

Interleukin (IL)-4 Induces Production of Cytokine-induced Neutrophil Chemoattractants (CINC) and Intercellular Adhesion Molecule (ICAM)-1 in Lungs of Asthmatic Rats*

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Summary: The present study aimed to examine the effect of interleukin (IL)-4 on neutrophil chemotaxis in airway inflammation in asthmatic rats and the possible mechanism. Male Wistar rats were intranasally instilled with recombinant rat (rr) IL-4 (rrIL-4) at different doses [2, 4 or 8 µg/animal, dissolved in 200 µL normal saline (NS)] or rrIL-4 at 4 µg/animal (dissolved in 200 µL NS). NS (200 µL) and LPS (6 mg/kg/animal, dissolved in 200 µL NS) were intranasally given respectively in the negative and positive control groups. Moreover, the asthmatic lung inflammation was induced in rats which were then intranasally treated with rrIL-4 (4 µg/animal) or LPS (6 mg/kg/animal). The normal rats treated with different doses of rrIL-4 and those asthmatic rats were sacrificed 6 h later. And animals instilled with rrIL-4 at 4 µg were sacrificed 6, 12 or 24 h later. The bronchoalveolar lavage fluid (BALF) and lungs were harvested for detection of leukocyte counts by Wright-Giemsa staining and lung histopathology by haematoxylin-eosin (HE) staining. The levels of cytokine-induced neutrophil chemoattractant (CINC)-1 and intercellular adhesion molecule (ICAM)-1 in BALF were determined by ELISA. Real-time PCR was used to measure the mRNA expression of CINC-1, CINC-2α, CINC-2β, CINC-3 and ICAM-1 in lung tissues. The results showed that the intranasal instillation of IL-4 did not induce a recruitment of neutrophils in BALF in rats. However, IL-4 could increase the CINC-1 level in BALF in a dose-dependent manner at 6 h. But the mRNA expression levels of CINC-1, CINC-2α, CINC-2β, CINC-3 were not significantly increased in lungs of IL-4-treated rats relative to NS negative control group. Moreover, IL-4 was found to augment the mRNA expression of ICAM-1 in lungs and the ICAM-1 level in BALF at 6 h. However, the increase in CINC-1 and ICAM-1 levels in BALF of IL-4-treated asthmatic rats was not significantly different from that in untreated asthmatic rats. These findings indicate that IL-4 does not directly recruit neutrophils in the rat lungs, but it may contribute to airway neutrophilia through up-regulation of CINC-1 and ICAM-1.

Key words: interleukin-4; cytokine-induced neutrophil chemoattractants; intercellular adhesion molecule-1; rat

Allergic lung diseases are driven and maintained by the persistence of Th2 cells sensitized against allergenic, occupational or viral antigens^[1]. Th2-type cytokines, such as IL-4, IL-5, IL-9 and IL-13, mediate allergic lung inflammation^[2, 3]. IL-4 is a pleiotropic immunomodulatory cytokine secreted by lymphocytes, eosinophils, mast cells, and basophils. It induces B-cell class switching to IgE, airway eosinophilia, and vascular cellular adhesion molecule (VCAM)-1 expression, which contribute to the airway hyperreactivity (AHR) and the airway remodeling^[4]. Neutrophils have been shown to be implicated in the pathogenesis of many inflammatory lung diseases,

including acute respiratory distress syndrome (ARDS), chronic obstructive pulmonary disease (COPD) and asthma. Although it is generally accepted that a Th2 type inflammation occurs in the conducting airways of asthmatic patients, and that neutrophils are the predominant cells in the airway secretions of many asthmatics, ranging from mild to severe, from child to adult^[5-9], little is known concerning the mechanism of neutrophils recruitment and their migration into the allergic lung.

Airway neutrophilia results from the ingress of neutrophils from blood to the inflammatory site. This process is directed by the sequential activation of adhesion molecules, such as selectins, intercellular adhesion molecule (ICAM)-1, VCAM-1, CXC chemokine^[10, 11]. Among them, CXCL-8 is a potent neutrophil recruiting and activating factor. CXCL-8 is also known to possess proangiogenic properties^[12], up-regulate matrix metalloproteinases (MMP)-2 and -9 production and mRNA ex-

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pression in endothelial cells^[13], which contributes to the asthmatic airway inflammation^[14, 15] and airway remodeling^[16, 17]. In the rat, the CXC chemokines that recruit and activate neutrophils are cytokine-induced neutrophil chemoattractants (CINC): CINC-1 (CXCL-1, GRO), CINC-2 α , CINC-2 β and CINC-3 (CXCL-2/3, MIP-2). Among them, CINC-2 α and -2 β differ from each other only in three amino acids at the C-terminus by alternate RNA splicing^[18, 19]. Rat CXCR2 on neutrophils is the unique receptor for four types of CINC, and CINC-1, -2 α , -2 β and -3 exert different biological activities through the common receptor^[20]. Neutrophil recruitment to inflamed vascular endothelia is initiated by selectins that facilitate cell rolling and activation of β_2 integrins (CD11/CD18) that mediate stable adhesion and migration across the blood vessel wall^[21]. Critical to the transition from cell rolling to arrest is a shift in β_2 integrins (CD18) from a low- to high-affinity state primed for binding ICAM-1, which is up-regulated on endothelium in response to cytokine stimulation^[22]. ICAM-1 is shown to play important roles in recruitment of neutrophils into the inflamed airways^[14, 23]. The expression of ICAM-1 on the membrane of bronchial epithelial cells is thought to be involved in leukocyte trafficking and activation during bronchial inflammation^[24].

