

Correlative changes of interferon- γ and interleukin-4 between cortical layer and pulmonary airway of sensitized rats

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results showed a significant interplay changes of Th1 and Th2 cytokine between central nervous system and pulmonary airway in the asthmatic inflammatory model.

ABSTRACT

AIM: To explore correlation of T helper 1 (Th1) and Th2 cytokine between the cortical layer and pulmonary airway in ovalbumin-induced rat asthma model. **METHODS:** Aerosol antigen-induced changes of inflammation in bronchoalveolar lavage fluids (BALF) and pulmonary histologic section in sensitized rats were investigated. Changes of interferon- γ (IFN- γ) and interleukin-4 (IL-4) in BALF and cortical layer homogenates were determined by ELISA. **RESULTS:** The number of inflammatory cells in BALF in antigen challenged group was significantly higher than control group ($P < 0.05$). Dexamethasone (DXM, 0.5 mg/kg, ip) markedly reduce total leukocyte numbers in BALF, and almost completely inhibited eosinophil, lymphocyte accumulation. The score of histological examination (eosinophil infiltration, mucous edema and epithelial lesion) in antigen challenged group was significantly higher than control group ($P < 0.05$). DXM (0.5 mg/kg, ip) reduced the numbers of eosinophils and improved mucous edema and epithelial lesion of bronchi and bronchioles. In addition, our results demonstrated decreased IFN- γ level accompanied by increased IL-4 level that resulted in a decreased IFN- γ /IL-4 ratio in the BALF after the sensitized rats were challenged with aerosol antigen. Meanwhile, there was a similar change of IFN- γ and IL-4 in cortical layer homogenates. DXM (0.5 mg/kg, ip) could reverse IFN- γ /IL-4 ratio in the BALF and cortical layer homogenates in the model. **CONCLUSION:** The

INTRODUCTION

The central nervous system (CNS) has traditionally been regarded as an immunologically privileged organ. This view has been based on four main arguments including the lack of lymphatic draining, the presence of a tight blood-brain barrier (BBB), the impaired rejection of transplants, and the low level expression of major histocompatibility complex (MHC) and adhesion molecules^[1]. However, accumulating evidence suggests that the concept of immunological privilege within the CNS needs re-evaluation. The CNS as an immunocompetent organ has received increasing attention. Recent evidence showed that microglia, astrocytes, and mast cells secrete numerous cytokines, it is widely accepted that these cells participate actively in an integrative communication between resident immune cells of the CNS and those of the periphery. The CNS can turn on, restrict, and regulate the immune system. These communications between cells of the immune and nervous systems occur via the coordinated use of surface structures (MHC class II, costimulatory and adhesion molecules, as well as receptors) and soluble mediators (hormones, cytokines, neurotrophic factors, and synaptic transmitters)^[2]. In acute multiple sclerosis (MS) or experimental allergic encephalomyelitis (EAE) models, early disruption in the integrity of the BBB precedes brain infiltration by inflammatory cells or any clinical evidence of disease. BBB permeability could be affected by vasoactive mediators and cytokines released from perivascular brain mast cells, microglia, and astrocytes^[3,4]. Although immune and inflammatory response of CNS has never been reported in animal asthma model, it is possible that the response can be produced in monkey and rat anaphylaxis shock

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model^{5,6}. Therefore, we hypothesized that immune and inflammatory response of CNS might occur in animal asthma model, and play an important role in pathological status of asthma. To investigate this, in the present study, the rats were sensitized with antigen ovalbumin together with aluminium hydroxide adjuvant, formed asthmatic inflammation model after antigen challenge by local airway inhalation. We studied the model to evaluate the correlation of Th1 and Th2 cytokine in cortical layer and pulmonary airway by measuring interferon- γ (IFN- γ) and interleukin-4 (IL-4) in BALF and cortical layer homogenates.

MATERIALS AND METHODS

Rats Sprague-Dawley rats of either sex weighing $180 \text{ g} \pm 5 \text{ g}$ were purchased from Laboratory Animal Center of Medical School of Zhejiang University (Grade II, Certificate No 220010014 conferred by Zhejiang Medical Laboratory Animal Administration Committee). Rats were fed by the technicians of Laboratory Animal Center of Medical School of Zhejiang University.

Drugs Dexamethasone sodium phosphate (DXM, Suzhou Sixth Pharmaceutical Factory), urethane (Shanghai Chemical Reagent Company), egg albumin grade V (Sigma, USA), heparin sodium (Xuzhou Biochemical Pharmaceutical Factory), IFN- γ and IL-4 ELISA kit (Jingmei Biotech Co Ltd, China) were commercially available.

Sensitization, treatment, and challenge regimens All of Sprague-Dawley rats were sensitized by subcutaneous injection (sc) on experiment d 0 with antigen ovalbumin 1 mg mixed with aluminium hydroxide adjuvant 100 mg in saline 1 mL, at footpad, neck, back, and groin.

