclease protection assay (RPA) according to the protocol. The amount of 5×10^5 cpm of the cRNA probes were hybridized with 20 μg total RNA at 56 ℃ overnight, and were then digested with RNase A/T1. After phenol/chloroform extraction and ethanol precipitation, the protected fragments were separated on a denaturing 10 % polyacrylamide/urea sequencing gel and were visualized by autoradiography at - 80 ℃ for one to three days. Results were quantified with an image analyzer (UVP). The densitometric quantitication of cytokines mRNA was expressed in arbitrary units as the ratio of cytokines/GAPDH levels. The amount of 6.0 μg yeast RNA was used as the controls without TNF-α, IL-1α, IL-1β, MCP-1, IL-8, TGF-β1, and GAPDH expressions.

Statistical analysis

All data were expressed as $\bar{x} \pm s$. One-way ANOVA was used to analyze the difference with SPSS 10.0 software.

Results

Expression of IL-13 mRNA

The expression of IL-13 mRNA was undetected in the Control group without LPS stimulation. It was induced as early as the 12th hour after HMC were activated with LPS, reached a peak level at the 48th hour, and remained a high level until the 72nd hour (Figures 1A and 1C). The IL-13 mRNA expression of the HMC increased as an increase of the LPS concentration (Figures 1B and 1D).

Effects of LPS on the level of IL-13 protein

The level of IL-13 protein was undetected in the supernatant of the controls without LPS stimulation. However activation of HMC with LPS resulted in an increase of IL-13 protein level in a dose and time-dependant way (Figure 2).

Effects of IL-13 on the proinflammatory cytokines, chemokines and profibrogenic cytokine mRNA expression

The HMC cultured by 1640 medium containing 20 % FCS without LPS stimulation were not found to express IL-1α and MCP-1 mRNA but they expressed TNF-α, IL-1β, IL-8, and TGF-β1. The expression of the TNF-α, IL-1α, IL-1β, MCP-1, IL-8, and TGF-β1 mRNA in the HMC increased significantly after LPS stimulation ($P < 0.01$). IL-13 had no effect on the expressions of these cytokines in the HMC without stimulation but it decreased significantly in the expressions of TNF-α, IL-1α, IL-1β, MCP-1, IL-8, and TGF-β1 mRNAs in the HMC induced by LPS in a dose-dependent way (Figure 3).

Effects of endogenous IL-13 on the proinflammatory cytokines, chemokines and profibrogenic cytokine mRNA expressions

The expressions of TNF-α, IL-1α, IL-1β, MCP-1, IL-8, and TGF-β1 mRNA in the HMC with LPS and anti-IL-13 mAb stimulations were higher than those in the HMC stimulated with LPS alone (Figure 4).

Discussion

It has been reported that the expression of IL-13 mRNA and the production of IL-13 protein in periph-

eral blood mononuclear cells (PBMC) are increased significantly in childhood nephrotic syndrome[2]. In this study, it was shown that the IL-13 mRNA expression was associated with IL-13 protein production. The LPS induced IL-13 synthesis in a dose-dependent way and the maximal production of IL-13 occurred at the highest dosage of LPS. The IL-13 mRNA expression in the HMC was induced as early as 12 hours after LPS stimulation, reached a peak at 48 hours and remained at high level until 72 hours. It is suggested that LPS could induce HMC to synthesize and release IL-13.

The anti-inflammatory properties of IL-13 are well recognized. In vitro, IL-13 can inhibit the production of proinflammatory cytokines by activating macrophages and monocytes, can inhibit the expression of the inducible isoform of nitric oxide synthase (iNOS) and cyclooxygenase-2 and prostaglandin E_2 (PGE_2) produced by mesangial cells and inhibit vascular permeability factor released by PBMC from patients with lipoid nephrosis[1,3,4]. In vivo, IL-13 can protect animals from LPS-induced lethal endotoxemia and from IgG immune complex-induced lung injury[5]. In these models, the protective effects of IL-13 were associated with reduced production of proinflammatory cytokines. In this study, it was shown that human IL-13 strongly inhibited IL-1α, IL-1β, TNF-α, IL-8, MCP-1 and TGF-β1 expressions of LPS-induced HMC in a dose-dependent way. IL-13 with the concentration of 100 ng/ml reduced IL-β1, IL-8, MCP-1, and TGF-β1 synthesis by more than 80%. The inhibitory effects on IL-1α and TNF-α synthesis were somewhat less. These results were in agreement with the negative modulatory effect of IL-13 on the synthesis of IL-1 and TNF observed in human monocytes[1]. However, it has been reported that IL-13 can increase IL-8 and MCP-1 expressions in human bronchial epithelial cells and human vascular smooth muscle cells and that it had no effect on MCP-1 secretion in myelomonocytic cells[6]. It is suggested that cell type specificity is critical in interpreting the effects of LPS stimulation.

The ability of neutralizing the IL-13 activity of the anti-human IL-13 antibody has been confirmed previously[7]. In this study, it was shown that even when the production of IL-13 by LPS-stimulated HMC was low, it was sufficient to exert autoregulatory effects on the proinflammatory cytokines synthesized by these HMC. The expression of cytokines mRNA in the HMC cultured with LPS and anti-IL-13 mAb was higher than that in the HMC cultured with LPS alone. It is suggested that HMC could express IL-13 after immune induction or non-immune injury and that, in turn, IL-13 can inhibit synthesis and release of proinflammatory cytokines, chemokines and profibrogenic cytokine.