In a previous study, we found that IL-4 causes neutrophils infiltration by increasing the mRNA expression of CXCL-8, E-selectin and VEGF in equine pulmonary artery endothelial cells (ECs)^[25]. We also reported that IL-4 similarly induces neutrophil recruitment and migration by promoting the expression of CXCL-8 in human pulmonary artery ECs^[26]. However, these effects do not occur in human microvascular ECs and umbilical vein ECs^[26]. It is known that in the pulmonary circulation, there are large numbers of marginated polymorphonuclear leukocytes (PMN) present in the capillaries, and their migration preferentially occurs at that level. Thus, we hypothesized that IL-4 present in the airway lumen directly promotes airway neutrophilia in the rat lungs. Therefore, the aim of this study was to investigate the effect of IL-4 on the inflammatory cells burden in the bronchoalveolar lavage fluid (BALF) and lungs, the CINC and ICAM-1 levels in BALF supernatant and their mRNA expression in lung tissues.

1 MATERIALS AND METHODS

1.1 Animals

Male Wistar rats (200–250 g) were purchased from Hubei Research Center of Experimental Animals [Wuhan, China, certificate number: SCXK (e) 2008-0005]. For animal welfare, the Animal Act “On the Kind Guidance in Experimental Animals” (2006) of Ministry of Science and Technology of China was strictly followed. Rats were housed in groups of five per cage at Animal Center of Tongji Hospital with free access to food and water, and stayed at least 5 days before treatment to allow them to adjust to the environment.

1.2 Experimental Groups

Based on results of preliminary experiments and published references on the model of lung asthmatic inflammation^[27, 28], 3 sessions of experiment were designed:

a dose dependence session, a time dependence session and an asthmatic model session. In the dose dependence session, rats were intranasally instilled with different doses of recombinant rat (rr) IL-4 (rrIL-4) [2, 4 or 8 μ g/animal, dissolved in 200 μ L normal saline (NS), Peprotech, UK] and the animals were sacrificed 6 h later. In the time dependence session, rrIL-4 (4 μ g/animal, dissolved in 200 μ L NS) was similarly instilled intranasally into rats and animals were then sacrificed after 6, 12 and 24 h, respectively. NS (200 μ L) and LPS (6 mg/kg/animal, dissolved in 200 μ L NS, Escherichia, Sigma, USA) were intranasally instilled respectively in the negative and positive control groups^[29]. In the asthmatic model session, the asthmatic lung inflammation was induced in rats as previously reported^[30]. In brief, the rats were sensitized on day 1 and 8 by the subcutaneous administration of 20 mg of ovalbumin (OVA, Sigma, USA) emulsified in 100 mg aluminum hydroxide (Sigma, USA) in a total volume of 1 mL NS; from day 15 the rats were challenged with 2% OVA/NS for 30 min by using the ultrasonic nebulizer once a day for 7 days. After the last challenge with OVA, 200 μ L rrIL-4 (4 μ g/animal, OVA/IL-4 group), LPS (6 mg/kg/animal, OVA/LPS group) and NS (OVA/NS group) was intranasally instilled, respectively. The negative control groups (NS/NS, NS/IL-4 and NS/LPS groups) were only NS sensitized and challenged and then intranasally given 200 μ L NS, rrIL-4 (4 μ g/animal) and LPS (6 mg/kg/animal), respectively. Rats were sacrificed 6 h later.

1.3 Leukocyte Counts in BALF

Bronchoalveolar lavage (BAL) was performed in the left lung of all the rats. Briefly, the rats are anesthetized intraperitoneally with 6% chloral hydrate (300 mg/kg body weight, Tongji Hospital, China); BALF was collected and centrifuged at 1200 r/min for 10 min at 4°C. The supernatant was stored at -70°C and the cell pellet was resuspended in PBS solution in a suitable volume to maintain the cells at a density of 10^6 /mL. Cell supernatants of 100 μ L were centrifuged (1200 r/min for 10 min at 4°C) and then were stained with a Wright-Giemsa solution and 10 μ L supernatants were used for leukocyte counting by using a hemocytometer. The cells were classified as neutrophils, eosinophils, lymphocytes and monocyte-macrophages based on their normal morphology.

1.4 Lung Histopathology

Following BAL, the posterior lobe of the right lung was instilled and fixed with 4% paraformaldehyde overnight. Lungs were then embedded in paraffin, and 8- μ m thick sections were stained with haematoxylin-eosin (HE), and examined by light microscopy.

