Dexamethasone alleviates allergic asthma immature rat through Toll like receptor 4

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Abstract. – OBJECTIVE: The allergic asthma model induced by ovalbumin (OVA) was established in the immature rat. Dexamethasone (DXM) was adopted for intervention to analyze the treatment effect and to explore the relationship with toll-like receptor 4 (TLR4).

MATERIALS AND METHODS: Immature SD rat was treated by OVA to construct allergic asthma model and intervened by DXM. The rats were randomly divided into model group, experimental group, and control group. The changes in lung tissue were observed by light microscope. The EOS infiltration and reactivity of airway wall were compared. The expressions of TLR2 and TLR4 protein and mRNA in the lung tissue were tested by Western blot and RT-PCR.

RESULTS: The lung tissue in the model group was infiltrated by a lot of inflammatory cells, and mucous membrane edema was observed, compared with that in the control group. There were only a few inflammatory cells in the interstitial tissue and pulmonary alveoli in the experimental group compared with that in the model group. EOS count of airway wall and airway reactivity decreased in the experimental group. The levels of TLR2 and TLR4 were significantly elevated in the third week compared with the first week (p<0.05).

CONCLUSIONS: The treatment of DXM can alleviate the pathological changes of the lung tissue in SD immature rat with allergic asthma, reduce EOS infiltration in the airway wall, decrease airway reactivity, and elevate expressions of TLR2 and TLR4.

Key Words
Dexamethasone, Allergic asthma, TLR4.

Introduction

Bronchial asthma is a type of common respiratory disease in children associated with multiple cells and components. Its pathological basis is accounted for cell injury and inflammation1. It is generally considered that the main pathogenesis of childhood asthma is associated with eosinophils (EOS)-induced airway inflammation2. Toll-like receptor (TLR) belongs to the family of pattern recognition receptor that can timely recognize the pathogen-associated molecular pattern and further modulates the activation of the immune response of host. TLR contains a large category of molecular models and exits in various sorts of cells3,4. The current study found that there are 11 types of TLR in the mammalian. All of them are characterized by biological function of pattern recognition receptor and participate in the regulation of multiple signaling pathways in the body5. Neutrophils, mast cells, and macrophages are stimulated by infection, immune, or inflammation may secrete cytokines, leading to the imbalance of Th1/Th2 immune response that deviates to Th26. At present, the application of glucocorticoid is still the most effective reagent to treat asthma in clinic. Dexamethasone (DXM) is a kind of immunosuppressant and exerts to anti-inflammatory function7. However, it is still lack of report about the mechanism of glucocorticoid in regulating immune response. This study established allergic asthma model with immature rat and determined the curative effect of DXM and its relationship with TLR4.

Materials and Methods

Experimental Animals

A total of sixty female SD rats at three weeks old were bought from Shandong animal experiment center, Chinese Academy of Sciences (Jinan, Shandong, China). The rats were raised with temperature at 21 ± 1 ºC, relative humidity at 50-70%, and 12 h day/night cycle.
Rats were used for all experiments, and all procedures were approved by the Animal Ethics Committee of Dezhou People’s Hospital.

**Main Reagents**

Ovalbumin (OVA) and DXM were got from Sigma (Temecula, CA, USA). TLR2 and TLR4 in situ hybridization kits were obtained from Boster (Wuhan, Hubei, China).

**Experimental Methods**

**Ova Allergic Asthma Model Establishment**

The rats were randomly equally divided into three groups. The rats were intraperitoneally injected with 0.5 ml normal saline containing 10 μg OVA and 1 mg Al(OH)$_3$ on the first day. Next, the rats were treated by 0.5 ml normal saline containing 10 μg OVA aerosol for 30 min inhalation. At last, the rat received 50 μl OVA aerosol via inhalation at 2 mg/ml for continuous fifteen days.

**Experimental group:** The rat received 1 ml normal saline containing 100 μg DXM via intraperitoneal injection at 8 am every day for continuous three weeks.

**Model group:** The rat received 1 ml normal saline by intraperitoneal injection at 8 am every day for continuous three weeks.

**Control:** The healthy rat received equal amount of normal saline during model rat establishment, and then received 1 ml normal saline by intraperitoneal injection at 8 am every day for continuous three weeks.

