

Effect of IL-12 on Experimental Allergic Otitis Media with Effusion

Jie Li^{1,2}, Shouqin Zhao¹, Yubin Xue³, Ying Li¹ and Chenyi Wei¹

¹Department of Otolaryngology Head and Neck Surgery, Beijing Tong Ren Hospital, Key Laboratory of Otolaryngology Head and Neck Surgery, Capital Medical University, Beijing, PR China

²Department of Otolaryngology Head and Neck Surgery, Fu Xing Hospital, Capital Medical University, Beijing, PR China

³Department of Otolaryngology Head and Neck Surgery, Beijing Tian Tan Hospital, Capital Medical University, PR China

*Corresponding author: Shouqin Zhao, Department of Otolaryngology Head and Neck Surgery, Beijing Tong Ren Hospital, Capital Medical University, Beijing, 100730, PR China, Tel: +861083911000; E-mail: shouqinzhao@163.com

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Abstract

Objective: To investigate the role of IL-12 in the pathogenesis of Otitis media with effusion (OME) in animal model induced by ovalbumin.

Methods: Twenty-four Sprague-Dawley (SD) rats were randomly divided into three group as OME group, IL-12 group, and control group. The rats of OME group were sensitized to OVA via intraperitoneal injection once a week for two weeks and then challenged transtympanic. The rats of IL-12 group and control group were sensitized and challenged in the same way, but before challenge they were injected IL-12 and PBS transtympanic respectively. The histomorphologic changes of middle ear mucosa were observed with histochemical technique. The level of IL-4 and IL-5 was measured by immunohistochemistry.

Results: Tympanal mucosa in OME group and control group rats thickened significantly. Meanwhile, inflammatory cells infiltrated obviously. Such histological findings were abated to absent in IL-12 group. The level of IL-4 and IL-5 in OME group and control group was higher significantly than in IL-12 group.

Conclusion: The local application of IL-12 is effective in attenuating allergic inflammation of middle ear. It can be used as a novel treatment for allergic OME.

Keywords: Otitis media with effusion; Allergy; Ovalbumin; IL-12; Animal model

Introduction

Otitis media with effusion (OME) is defined as inflammation of the middle ear in which a collection of liquid is present in the middle ear space. Signs and symptoms of acute infection, such as otalgia and fever, are absent and the tympanic membrane is intact. OME is highly prevalent among young children, and is one of the most common cause of hearing impairment in childhood. The pathogenesis of OME is multifactorial, and the precise mechanism for the development of the disease is still unknown.

In recent years, many investigators found that allergy is an important factor in the development of OME, but the precise mechanism of allergy is still controversial. Many studies revealed that like other parts of respiratory mucosa, the mucosa lining the middle-ear cleft is capable of an allergic response mediated by Th2 cytokines (IL-4, IL-5, IL-9 and et al) as asthma and allergic rhinitis. Interleukin-12(IL-12) is a key cytokine involved in regulating the balance between Th1 and Th2 cells, it can promote Th1-type cell-mediated immune function and also inhibit Th2 cytokines. The purpose of the study is to investigate the role of IL-12 in the pathogenesis of animal model of OME induced by ovalbumin.

Materials and Methods

Twenty-four male Sprague-Dawley(SD) rats weighing between 200 g to 250 g were used. The SD rats were randomly divided into three groups: the model Group (group), the IL-12 treatment Group (group) and the control Group (group). In the experimental group, rats were sensitized to ovalbumin(OVA) (A5503; Sigma chemical Co, St Louis, MO) via intraperitoneal injection of 1.2 mg OVA in 0.6 ml phosphate-buffered saline solution (PBS) mixed with 5.14 mg aluminum hydroxide in 0.6 ml PBS as a local adjuvant on days 1 and 8.

On days 15 they were anesthetized with 40 mg/kg body weight of pentobarbital sodium. Tympanic membranes were visualized using an operating microscope to exclude pre-existing pathologic conditions. Subsequently, 0.1 mg OVA in PBS, total volume of 35 µl, was transtympanically injected into the middle ear cavity with a 50-gauge needle. Rats in the IL-12 treatment group received a 25 µl injection of 0.5 µg recombinant rat IL-12 (1760-RL/CF, R&D Systems) 2 hours before transtympanically injection of OVA. Rats in the control group received an injection of 25 µl PBS before transtympanically injection of OVA. Twenty-four hours later, the tympanic membranes were again visualized with the operating microscope and all the rats were challenged again. Forty-eight hours after the last challenge, the whole rats were killed.

Histologic Analysis

Postmortem, the tympanic bulla was isolated and fixed for at least 12 hours in 4% paraformaldehyde in PBS. After fixation, the middle ear was decalcified in 10% EDTA in PBS for 20 days dehydrated in increasing concentrations of ethanol, and suspended and embedded in paraffin blocks. Then 4 μm sections were cut from each block with a standard microtome until sections through the Eustachian tube were obtained. Sections were mounted on glass slides, stained with H&E.

Immunohistochemistry for IL-4 and IL-5 was performed using rabbit polyclonal antibody specific for rat IL-4 and IL-5.