1.5 ELISA for CINC-1 and ICAM-1 in the BALF

CINC-1 and ICAM-1 were quantified in BALF supernatants using ELISA (Shanghai Westang Biotechnology Co., Ltd, China). Chromophore absorbance (*A* value) at 450 nm was determined in duplicate microtiter wells. CINC-1 and ICAM-1 levels (pg/mL) were obtained from standard curves, using the quantitative “sandwich” enzyme immunoassay technique according to manufacturer’s instructions.

1.6 Lung CINC and ICAM-1 Gene Expression by Real-time RT-PCR

The anterior and middle lobes of the right lung were collected. Total RNA was extracted from 100 mg lung

tissue by the TRIzol reagent method, according to the manufacture protocol (Takara, Japan). The protein- and DNA-free RNA was reverse-transcribed to cDNA using the PrimeScript RT Master Mix Perfect Real Time (Takara, Japan). Reverse transcription reaction mixture (20 μ L) contained 1 μ g RNA, 4 μ L PrimeScript RT Master mix (5 $\times$), and RNA-free ddH₂O. The thermal profile for reverse transcription consisted of 15 min at 37°C, 5 s at 85°C and 4°C forever.

Real-time PCR was performed on ABI7900HT Fast Real-Time PCR System (Applied Biosystems, USA) using SYBR Premix Ex TaqTM kit (Takara, Japan). PCR

mixture contained 2 μ L of reverse transcription product, SYBR Premix Ex Taq (2 $\times$), each gene-specific forward and reverse primers of 0.2–0.4 μ mol/L, and sterile ddH₂O in a total volume of 20 μ L. The thermal profile for β -actin, CINC-1, and ICAM-1 consisted of 30 s at 95°C followed by 40 cycles for 30 s at 95°C and 30 s at 60°C; that for CINC-2 α , - β and -3 consisted of 30 s at 95°C followed by 42 cycles for 30 s at 95°C, 30 s at 58°C and 40 s at 72°C. Gene-specific oligonucleotide primers were listed in table 1. Gene expression was quantified using a modification of 2^(- $\Delta\Delta C_t$) method^[31].

Table 1 Rat gene-specific oligonucleotide primer sequences

Genes	Sequences	Size (bp)	Temperature (°C)
β -actin	Forward GGAGATTACTGCCCTGGCTCCTA	150	60
	Reverse GACTCATCGTACTCTGCTTGCTG		
CINC-1	Forward AGGCTTGCCTTGACCCTGAA	106	60
	Reverse ACGCCATCGGTGCAATCTATC		
CINC-2 α	Forward CCAGCTGAGCTGGGAAAGG	75	58
	Reverse GGATCGCTGCTCTGCTTCA		
CINC-2 β	Forward GAGACGGGAATGCAATTTGTTT	66	58
	Reverse GGTCTGCTAGGAATGTTGTCGAT		
CINC-3	Forward CGGGCAGAATCAAAGAGAAAA	77	58
	Reverse CTCAGACAGCGAGGCACATG		
ICAM-1	Forward CAAACGGGAGATGAATGG	183	60
	Reverse TGGCGGTAATAGGTGTAAAT		

1.7 Data Analysis

Data are presented as $\bar{x} \pm s_{\bar{x}}$. Groups were compared using one-way analysis of variance (ANOVA) followed by Students-Newman-Keuls (SNK) test for post hoc analyses or a nonparametric Kruskal-Wallis ANOVA with Dunn's test as a multiple-comparison method as appropriate. A *P* value less than 0.05 was considered to be statistically significant. In the time dependence session, the variables measured at three time points were compared between each two time points.

2 RESULTS

2.1 Effect of rrIL-4 on Cell Recruitment in BALF

As shown in fig. 1 (a–f), macrophages were the main cells present in BALF in both normal rats and rrIL-4-treated rats. However, in asthmatic and rrIL-4-treated asthmatic rats, eosinophils, neutrophils, lymphocytes and monocytes were found abundant in BALF. Neutrophils were the predominant cells in BALF in normal and asthmatic rats exposed to LPS.