**Airway Reactivity Measurement**

The trachea was routinely incised and connected to the T-branch pipe. One pipe was connected to the breathing machine, while the other was connected to piezometer tube. The PBS was injected to the trachea cannula with 0.01 g/L histamine. The pressure was monitored.

**Sample Collection**

The rat was fixed on the table and the eyelash was cut off. A capillary tube was vertically inserted to the inner canthus to collect the blood. The head of the SD rat was inverted to obtain more blood. At last, the blood was stored at -80 °C and the lung tissue was collected and saved at -80 °C.

**Lung Tissue Morphology Changes**

The lung tissue was embedded and sectioned. After stained by hematoxylin and eosin, the slice was dehydrated and hyalinized to be observed under the microscope.

**Airway Wall EOS Counting**

The airway wall with integrated structure was observed under the microscope. Mean number of EOS on the airway wall within five visual fields were counted.

**Western Blot**

Total protein was separated by 8% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and blocked. Next, the membrane was incubated with primary antibody (TLR2 and TLR4 1:200, β-actin 1:500) for 30 min and then incubated in secondary antibody (1:2000) for 1 h (Abcam, Cambridge, MA, USA). After developed by chemiluminescence kit (Bio-rad, Hercules, CA, USA), the result was analyzed.

**RT-PCR**

Total RNA was extracted from the lung tissue. The RNA was reverse transcribed to cDNA for PCR amplification (TaKaRa, Kusatsu, Shiga, Japan). The primers used were listed in Table I. The PCR reaction contained 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The products were then analyzed on a 1.5% agarose gel.

<table>
<thead>
<tr>
<th>Table I. Primers sequences.</th>
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<tr>
<td><strong>Gene</strong></td>
<td><strong>Sequence</strong></td>
</tr>
<tr>
<td>TLR2</td>
<td>5’-AAA CGG TAA CAA TAC GGA G-3’</td>
</tr>
<tr>
<td>Forward</td>
<td>5’-TGA CAA CTG TCG GGC ATA-3’</td>
</tr>
<tr>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>5’-CAG AGC CGT TGG TGT ATC-3’</td>
</tr>
<tr>
<td>Forward</td>
<td>5’-CCC TGT GAG GTC GTT GA-3’</td>
</tr>
<tr>
<td>Reverse</td>
<td></td>
</tr>
<tr>
<td>U6</td>
<td>5’-AGT TGC GGT ACA CCC TTT C-3’</td>
</tr>
<tr>
<td>Forward</td>
<td>5’-CAC CTT CAC CGT TCC AGT-3’</td>
</tr>
<tr>
<td>Reverse</td>
<td></td>
</tr>
</tbody>
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by 30 cycles of 95 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s, and 72 °C for 10 min at last.

**Data Analysis**

SPSS 17.0 software was applied for statistical analysis. The enumeration data were compared by chi-square test, while the measurement data among groups were compared by one-way ANOVA followed by Fisher’s LSD tests. p < 0.05 was considered as statistical significance.

**Results**

**Lung Tissue Observation Under the Microscope**

A large amount of inflammatory cells were found infiltrating in bronchus, alveolar space, and blood vessel of the rats in the model group. Edema and ruffle were shown in the mucosa. Epithelial cells fell off, together with a lot of mucus plugs. However, in the experimental group, there were relatively few inflammatory cells located in the bronchus, interstitial tissue, and alveolar space. No inflammatory cells infiltration was found in control (Figure 1).

**Airway wall EOS Infiltration and Airway Reactivity Comparison**

The EOS count of airway wall was significantly elevated in model group compared with control, whereas EOS count and airway reactivity were decreased in the experimental group compared with the model group (p < 0.05) (Table II).

**TLR2 and TLR4 Protein Expressions Comparison**

TLR2 and TLR4 protein levels were higher in experimental group than those in model group and control. Moreover, their expressions were markedly higher in the third week compared with that in the first week (p < 0.05) (Figure 2-3).

