DOI: 10.13656/j.cnki.gxkx.1997.02.022
广西科学 Guangxi Sciences 1997, 4 (2): 150- 154

Effect of Interferon-gamma on Antigen-induced Eosinophil Infiltration into the Asthmatic Airway

干扰素-γ 对哮喘患者抗原引起气道嗜酸性粒细胞浸润的影响*

Li Guoxiu Liu Guangnan Qin Shouming Xiao Changqing Chen Yiqiang Xu Hui Shi Huanzhong
李国秀 柳广南 覃寿明 肖常青 陈一强 许 辉 施焕中

(Department of Internal Medicine, First Affiliated Hospital, Guangxi Medical University, 6 Binhulu, Nanning, Guangxi, 530021)
(广西医科大学一附院 南宁市滨湖路6号 530021)

Abstract To investigate whether human recombinant interferon-gamma (hrIFN-γ) is capable of inhibiting the antigen-induced eosinophil infiltration into the airways of the patients with allergic bronchial asthma, ten atopic asthmatic subjects were underwent an initial bronchoscopy when the hrIFN-γ plus dust mite were instilled into a subsegment of the lingula or right middle lobe and diluent plus dust mite were instilled into a subsegment of the opposite lung (middle lobe, or lingula). The second bronchoscopy was performed 24 h later, and bronchial mucosa from each treated sites were taken separately. Immunohistochemical technique was used to evaluate the presence of eosinophils and some subsets of T lymphocytes including $CD4^+$, $CD8^+$ and $CD25^+$ cells. Immunostaining revealed decreases in the numbers of total eosinophils (BMK-13$^+$ cells, $P < 0.01$), $CD4^+$ lymphocytes ($P < 0.01$) and $CD25^+$ cells ($P < 0.05$) from IFN-γ treated biopsies compared with control biopsies. However, no difference was observed in the numbers of $CD8^+$ cells. Locally administered hrIFN-γ could prevent the antigen-induced eosinophil recruitment into the asthmatic airways by inhibiting $CD4^+$ T cell infiltration as well as T cell activation. These results suggest that hrIFN-γ may be of value in obtaining clinical improvement in asthmatics.

Key words human recombinant interferon-gamma (hrIFN-γ), eosinophils, asthma

摘要 为探讨人重组γ-干扰素 (hrIFN-γ)能否抑制抗原引起嗜酸性粒细胞 (EOS)对过敏性支气管哮喘患者气道的浸润，选择10例过敏性哮喘患者以纤支镜直接将 hrIFN-γ 及过敏原注入左舌肺叶或右中肺叶的段支气管，将生理盐水及过敏原注入对侧肺相应的段支气管作为对照。24 h后复以纤支镜取出支气管粘膜组织，然后以免疫组化技术探测 EOS $CD4^+$、$CD8^+$ 以及 $CD25^+$ 淋巴细胞的数量。结果发现 IFN-γ 组支气管粘膜组织中 EOS总数，$CD4^+$ 及 $CD25^+$ 细胞数明显低于对照组，而 $CD8^+$ 数则无明显变化。说明 hrIFN-γ 局部用药可以通过抑制 $CD4^+$ 细胞的浸润及 T细胞的活化而抑制抗原引起哮喘气道 EOS的浸润。

关键词 干扰素-γ 嗜酸性粒细胞 哮喘
中图法分类号 R562.2; R446.63

Interferon-gamma (IFN-γ) is an immunomodulatory cytokine known to inhibit IgE synthesis and Th2 lymphocyte proliferation and function[1]. These observations suggest a potential role for IFN-γ in treatment of allergic diseases. Our previous study has demonstrated that IFN-γ could prevent antigen-induced eosinophils recruitment into the airway of

1997-03-17收稿。
* 广西区科委自然科学基金资助项目。

sensitized mouse accompanied by a decrement of levels of interleukin (IL)-5 with a dose-related response[2]. Our results suggested that IFN-γ might be of value in treating atopic bronchial asthma in humans. We performed the present study to investigate the effect of human recombinant (hr) IFN-γ on the eosinophil infiltration of the airways by antigen instillation topically in the patients with mild allergic asthma.