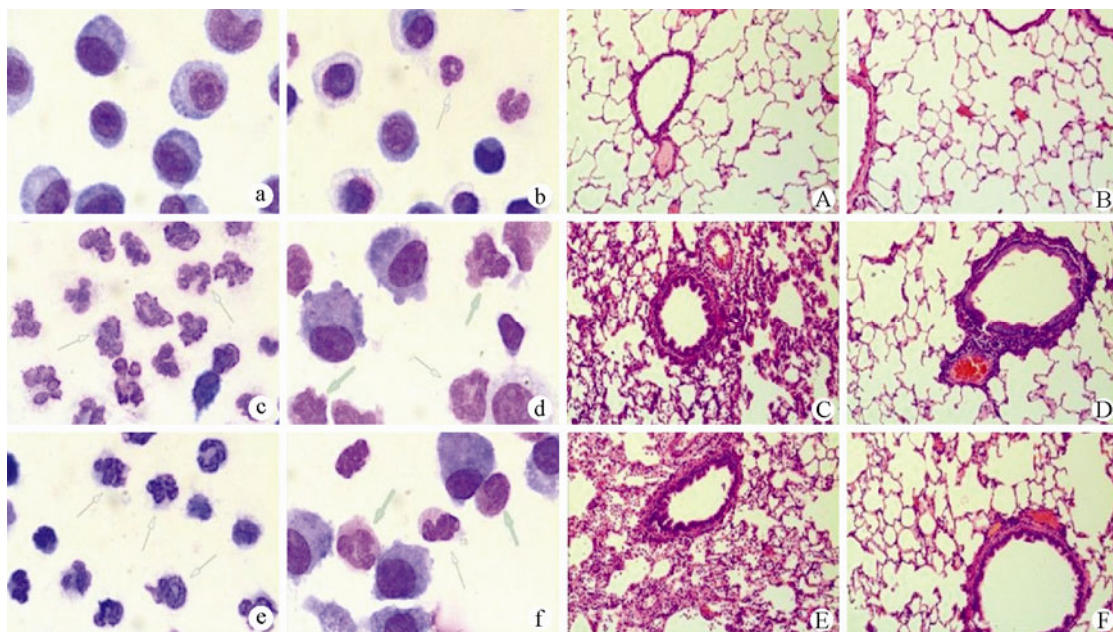


Fig. 1 Effect of rrIL-4 on leukocytes in BALF (a–f, Wright-Gimesa staining, $\times 1000$) and lungs (A–F, HE staining, $\times 100$) in rats a and A: NS negative control group; b and B: rrIL-4-treated group; c and C: LPS-treated group; d and D: OVA/NS group; e and E: OVA/LPS group; f and F: OVA/IL-4 group. Thin arrows indicate the neutrophils and thick arrows the eosinophils.

The total cell number in BALF and the percentages of neutrophils, macrophages, lymphocytes and eosinophils in BALF were not increased in rrIL-4-treated group in comparison to NS negative control group. However, the total cell number and the neutrophil percentage in rats exposed to LPS were increased significantly (fig. 2 and 3, table 2). The total cell number and the percentage

of neutrophils, lymphocytes and eosinophils were significantly increased in OVA/NS and OVA/IL-4 groups when compared with NS/NS group (fig. 2 and 3). LPS treatment substantially enhanced the percentage of neutrophils in asthmatic rats. Moreover, there was no significant difference in the percentage of neutrophils between the OVA/NS and OVA/IL-4 groups (fig. 3).

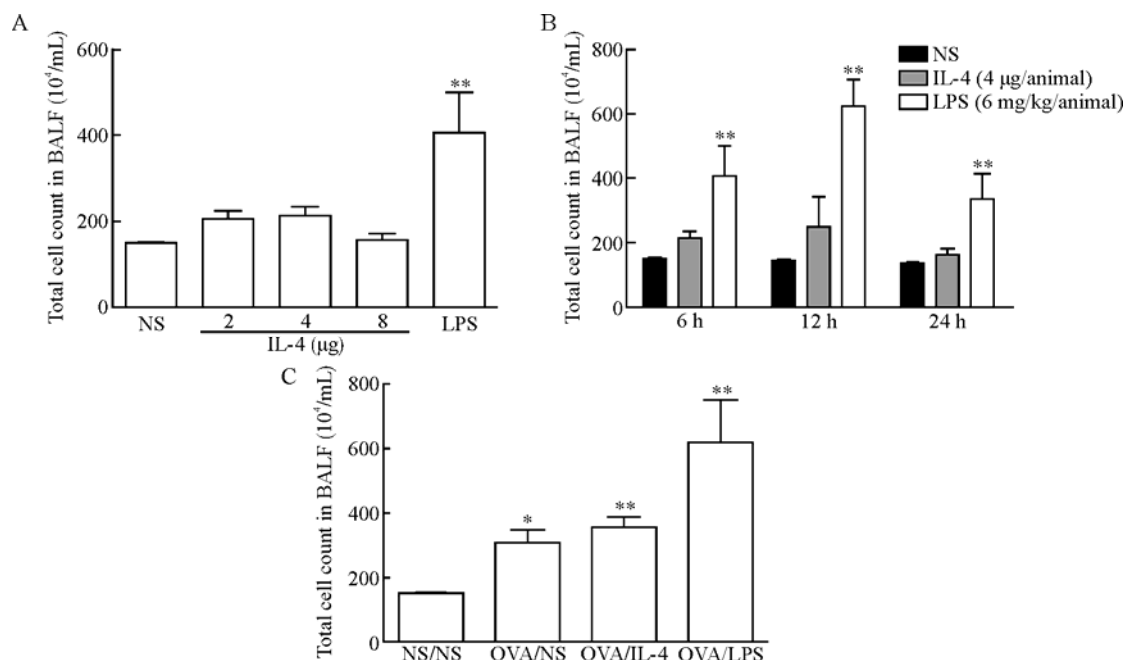


Fig. 2 Effect of rrIL-4 on total cell number in BALF in rats

The total cell number in BALF is shown in the dose dependence session (A), time dependence session (B) and asthmatic model session (C), respectively. Data are expressed as $\bar{x} \pm s_{\bar{x}}$ of more than 8 rats and tested with a nonparametric Kruskal-Wallis ANOVA with Dunn's test as a multiple-comparison method. * $P < 0.05$, ** $P < 0.01$ vs. NS or NS/NS group