In summary, the data obtained in this study demonstrated that LPS can induce the expression of IL-13 in HMC, and that IL-13 can inhibit the production of proinflammatory cytokines, chemokines, and profibrogenic cytokine synthesized by HMC. IL-13 produced by HMC may play an important anti-inflammatory role in the inflammatory process of glomerulonephritis.

(Figures are on the inside front cover)

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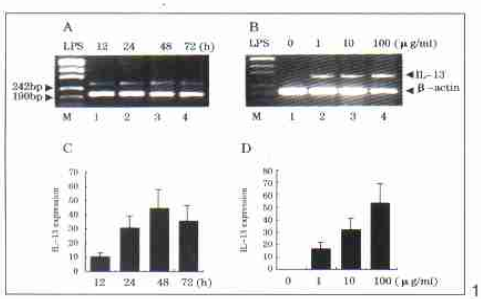
Effect of mesangial cell-derived interleukin-13 on expression of cytokines synthesized by human mesangial cells

(These figures refer to the paper on page 85)



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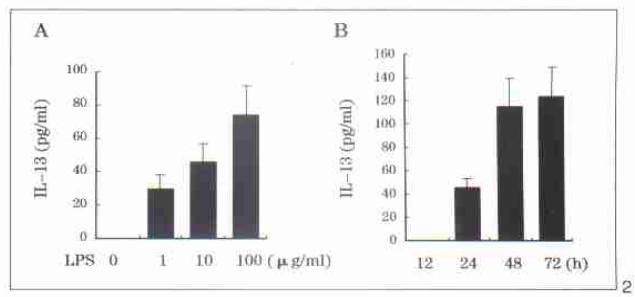

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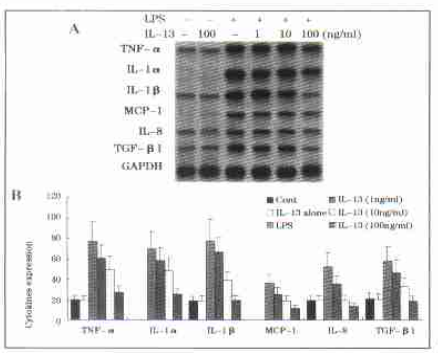

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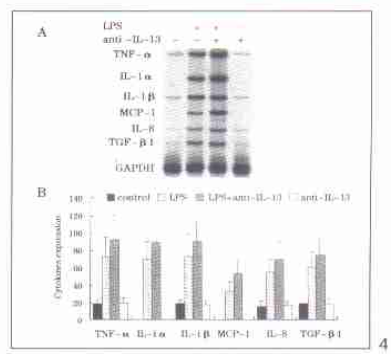

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Figure 1 Expression of IL-13 in the HMC

Figures 1A and 1C IL-13 expression of HMC at different time after being stimulated with 10 μg/ml LPS

Figures 1B and 1D IL-13 expression of HMC after being stimulated with different concentrations of LPS for 24 hrs

Figure 2 Level of IL-13 protein in the HMC culture supernatant

Figures 2A Level of IL-13 protein after the HMC were cultured with different concentrations of LPS (0-100 μg/ml) for 24 hrs

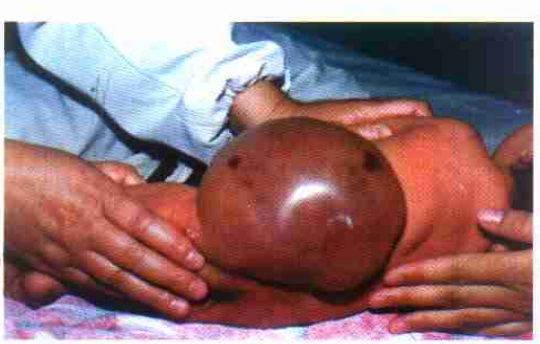
Figures 2B Level of IL-13 protein at different time after the HMC were cultured with 10 μg/ml of LPS

Figure 3 Expression of cytokines of LPS stimulated HMC with LPS with or without IL-13 pretreatment (1-100 ng/ml)

Figure 4 Expression of cytokines of LPS stimulated HMC with or without anti-IL-13 mAb treatment

新生儿巨大海绵状血管瘤 1 例

（正文见第 120 页）



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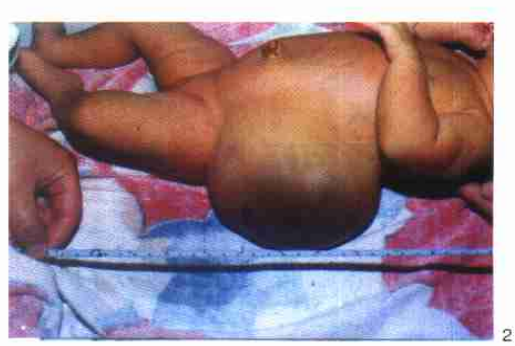

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图 1，2 新生儿巨大海绵状血管瘤

左侧腹壁可见 17 cm × 12 cm 大小肿物，触之软，似海绵，皮色紫红，薄而亮