1 Materials and Methods

1.1 Subjects

10 nonsmoking asthmatics (6 males, 4 females; 19 to 49 year of age), who fulfilled the criteria for a diagnosis of asthma as defined by The Respiratory Branch of Chinese Medical Association in 1992[3] were enrolled in this study. All patients had mild atopic asthma, with baseline FEV_1 greater than 70% of predicted value, requiring only intermittent use of inhaled β_2-agonists. Each patient had a positive skin prick test responses to dust mite and/or one or more other aeroallergens, but none was received either corticosteroids medication or other immunotherapy in 3 months.

1.2 Fiberoptic Bronchoscopy

Subjects were premedicated with 0.5 mg atropine subcutaneously. Oxygen at 4 L/min was given via nasal cannula throughout the procedure. After the nose and throat were anesthetized with a topical lidocaine spray, a fiberoptic brochosocope of Olympus model BF 1T20 was inserted into the lower airways. Local anesthesia was supplemented with 2% lidocaine. An IFN-γ treatment was performed by instilling 1: 5000 dust mite 1 mL (Shanghai Xudong Haipu Pharma. Ltd.) plus hrIFN-γ 1.0×10^6 U (dissolved in 5 mL normal saline, obtained from Zhuhai Lizhu Pharma. Lid.) into a subsegment of the lingula or right middle lobe. A control procedure was performed by instilling 1: 5000 dust mite 1 mL plus 5 mL normal saline into a subsegment of the opposite lung (middle lobe, or lingula). 24 h later, a second bronchoscopy was carried out when Olympus alligator forceps (model FB15C) were used to take four to six bronchial mucosal from each of the treated lingula or middle lobe separately.

1.3 Biopsy Samples

The bronchial mucosal biopsies were taken in phosphate-buffered saline placed in OCT embedding medium and snapfrozen in isopentane precooled in liquid nitrogen. Six-micron cryostat sections were cut and the sections were placed on microscope slides. They were air-dried for 1 h, fixed in equal parts of acetone and methanol for 5 min, and further air-dried for 1 h. The slides were then wrapped in aluminum foil and stored at -20℃ prior to immunostaining.

1.4 Immunohistochemistry

The following monoclonal antibodies were used: an antibody stained both resting and activated eosinophils (BMK-13 obtained from Nichirei Co., Tokyo, Japan), CD4 (leu3a), CD8 (leu2a), CD25 (anti-IL-2 receptor) (all purchased from Dako Ltd., Santa Barbara, CA). An Immunostaining was detected by the alkaline phosphatase-antialkaline phosphatase method with a commercial kit (APAAP kit, Dako Ltd.) according to the procedures recommended by manufactures. System and specificity controls were included in each staining run, using human lymphadens with eosinophilic lymphodenitis and a mouse IgG2a myeloma protein (Dako Ltd.) as a negative control. The numbers of positive stained cells were counted in a zone 115μm deep (as defined by squared eyepiece graticule) along the length of the epithelial basement membrane (BM). A calibrated and computerized graphics table was employed to determine the length of BM. At least two-step sections (18μm apart) were stained and counted for each antibody and the average count taken as the value of biosy.

1.5 Statistical Analysis

All data were presented as $\bar{x}\pm s$. Analysis of paired t test was used to determine the significance in the matched comparison for the results obtained from bronchial mucosal biopsies. P values < 0.05 were considered significant.

2 Results

Positive staining for eosinophils with BMK-13 was observed in the bronchial epithelium and lamina propria. In the lamina propria, the infiltration was more prominant. In some sections, especially those not to be treated with IFN-γ, eosinophils were also observed around vessels, and scattered throughout the deep layer of lamina propria. A representative

example of the immunostaining pattern for eosinophils is showed in figure 1. As showing in figure 2, the number of eosinophils in the bronchial mucosa exposed to hrIFN-γ (9.4± 1.4/mm) were significantly lower those that in the control mucosa (18.9± 3.4/mm, $P < 0.01$). It suggests that hrIFN-γ could prevent the eosinophils infliltration

(A)

(B)

by one-second. Fig. 1 Immunostaining patterns with BMK-13 in bronchial mucosa by the alkaline phosphatase-antialkaline phosphatase method (original magnification× 400). (A) control mucosa exposed to normal saline; (B) bronchial mucosa exposed to hrIFN-γ.