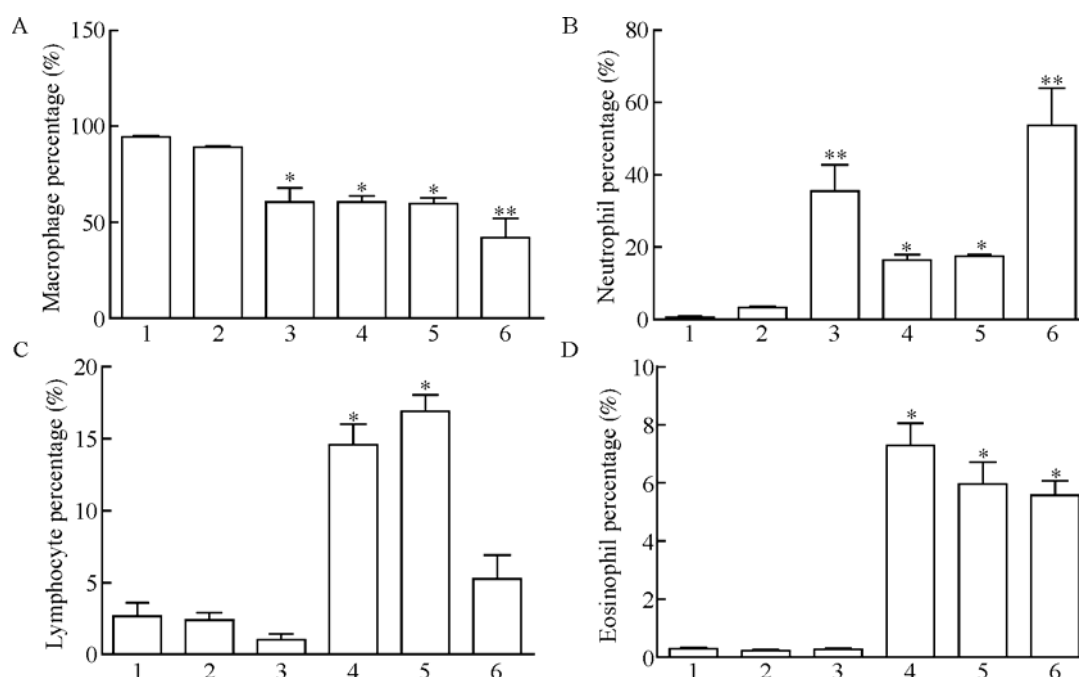


Fig. 3 Effect of rrIL-4 on cell percentage in BALF in rats

A: Macrophage percentage; B: Neutrophil percentage; C: Lymphocyte percentage; D: Eosinophil percentage. Data are expressed as $\bar{x} \pm s_{\bar{x}}$ of more than 8 rats. Data were tested with a nonparametric Kruskal-Wallis ANOVA with Dunn's test as a multiple-comparison method. 1: NS/NS; 2: NS/IL-4; 3: NS/LPS; 4: OVA/NS; 5: OVA/IL-4; 6: OVA/LPS. * $P < 0.05$, ** $P < 0.01$ vs. NS/NS group

Table 2 Effect of LPS on neutrophil percentage in BALF

Groups	Neutrophil percentage (%)		
	6 h	12 h	24 h
NS	0.40±0.18	0.52±0.15	1.95±1.46
LPS	35.44±7.40*	51.33±6.05*	47.14±14.50*