Results

Histology

We examined histology of the middle ear and analyzed cells infiltrating into middle ear on the same specimen (Figures A1-A5). The sections from the group I and group III are characterized by extensive mucosal edema, infiltration of inflammatory cells, and a marked increase in eosinophils and mast cells. The sections from group II showed a decreased inflammatory reaction and inflammatory cells. Furthermore, the number of eosinophils and mast cells in the submucosa of group I and group III is significantly higher than group II, the difference is statistically significant (Table 1, $P < 0.05$).

Groups	Ears	Eosinophils	Mast cells
I	16	17.54 ± 4.2	10.3 ± 4.8
II	16	6.37 ± 3.6	3.7 ± 0.9
III	16	18.42 ± 3.5	8.7 ± 3.5

Table 1: The number of eosinophils and mast cells in every group ($x \pm s$).

Immunohistochemistry

Immunohistochemistry for IL-4 and IL-5 revealed that the number of IL-4 and IL-5 expressing cells were significantly increased in the group I and group III, while in group II, the number was significantly decreased (Figures A6-A9, Table 2, $P < 0.05$).

Groups	Ears	IL-4 expressing cells	IL-5 expressing cells
I	16	25.4 ± 8.7	15.7 ± 6.9
II	16	9.3 ± 3.4	8.4 ± 3.6
III	16	28.5 ± 9.2	17.2 ± 2.4

Table 2: The number of IL-4 and IL-5 expressing cells in every group ($\%$, $x \pm s$).

Discussion

Allergy as an etiological factor was first suggested by Lewis in 1929. Since that time, many other studies were performed to understand the relationship between allergy and OME. More and more studies found that the Th2 type cytokines are significantly higher in allergic patients than non-allergic ones [1,2]. Furthermore, Nguyen found that in atopic OME patients, the middle ear effusion has significantly higher levels of

eosinophils, T-lymphocytes, and Th2 type cytokines compared with nonatopic patients. Meanwhile the nasopharyngeal tissue biopsies revealed similar cellular and cytokine profiles.

The inflammatory cells and cytokines profile of asthma, and allergic rhinitis were well documented in the literature [3,4]. Eosinophils, basophils, and Th2 type cytokines are responsible for the late phase allergic response [5]. Given that the middle ear is contiguous with the upper airway, it is reasonable to hypothesize that allergy mediators play a role in the development of OME in atopic patients. Other studies and our precious study have found that in rat's allergen challenge to the middle ear could establish the allergic OME model.

IL-12 is a cytokine produced by antigen-presenting cells, it promotes Th1 responses and stimulates active T cells and NK cells to maximally produce IFN-γ, whereas it inhibits Th2-type cytokines such as IL-4 and IL-5.

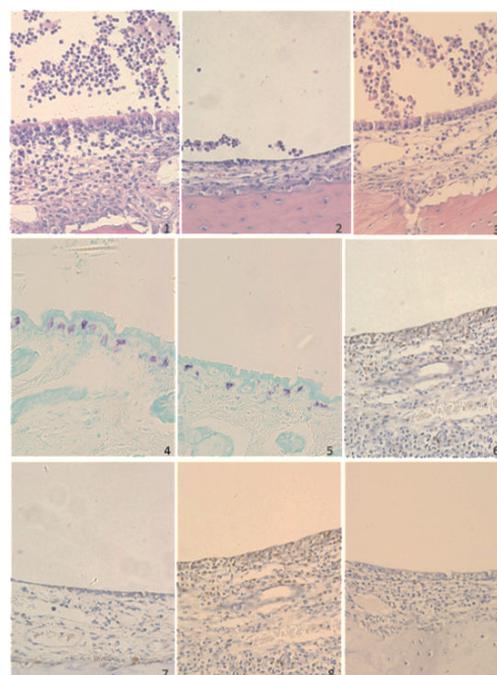


Figure A: Figure 1 The group I are characterized by extensive mucosal edema, infiltration of inflammatory cells, and a marked increase in eosinophils, and much effusion in the middle ear. Figure 2 The group II shows a decreased inflammatory cells and middle ear effusion. Figure 3 The group III has a similar inflammatory with the group I. Figure 4 Toluidin blue staining shows there are many mast cells infiltrating in the submucosa in the group I. Figure 5 Toluidin blue staining shows that the mast cells in the group II mucosa are less than the group I. Figure 6 IL-4 expression cells (presented with brown particles in the cells) in the mucosa of group I (immunohistochemistry×40). Figure 7 IL-4 expression cells in the mucosa of group (immunohistochemistry×40). Figure 8 IL-5 expression cells (presented with brown particles in the cells) in the mucosa of group I (immunohistochemistry×40). Figure 9 IL-5 expression cells in the mucosa of group II (immunohistochemistry×40).

In this study, we demonstrate that allergic otitis media with effusion induced by allergen challenge are associated with a Th2 pattern of cytokine expression in the rats' middle ear. IL-12 administration prevented the development of these responses in conjunction with suppressing the allergen-induced increases in expression of the Th2 cytokines IL-4 and IL-5 [6-8].

Suppression of these two cytokines is particularly important because they have been implicated in the pathogenesis of allergic responses via their role in mediating IgE production, mast cell activation, and eosinophilia. These results support the concept that Th2 cytokines play a key role in allergen-induced allergic otitis media with effusion, and IL-12 can suppress the allergic response induced by allergen. In conclusion, this study showed that local administration of IL-12 might provide a novel immunotherapy for the treatment of allergic otitis media with effusion.

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