A prominant lymphocyte in infiltratation was also observed in the bronchial mucosa of all subjects studied (Figure 3). The instillation of hrIFN-γ significantly decreased antigen-induced $CD4^+$ cells infiltration in the bronchial mucosa of atopic asthmatics by 53.1± 7.2% (control, 20.7± 3.8/mm versus IFN-γ, 9.7± 1.9/mm, $P < 0.01$). A decrease in $CD25^+$ cells, presumed activated T lymphocytes, was also observed when IFN-γ treatment was compared with diluemt instillation (1.6± 0.3/mm versus 4.1± 0.9/mm, $P < 0.01$). In contrast, IFN-γ treatment did not result in an overall decrement in the numbers of total $CD8^+$ cells (5.3± 1.0/mm) as compared with normal saline treatment (5.9± 1.2/mm, $P < 0.05$).

3 Discussion

The previous study has demonstrated that segmental antigen challenge was capable of inducing the eosinophil recruitment in the airway of allergic subjects[4]. In the present study, we used this inflammatory model to investigate the effect of hrIFN-γ on the eosinophil infiltration of the bronchial mucosa with atopic asthmatics. The patients with atopic dermatitis treated with subcutaneous hrIFN-γ demonstrated clinical improvement along with a marked decrease in circulating eosinophil counts[5], however, the patients with steroid-dependent asthma treated with hrIFN-γ showed no improvement, although they did have an observed decrease in circulating eosinophil counts[6]. One explanation for this might be that the subcutaneous hrIFN-γ did not exert a biologic effect in the airways. Indeed, Jaffe et al.[7] have shown that subcutaneously administered hrIFN-γ did not reach the respiratory epithelial surface. Therefore, topical instillation

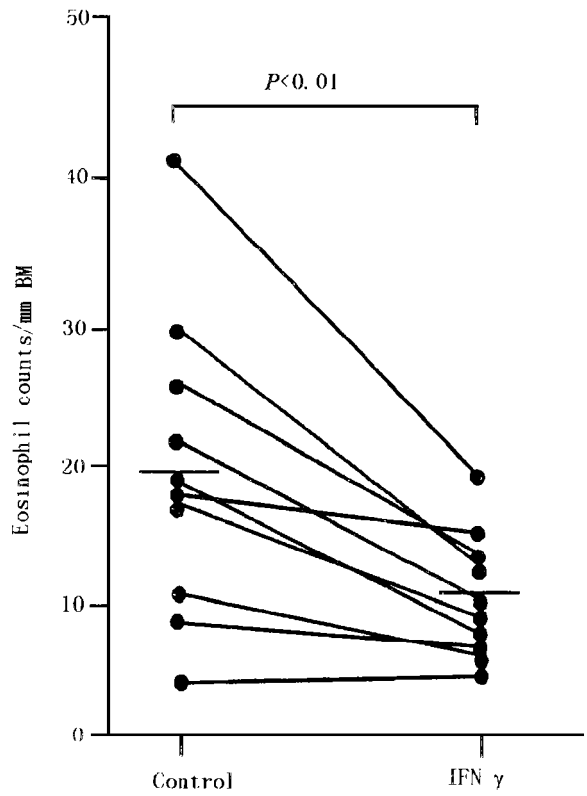

Fig. 2 Comparison of numbers of total eosinophils (BMK-13$^+$ cells) within the bronchial mucosa obtained from control segments (coutrol) or segments treated with hrIFN-γ (IFN-γ), expressed per mm length of basement membrane (BM). Sections were stained immunohistochemically by the alkaline phosphatase-antialkaline phosphatase method. Horizontal bars represent group mean values