**P*<0.05 vs. NS group

2.2 Effect of rrIL-4 on Lung Histopathology

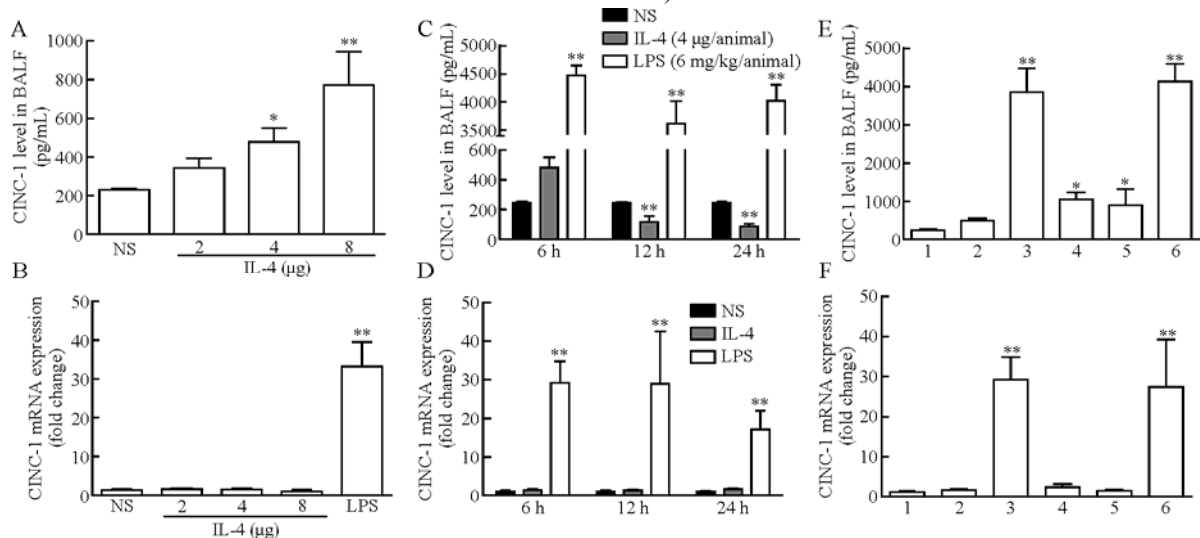
Histopathologic analysis showed that lungs from NS-treated rats had normal alveolar space and alveolar septum (fig. 1A). Moreover, it was found that rrIL-4 at different doses and time points failed to induce the accumulation of neutrophils, lymphocytes and eosinophils in lungs (fig. 1B). However, neutrophils were observed in alveolar spaces in LPS-treated rat lungs, as well as peribronchial inflammation, perivascular edema, ruptured and thickened alveolar walls (fig. 1C).

There was obvious infiltration of inflammatory cells, mainly mast cells, eosinophils, neutrophils, and lymphocytes, surrounding the airways of asthmatic rats (fig. 1D) and rrIL-4-treated asthmatic rats (fig. 1F). The lungs of LPS-treated asthmatic rats were shown to have the predominant presence of neutrophils in alveolar spaces (fig. 1E).

2.3 CINC-1 and ICAM-1 Protein Expression in Rats

ELISA showed that the expression of CINC-1 was increased significantly at 6 h in rats stimulated with rrIL-4 (4 µg/animal) when compared with the NS group. Moreover, at 6 h, rrIL-4 induced the increase in CINC-1 in a dose-dependent manner (fig. 4A). However, the expression of CINC-1 was decreased significantly at 12 and 24 h in rrIL-4-treated rats in comparison with NS-treated ones (fig. 4C). In addition, intranasal instillation of LPS increased the expression of CINC-1 at 6, 12 and 24 h compared with NS-treated rats (fig. 4C).

The expression of CINC-1 in BALF was also increased in asthmatic rats and in LPS-treated asthmatic rats compared with NS-treated rats. However the expression of CINC-1 in rrIL-4-treated asthmatic rats was not significantly different from that in asthmatic rats (fig. 4E).

**Fig. 4** Effect of rrIL-4 on CINC-1 expression in lungs of rat

The graphs show CINC-1 protein level in BALF (A, C, E) and CINC-1 mRNA expression in rat lungs (B, D, F). Data are expressed as $\bar{x} \pm s_{\bar{x}}$ of more than 8 rats. ANOVA followed by SNK test was used to assess significance. 1: NS/NS; 2: NS/IL-4; 3: NS/LPS; 4: OVA/NS; 5: OVA/IL-4; 6: OVA/LPS. **P*<0.05, ***P*<0.01 vs. NS or NS/NS group

The expression of ICAM-1 in BALF was increased in rats stimulated with rrIL-4 at the dose of 4 and 8 µg at 6 h (fig. 5A). However, it returned to basal level at 12 h, and decreased significantly at 24 h (fig. 5C) when compared with NS-treated rats. A significant increase of ICAM-1 level in BALF was also detected at 6 and 24 h in LPS-administrated rats (fig. 5A and 5C).

The protein expression of ICAM-1 in asthmatic rats and asthmatic rats treated with rrIL-4 or LPS was substantially increased in comparison to NS-treated rats. However, the protein expression of ICAM-1 was not significantly different between asthmatic rats and rrIL-4-treated asthmatic rats (fig. 5E).

2.4 CINC-1 and ICAM-1 mRNA Expression in Rats

The lung mRNA expression of CINC-1, -2α, -2β and -3 in rats was not induced by rrIL-4 alone, at either

different doses or different time points. Similarly, they were not up-regulated in asthmatic rats and rrIL-4-treated asthmatic rats in comparison to NS-treated rats. Moreover, the mRNA expression of CINC-1, -2α, -2β and -3 in asthmatic rats was not significantly different from those in rrIL-4 treated asthmatic rats (fig. 4B, 4D and 4F, fig. 6). However, the lung mRNA expression of CINC-1, -2α and -3 was enhanced in LPS-treated normal and asthmatic rats compared with NS-treated rats (fig. 4B, 4D and 4F, fig. 6).