One time every day for 6 d and 1 h before antigen challenge, rats in treatment group were injected intraperitoneally (ip) with DXM 0.5 mg/kg. Rats in control and model group were injected ip with saline.

After treatment, rats were placed in a 45 cm \times 45 cm \times 15 cm plastic box and challenged by exposure to an aerosol of ovalbumin (10 g/L in saline) which was generated in a jet nebulizer (particle size 1 - 5 μm ; BARI, MASTER, Germany) for 20 min, and one time every day for 6 d. Control rats were similarly exposed to an aerosol of saline.

Cell recovery and counts Cell recovery from the airway in rats at 24 h after the last time antigen challenge, rats were anesthetized with urethane (2 g/kg,

ip) and the trachea was cannulated. Bronchoalveolar lavage was performed by flushing the airways with saline 5 mL/kg containing 1 % bovine serum albumin and 1 kU/L heparin sodium through the tracheal cannula for three flushing. BALF were pooled and centrifuged (Eppendorf Centrifuge 5804R, Germany) at $500 \times g$ for 10 min at 4 $^{\circ}\text{C}$, the supernatant was collected and stored at -80 $^{\circ}\text{C}$ for assaying IFN- γ and IL-4, and the cells resuspended in 1 mL saline containing 10 % bovine serum albumin. Counts of total number of leukocytes recovered in BALF were made using a Neubauer chamber, and differential cell analysis made under light microscopy after Wright-Giemsa staining.

Lung tissue histology The lungs of rats were collected intact for semi-quantum assessment of airway eosinophilia. The lungs were gently inflated by tracheal cannula with 10 % buffered formalin until no pleural creases were visible, and the trachea was ligated followed by immersion in 10 % buffered formalin. The lungs were sectioned longitudinally to include trachea, airways, and both right and left lungs. The tissue was paraffin embedded and sectioned at 5 μm thickness followed by hematoxylin and eosin (HE) stain. The semi-quantum assessment of histological changes was made under light microscopy. Score of eosinophil infiltration, mucous edema, and epithelial lesion was as absent (scale 0), rare (scale 1), mild (scale 2), moderate (scale 3), and severe (scale 4) by an observer blinded to the experimental groups⁽⁷⁾.

Brain homogenates preparation The brain cortical layer was removed, pooled both were homogenated in ice-cold Hanks' buffer (pH 7.5). Therefore, the homogenates were centrifuged at $3000 \times g$ for 10 min at 4 $^{\circ}\text{C}$. The supernatant were collected and stored at -80 $^{\circ}\text{C}$ for assaying IL-4 and IFN- γ .

Assay of IL-4 and IFN- γ The IL-4 and IFN- γ levels in the BALF and cortical layer homogenates were measured by an IL-4 and IFN- γ ELISA kit. Briefly, 100 μL of BALF, cortical layer homogenates, recombinant IL-4 and IFN- γ -standard were added to a 96-well microtitre plate precoated with a specific antibody for IL-4 and IFN- γ and incubated for 2 h at room temperature. After washing for 4 times, 100 μL of biotin-antibody was added and after 1 h incubation, the plate was washed for 4 times. Then horseradish-peroxidase goat anti-rat IgG conjugated was added and after 30 min incubation the plate was washed for 4 times. Then 3, 3', 5, 5'-tetramethylbenzidine (TMB) was added. Finally, after 15 min incubation, 50 μL of

terminate liquid was added and each sample was scanned by ELISA reader (BIO-TEK, ELX800, USA) at 450 nm absorbance.

Statistical analysis Statistical difference was determined using one-way analysis of variance followed by the Student-Newman-Keuls method for multiple comparisons between groups. Data were presented as $\bar{x} \pm s$.

RESULTS

Antigen-induced changes of airway inflammatory cells in BALF, and eosinophil infiltration and histological change of pulmonary tissue In sensitized rats, after 6 × ovalbumin-aerosol challenge, antigen induced a significant increase of eosinophil, neutrophil, and lymphocyte. The number of inflammatory cells in BALF in antigen challenged group was significantly higher than that in control unchallenged group ($P < 0.05$). DXM (0.5 mg/kg, ip) markedly reduced total leukocyte numbers in BALF, and almost completely inhibited eosinophil and lymphocyte accumulation, but increased neutrophil numbers (Tab 1). Pulmonary histologic examination found characteristic of inflammatory cell infiltration around the airways and blood vessels. Eosinophil numbers in the epithelium and

subepithelial connective tissue of bronchi, bronchioles, and peripheral small vascular were increased. The mucous edema and epithelial lesion of bronchi and bronchioles were also observed. The score of histological examination (eosinophil infiltration, mucous edema, and epithelial lesion) in antigen-challenged group was higher than that in control unchallenged group ($P < 0.05$). DXM (0.5 mg/kg, ip) reduced the numbers of eosinophils and improved mucous edema and epithelial lesion of bronchi and bronchioles (Tab 2).