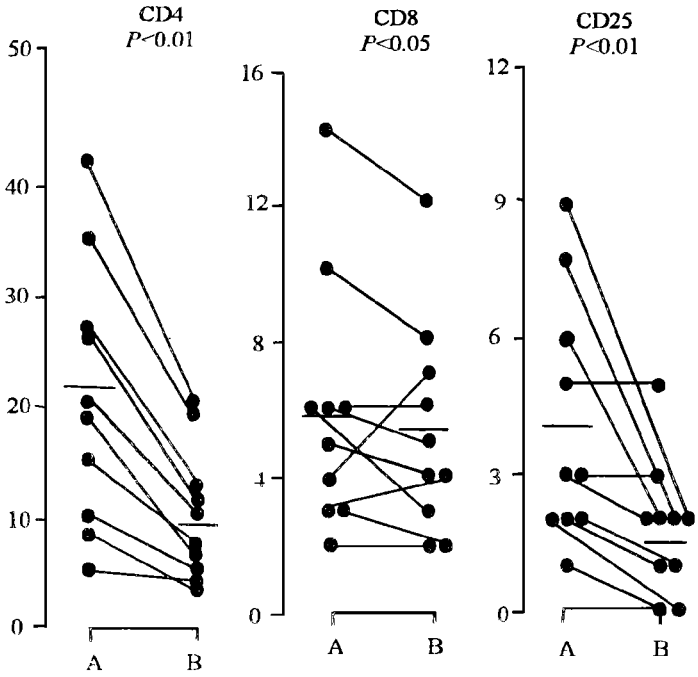

Fig. 3 Numbers of $CD4^+$, $CD8^+$ and $CD25^+$ cells within the bronchial mucosa obtained from control segments (A) or segments treated with hrIFN-γ (B), expressed per mm length of BM. Sections were stained immunohistochemically by the alkaline phosphatase-antialkaline phosphatase method. Horizontal bars represent group mean values

of hrIFN-γ was adopted in this pilot study to obtain a practical effect.

In this study, it showed that the topical administration of hrIFN-γ decreased the eosinophil recruitment into the airways induced by antigen challenge in the patients with mild atopic asthma. We also found that the inhibition of the eosinophil infiltration was associated with the decrement of $CD4^+$ and $CD25^+$ but not $CD8^+$ T cell infiltration.

It has been suggested that $CD4^+$ helper (Th) lymphocytes can be divided into two subsets, Th1 and Th2 cells, on the basis of their different pattern of cytokine secretion[8]. Th1 cells produce IL-2, IFN-γ, and lymphotoxin, etc., Th2 cells produce IL-4, IL-5, IL-6 and IL-10, etc.. Nakajima et al.[9] demonstrated that the in vivo depletion of $CD4^+$ cells by pretreatment with anti-$CD4^+$ monoclonal antibody significantly decreased the eosinophil infiltration induced by antigen inhalation in the airways of sensitized mice. However, the in vivo depletion of $CD8^+$ cells by pretreatment with anti-$CD8^+$ monoclonal antibody had no significant effect on the antigen-induced eosinophil infiltration. Furthermore, pretreatment with anti-murine IL-5 monoclonal antibody also decreased the antigen-induced eosinophil infiltration. Therefore, It could be concluded that $CD4^+$ but not $CD8^+$ cells mediate the antigen-induced eosinophil recruitment in the airway, and that IL-5 also mediates the eosinophils recruitment.

IFN-γ is a Th1-cell-derived cytokine that has been known to inhibit Th2 cell proliferation in vitro[1] and antagonizes in vivo Th2 type responses[10,11]. It has been reported that the inhibitory effect of IFN-γ on the in vivo Th2-type responses occurs at the time of initial $CD4^+$ cells activation during the antigen presentation[12]. Although resting T cells are known to express small numbers of low-affinity IL-2 receptor, the expression of the high-affinity receptor, required for growth and proliferation, is found only after stimulation by appropriate antigen, anti-CD3 monoclonal antibody or lectin[13]. Positive staining is likely to represent only recently activated T cells because IL-2 receptor expression is transient and disappears in the absence of continued stimulation. Thus, the decrease of $CD25^+$ cells indicats that the activation of T cells is inhibited. In this study, we found that hrIFN-γ prevented the antigen-induced eosinophil recruitment into the airways accompanied by decrements of infiltrating $CD4^+$ and $CD25^+$ cells. Taken together with the results of our previous study[2], these results suggested that the inhibitory effect of hrIFN-γ on the antigen-induced eosinophil infiltration into the airways might be due to that hrIFN-γ inhibited $CD4^+$ cell infiltration into the airways, and that hrIFN-γ inhibited T cells (possibly Th2 cells) activation for secreting IL-5 at the time of antigen challenge. On the contrary, it seems unlikely that IFN-γ derectly acted on eosinophils and thereby inhibited the IL-5-dependent eosinophil infiltration because it has been demonstrated that IFN-γ is an activator for eosinophils to prolong the survival and enhance the cytotoxicity[14].

For practical and ethical reasons, it was not possible to perform a time-course study of bronchial mucosal changes after allergen challenge and IFN-γ

administration. We chose the 24-h time point for biopy in order to avoid the peak bronchoconstriction associated with the late asthmatic response. Actually, the challenge of specific antigen in allergic asthmatics caused a progressively increasing late-phase bronchial abstruction which peaked around 7 to 8 h and in some cases persisted for several days[15], with the prominant cellular changes were apparent at 24 h after challenge[16,17].