The lung mRNA expression of ICAM-1 was up-regulated in rats treated with rrIL-4 at the dose of 4 and 8 µg at 6 h (fig. 5B). Intranasal instillation of rrIL-4 (4 µg/animal) caused an up-regulation of the lung mRNA expression of ICAM-1 in rrIL-4-treated rats at 6 h, which returned to basal level at 12 and 24 h (fig. 5D). The lung

mRNA expression of ICAM-1 was not up-regulated in asthmatic rats and rrIL-4-treated asthmatic rats compared

with NS-induced rat (fig. 5F).

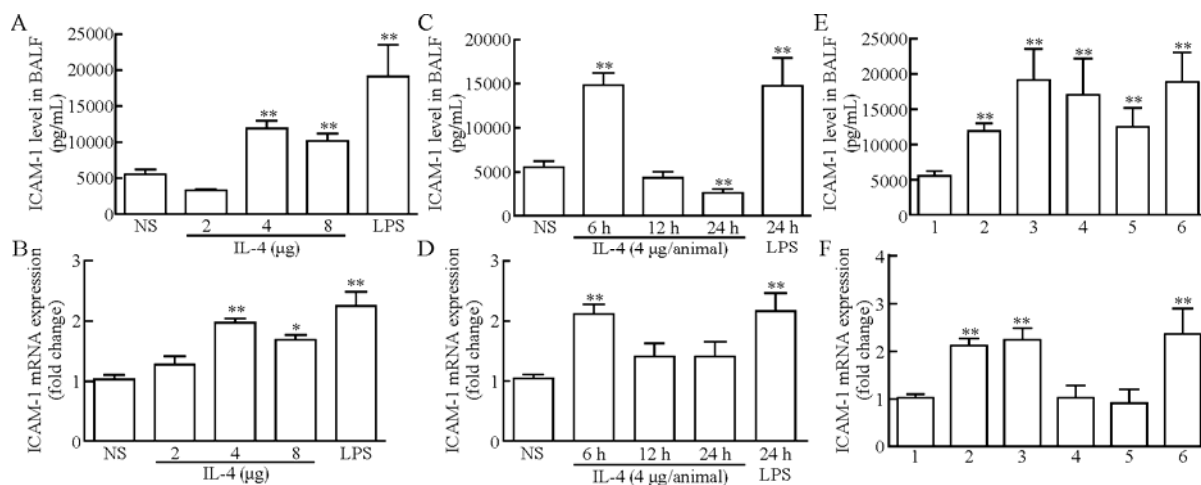


Fig. 5 Effect of rrIL-4 on expression of ICAM-1 in rat lungs

The graphs show ICAM-1 level in BALF (A, C, E) and mRNA expression in rat lungs (B, D, F). Data are expressed as $\bar{x} \pm s_{\bar{x}}$ of more than 8 rats. ANOVA followed by SNK test was used to assess significance. 1: NS/NS; 2: NS/IL-4; 3: NS/LPS; 4: OVA/NS; 5: OVA/IL-4; 6: OVA/LPS. * $P<0.05$, ** $P<0.01$ vs. NS or NS/NS group