**TLR2 and TLR4 mRNA Expressions Comparison**

We also detected the expressions of TLR2 and TLR4 at mRNA levels. Our data showed

![Figure 1. Lung tissue morphological changes (×400). A, Control. B, Experimental group. C, Model group.](image)

![Figure 2. TLR2 and TLR4 protein expressions comparison. A, TLR2 protein. B, TLR4 protein. *p<0.05, compared with model group. # p<0.05, compared with control. & p<0.05, compared with the first week.](image)

<table>
<thead>
<tr>
<th>Group</th>
<th>EOS(/HP)</th>
<th>PC_{20}</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>3.24±1.11</td>
<td>0.446±0.010</td>
</tr>
<tr>
<td>Experimental</td>
<td>63.21±4.11*</td>
<td>0.102±0.006*</td>
</tr>
<tr>
<td>Model group</td>
<td>75.45±642*</td>
<td>0.036±0.011*</td>
</tr>
</tbody>
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Table II. Airway wall EOS infiltration and airway reactivity comparison (x ± s).
that similar to the protein expressions, the levels of TLR2 and TLR4 mRNA were also apparently higher in experimental group than those in model group and control, the expressions of which were significantly higher in the third week compared with the first week ($p < 0.05$) (Figure 4).

**Discussion**

Asthma is a common chronic airway inflammatory disease in clinic. Its incidence has been gradually increasing in children recently. The epidemiologic study proposed that the reduction of microbes exposure may be one of the important causes of asthma. It is confirmed that a large amount of inflammatory cells and components are involved in the occurrence of asthma. The pattern recognition function of innate immune system triggers the T cell-mediated immune response in the body, while TLR belongs to one type of PRR in innate immune system. It is pointed out that TLRs have a critical role in the pathogenesis of asthma by bridging the innate immunity to acquired immunity. Up to now, there are 13 types of TLR found by the researchers. Among them, only TLR2 and TLR4 can trigger transcription factor translocation to activate immune-related genes and induce inflammatory response, which is associated with TLR level. External stimuli activate TLR to trigger downstream molecules, resulting in the occurrence and development of inflammation. Researchers suggested that TLR elicits the release of inflammation factors and aggravates the inflammatory response.

Asthma mainly includes airway inflammation and reconstruction, which are different from other upper respiratory tract diseases. In this study, OVA was used to establish animal model of allergic asthma. The rats in the model group present severe inflammatory response with a large amount of inflammatory cell infiltration in bronchus, alveolar space, and blood vessel. However, the treatment of DXM remarkably alleviated the pathological changes of the lung tissue, reduced eosinophil infiltration in airway wall, and decreased airway reactivity caused by allergic asthma, along with the rise of TLR2 and TLR4 expressions.

Macrophages play the fastest biological function in the first line of defense against the pathogenic infection. As TLR2 and TLR4 participate in the innate immunity, their mRNA mostly express in macrophages. Once the specific pathogenic pattern was recognized by TLR2 or TLR4, macrophages are activated to release a large amount of proinflammatory factors, leading to the activation of the innate immune and acquired immune response. Consistently, this study found that TLR2 and TLR4 protein and mRNA
levels were significantly higher in experimental group than those in model group and control, and increased in a time-dependent manner. Kumar et al\(^9\) proposed that serum TLR2 and TLR4 levels were significantly higher in children with asthma, especially in acute phase than that in children at remission phase or healthy children. Moreover, TLR4 exhibited certain regulatory effect in promoting the release of proinflammatory factors, such as interleukin. Accumulative evidence found that IL-6 and TNF-α expressions were decreased after the expression of TLR4 was inhibited\(^{20,21}\). It showed that the impact of IL-6 and TNF-α expressions was inhibited and increased in a time-dependent manner. Ku\(^-\)group than those in model group and control, and increased in a time-dependent manner. Ku\(^--\)expressed in promoting the release of proinflammatory factors, such as interleukin. Accumulative evidence found that IL-6 and TNF-α expressions were decreased after the expression of TLR4 was inhibited\(^{20,21}\). It showed that the impact of IL-6 and TNF-α expressions was inhibited and increased in a time-dependent manner. Ku\(^--\)protein is protection or damage. Chin J Gastroenterol Surg 2009; 12: 540-541.


4) Li GX LN INCOMPLETE. Toll like receptors on intestinal barrier is protection or damage. Chin J Gastroenterol Surg 2009; 12: 540-541.


Conclusions

We showed that the treatment of DXM can alleviate lung tissue pathological changes, reduce EOS infiltration, impede airway reactivity, and induce TLR2 and TLR4 expressions in the allergic asthma SD rat.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References


4) Li GX LN INCOMPLETE. Toll like receptors on intestinal barrier is protection or damage. Chin J Gastroenterol Surg 2009; 12: 540-541.