Antigen-induced changes of IFN- γ and IL-4 levels in bronchoalveolar lavage fluid and cortical layer homogenate Amounts of IL-4 from BALF and cortical layer homogenates in antigen-challenged rats obtained were markedly higher compared with samples from antigen-unchallenged rats ($P < 0.05$). In contrast, amounts of IFN- γ in antigen-challenged rats were less (BALF $P < 0.05$, cortical layer homogenates $P < 0.01$). Therefore, IFN- γ /IL-4 ratio was lowered ($P < 0.01$, Tab 3 and Tab 4). DXM-treated rats had less IL-4 amounts in cortical layer homogenates and higher IFN- γ amounts compared with that of model rats. The down-regulation of IFN- γ /IL-4 ratio in antigen-challenged rats was recovered by DXM (BALF $P < 0.05$ and cortical layer homogenates $P < 0.01$). The results was repeated two experiments.

Tab 1. Antigen-induced changes of airway inflammatory cells in bronchoalveolar lavage fluids and inhibitory effects of dexamethasone. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs sensitized + ovalbumin.

Groups	Dose	n	$10^{-8} \times$ Total leukocyte /L ⁻¹	$10^{-8} \times$ Eosinophil /L ⁻¹	$10^{-8} \times$ Neutrophil /L ⁻¹	$10^{-8} \times$ Lymphocyte /L ⁻¹
Sensitized	Saline 4 mL/kg × 6 d	9	1.4 ± 0.8^c	0.03 ± 0.20^c	0.05 ± 0.04^c	1.3 ± 0.8^b
Sensitized + ovalbumin	Saline 4 mL/kg × 6 d	12	3.8 ± 2.4	0.7 ± 0.7	0.19 ± 0.22	2.9 ± 2.5
Sensitized + dexamethasone + ovalbumin	0.5 mg/kg × 6 d	10	1.5 ± 0.6^c	0.03 ± 0.21^c	0.5 ± 0.4^b	1.0 ± 0.3^b

Tab 2. Antigen-induced eosinophil infiltration and histologic change in pulmonary tissue of rats and inhibitory effects of dexamethasone. $\bar{x} \pm s$. ^c $P < 0.01$ vs sensitized + ovalbumin.

Groups	Dose	n	Eosinophil infiltration	Score Mucous edema	Epithelial lesion
Sensitized	Saline 4 mL/kg × 6 d	9	0.40 ± 0.17^c	0.0 ± 0.0^c	0.0 ± 0.0^c
Sensitized + ovalbumin	Saline 4 mL/kg × 6 d	12	3.7 ± 0.9	2.6 ± 0.5	2.2 ± 0.8
Sensitized + dexamethasone + ovalbumin	0.5 mg/kg × 6 d	10	0.5 ± 0.7^c	0.1 ± 0.3^c	0.1 ± 0.3^c

Tab 3. Antigen-induced reduction of IFN- γ level and increase of IL-4 level in bronchoalveolar lavage fluids and regulatory effect of dexamethasone. $n = 11$ rats. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs sensitized + ovalbumin. ^e $P < 0.05$ vs sensitized.

Groups	Dose	IFN- γ /ng·L ⁻¹	IL-4/ng·L ⁻¹	IFN- γ /IL-4 ratio
Sensitized	Saline 4 mL/kg × 6 d	376 ± 86 ^c	34 ± 18 ^b	11 ± 5 ^e
Sensitized + ovalbumin	Saline 4 mL/kg × 6 d	62 ± 33	57 ± 30	1.1 ± 1.1
Sensitized + dexamethasone + ovalbumin	0.5 mg/kg × 6 d	113 ± 32 ^{bc}	24 ± 12 ^b	4.7 ± 2.6 ^e

Tab 4. Antigen-induced reduction of IFN- γ level and increase of IL-4 level in cortical layer homogenates and regulatory effect of dexamethasone. $n = 11$ rats. $\bar{x} \pm s$. ^b $P < 0.05$, ^c $P < 0.01$ vs sensitized + ovalbumin. ^e $P < 0.05$ vs sensitized.