Recent investigations suggested that the specific targeting of T cells might be beneficial in the treatment of asthma, although corticosteroids are still the mainstay of treatment for asthma[18]. We have shown that a platelet-activating factor antagonist ONO-6240 was able to eliminate the airway allergic inflammation by inhibiting production of IL-5 and IL-2 from T cells[19]. Our findings in this study that the topical administered hrIFN-γ prevented the antigen-induced eosinophil recruitment into the asthmatic airways provided evidence that more specific targeting of $CD4^+$ cells (presumably Th2 cells) by IFN-γ would be useful in the treatment of asthma.

References

1 Gajewski T F, Fitch F W. Antiproliferative effect of IFN-γ in immune regulation: I. IFN-γ inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clone. J Immunol. 1988, 140: 4245.

2 Shi H Z, Zhong X N, Long X M et al. Effect of interferon-gamma on antigen-induced eosinophil infiltration into the mouse airway. Chinese Medical Journal, 1996, 109: 215.

3 The Respiratory Branch of Chinese Medical Association. The definition, diagnosis, degree of severity and criteria of curative effect for bronchial asthma. Chin J Tuberc Respir Dis. 1993, 16 (asthma suppl): 5.

4 Calhoun W J, Jarjour N N, Gleich G J et al. Increased airway inflammation with segmental versus aerosol antigen challenge. Am Rev Respir Dis, 1993, 147: 1465.

5 Hanifin J, Schneider L C, Leung DYM et al. Recombinant interferon-gamma therapy for atopic dermititis. J Am Acad Dermatol, 1993, 28: 189.

6 Boguniewicz M, Schneider L C, Milgrom H et al. Treatment of steroid-dependent asthma with recombinant interferon-gamma. Clin Exp Allergy, 1993, 23: 785.

7 Jaffe H A, Buhl R, Mastrangeli A et al. Organ specific cytokine therapy: local activation of mononuclear phagocytes by delivery of an aerosol of recombinant interferon-γ to the human lung. J Clin Invest, 1991, 88: 297.

8 Mosmann T R, Coffman R L. Th1 and Th2 cells: different patterns on lymphokines secretion lead to different functional propties. Annu Rev Immunol, 1989, 7: 145.

9 Nakajima H, Iwamoto I, Tomoe S et al. CD4+ T-lymphocytes and interleukin-5 mediate antigen-induced eosinophil infiltration into the mouse trachea. Am Rev Respir Dis, 1992, 146: 374.

10 Flinkelman F D, Katona I M, Mosmann T R et al. IFN-γ regulates the isotypes of Ig secreted during in vivo humoral immune responses. J Immunol, 1988, 140: 1022.

11 Belosevic M, Finbloom D S, van der Meide P et al. Administration of monoclonal anti-IFN-γ antibodies in vivo abrogates natural resistance of C3H/HeN mice to infection with Leishmania major. J Immunol, 1989, 143: 266.

12 Scott P. IFN-γ modulates the early development of Th1 and Th2 response in a murine model of cutaneous leishmaniasis. J Immunol, 1991, 147: 3149.

13 Smith K A. The IL-2 receptor. Immunol Today, 1987, 8: 13.

14 Valerius T, Repp R, Kalden JR et al. Effects of IFN on human eosinophils in comparison with other cytokines: a novel class of eosinophil activators with delayed onset of action. J Immunol, 1990, 145: 2950.

15 Newnwn-taylor A J, Davies R J, Hendrick D J et al. Recurrent nocturnal asthmatic reactions to bronchial provocation tests. Clin Allergy, 1979, 9: 213.

16 Varney S R, Jacobson M R, Sudderick R M et al. Immunohistology of nasal mucosa following allergen-induced rhinitis: identification of activated T lymphicytes, eosinophils, and neutrophils. Am Rev Respir Dis, 1992, 146: 170.

17 Frew A J, Key A B. The relationship between infiltrating $CD4^+$ lymphocytes, activated eosinophils and the magnitude of the allergen-induced late-phase cutaneous reaction in man. J Immunol, 1988, 141: 4158.

18 Barnes P J. A new approach to the treatment of asthma. N Engl J Med, 1989, 321: 1517.

19 Shi H Z, Xu H, Liang G R et al. Effects of a platelet-activating factor antagonist ONO-6240 on antigen-induced eosinophil infiltration into the airways. Chin J Inter Med. 1995, 34: 753.

(责任编辑: 蒋汉明)