Groups	Dose	IFN- γ /ng·g ⁻¹ wet weight	IL-4/pg·g ⁻¹ wet weight	IFN- γ /IL-4 ratio
Sensitized	Saline 4 mL/kg × 6 d	2.3 ± 0.5 ^b	69 ± 36 ^b	34 ± 12 ^c
Sensitized + ovalbumin	Saline 4 mL/kg × 6 d	1.6 ± 0.4	135 ± 32	12 ± 7
Sensitized + dexamethasone + ovalbumin	0.5 mg/kg × 6 d	3.7 ± 0.8 ^{bc}	89 ± 32 ^b	41 ± 14 ^c

DISCUSSION

An important advance has been the recognition that airway inflammation is a critical feature of asthma and there is a characteristic inflammatory process in asthma that involves several inflammatory and structural cells. These cells produce the complex acute and chronic pathophysiological features of asthma through the production of multiple inflammatory mediators, including many cytokines^[8,9]. Th1 and Th2 cells can be redefined as polarized forms of immune responses that not only represent a useful model for understanding the pathogenesis of asthma, but also could provide the basis for the development of immunotherapeutic strategies. Mechanisms that regulate the balance of Th1 and Th2 cells, such as cytokines, are of great interest because they can determine the outcome of disease. For example, IFN- γ promotes the development of Th1 cells, whereas IL-4 leads to the expansion of Th2 cells^[10,11].

In addition, the CNS as an immunocompetent organ has received increasing attention. Top down CNS influences the immune system and bottom up immune system influences the CNS take part in a complex feedforward and feedback loop which may be responsible for initiating events and perpetuating circumstances in the course of neuropsychiatric as well as immune system diseases. The interplay between neurologic and immune systems may help to uncover the pathophysiology of certain neuropsychiatric systems. This may provide new

strategies for pharmacologic anti-inflammatory treatments^[12]. In the present studies, we studied correlation between of Th1 and Th2 cytokine in cortical layer and pulmonary airway in the asthma model. Results demonstrated that the decreased IFN- γ level accompanied by increased IL-4 level which resulted in a decrease of IFN- γ /IL-4 ratio in the BALF after the sensitized rats were challenged with aerosol antigen. Meanwhile, there was a similar change of IFN- γ and IL-4 in cortical layer homogenates. The evidence showed a significant interplay between cortical layer and pulmonary airway in the asthmatic inflammatory model.

Glucocorticoids are therapeutically useful agents for a variety of inflammatory and immune diseases. It is widely accepted that their potent anti-inflammatory and immuno-modulatory actions are due to inhibition of the activity of transcription factors, such as bradykinin B1 receptor (B κ B1) actioned nuclear factor κ B (NF- κ B), that are involved in activation of pro-inflammatory genes^[13]. Glucocorticoids are also the most effective therapeutic drugs in the long-term control of inflammatory and immune cells within the airway especially in asthma^[14]. In the present studies, DXM was applied as tool drug to observe cytokine changes in CNS and BALF in asthmatic inflammatory model. DXM inhibited IL-4 level accompanied by increased IFN- γ level that resulted in a reversion of IFN- γ /IL-4 ratio.

Though it is unclear what reason and what mechanism lead to the reduction of IFN- γ level and the

increase of IL-4 level in CNS and how the inflammatory mediators of CNS influence asthma pathological status, our results showed correlation of Th1 and Th2 cytokine in CNS and pulmonary airway indeed.

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致敏大鼠脑皮层和肺气道中干扰素-γ 和白介素-4 相关性的变化

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关键词 细胞因子类; 干扰素 II 型; 白介素-4; 中枢神经系统; 炎症; 哮喘; 大鼠

目的: 探讨致敏大鼠抗原攻击后脑皮层和肺气道中干扰素-γ(IFN-γ)和白介素-4(IL-4)出现的相关性变化。方法: 观察致敏大鼠吸入抗原诱导的支气管肺灌洗液(BALF)和肺组织切片炎症变化, 用 ELISA 法测定 BALF 和脑皮层 IFN-γ 和 IL-4 水平变化。结果: 抗原攻击组 BALF 中的炎症细胞数目明显高于对照未攻击组($P < 0.05$)。地塞米松(DXM, 0.5 mg/kg, ip)明显减少 BALF 中的白细胞总数, 几乎完全抑制嗜酸性粒细胞(EOS)和淋巴细胞的聚集, 但增加中性粒细胞数目。抗原攻击组的组织学检查积分(EOS 浸润、粘膜水肿和上皮损伤)也明显高于对照未攻击组($P < 0.05$)。DXM (0.5 mg/kg, ip)减少支气管和细支气管的 EOS 数目, 改善粘膜水肿和上皮损伤。致敏大鼠抗原攻击后, BALF 中的 IFN-γ 水平降低伴随 IL-4 升高导致了 IFN-γ/IL-4 比例下降。与此同时, 脑皮层匀浆中也出现相似的改变。DXM (0.5 mg/kg, ip)能反转 BALF 和脑皮层匀浆中的 IFN-γ/IL-4 比例下降。结论: 致敏大鼠抗原攻击后脑皮层和肺气道中的 IFN-γ 和 IL-4 出现相关性变化。

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