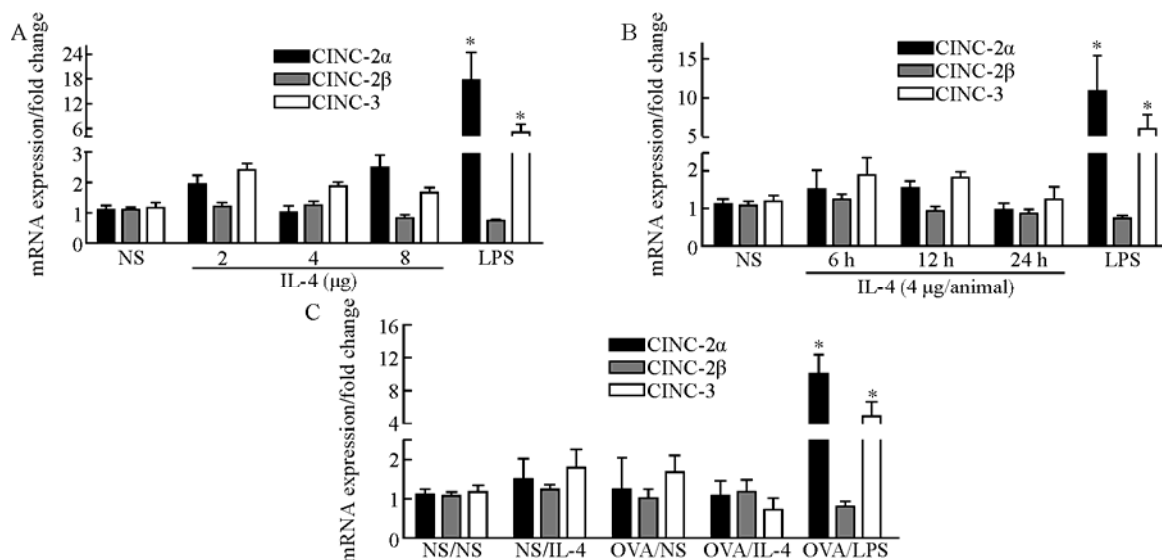


Fig. 6 Effect of rrIL-4 on mRNA expression of CINC-2α, -2β and -3 in rat lungs

A: Effect of rrIL-4 at different doses on mRNA expression of CINC-2α, -2β and -3; B: Effects of rrIL-4 treatment (4 μg/animal) for different time on mRNA expression of CINC-2α, -2β and -3; C: Effect of rrIL-4 (4 μg/animal) on CINC-2α, -2β and -3 mRNA expression in asthmatic rat lungs. Data are expressed as $\bar{x} \pm s_{\bar{x}}$ of more than 8 rats and were tested with a nonparametric Kruskal-Wallis ANOVA with Dunn's test as a multiple-comparison method. * $P<0.05$ vs. NS or NS/NS group

3 DISCUSSION

In the present study, we demonstrated that rrIL-4 neither directly recruited neutrophils, nor macrophages, lymphocytes and eosinophils in the normal and asthmatic rat lungs. However, rrIL-4 induced a dose-dependent increase in CINC-1 expression at post-transcriptional level 6 h after instillation in normal rats. The mRNA expression of CINC-1, -2α, and -3 was not elevated in lungs of rrIL-4-stimulated normal and asthmatic rats. In addition, rrIL-4 induced the lung ICAM-1 expression at both transcriptional and post-transcriptional level at 6 h

in normal rats. Taken together, these results indicate that while IL-4 fails to directly recruit neutrophils in the healthy or asthmatic lungs, it may contribute to airway neutrophilia by increasing the release of CINC-1 and ICAM-1.

IL-4 is a member of CD132-dependent cytokines that have a pleiotropic role in regulation of inflammation. For instance, it was found to inhibit prostaglandin (PG) E2 and cyclooxygenase (COX)-2 protein expression induced by LPS in neutrophils^[32]. It was also reported to promote the production of IL-1 receptor antagonist (IL-1Rα) in neutrophils^[33] and to antagonize the biological action of IL-1 by inducing the expression and release

of IL-1 type II decoy receptor^[34]. In contrast, IL-4 was also found to possess proinflammatory functions, including the recruitment of inflammatory cells by increasing the expression of VCAM-1 on the endothelial surface^[35]. It could also promote chemoattraction of eosinophils, macrophages and B cells^[36–38], enhance phagocytic and bactericidal activity of the mature neutrophil^[39], and induce the mRNA expression of CXCL-8 in neutrophils and the release of chemotactic factors, such as TNF- α ^[40].

Very few studies, however, have investigated the ability of IL-4 itself to promote a neutrophilia response *in vivo*. In the present study, we found that intranasally instilled rrIL-4 did not directly induce airway neutrophilia, or attenuate the airway neutrophilia induced by OVA inhalation challenge. The effects of IL-4 on inflammation appears to be agonist specific, as contrary to our results, that intratracheal administration of IL-4 attenuated the degree of inflammation in the lung of rats challenged with immuno-complexes^[41]. In a murine air pouch model, IL-4 either inhibited the migration of PMN stimulated by IL-1 β ^[42] or induced the migration of neutrophils by an indirect mechanism involving the production of several chemokines, such as CCL2 by at least some of the resident cells^[28]. Our results, however, do not negate an indirect role of IL-4 in airway neutrophilia, as IL-4 was found to promote the expression of CINC-1, a potent neutrophil chemokine, and ICAM-1, which is required for neutrophil transendothelial migration, in the normal rat lungs. These findings are in agreement with the IL-4-induced increased expression of ICAM-1 in various cell lines^[43–47], including human bronchial epithelial cells^[24]. However, this effect is not universal as IL-4 has no effect on the expression of ICAM-1 in human lung fibroblasts^[48] and even decreases its expression in WI-26 epithelial cells^[49].

Crosstalks between neutrophils, neutrophil chemokines, adhesion molecules in asthma had been reported previously. Neutrophil numbers in induced sputum are significantly increased in severe asthma compared with mild asthma and normal controls^[50]. CXCL-8 in induced sputum and BALF is increased in asthmatic patients, with the highest levels observed in severe asthma^[50–52] and acute asthma^[6]. *In vivo*, ICAM-1 expression by bronchial epithelial cells was significantly increased in asthmatic subjects^[49]. Our study showed that neutrophil percentages, CINC-1 and ICAM-1 protein levels in BALF were increased in asthmatic rats and LPS-treated asthmatic rats compared with NS-induced rats. However, the expression of CINC-1 and ICAM-1 in BALF was not increased in IL-4 treated asthmatic rats compared with asthmatic rats. Moreover, the mRNA expression of CINC-1, -2 α , -2 β , -3 and ICAM-1 was not up-regulated in asthmatic and IL-4-treated asthmatic rats in comparison to NS-induced rat. Our results provided evidence that neutrophils, as well as the expression of CINC-1 and ICAM-1 are involved in the persistence and development of allergic lung inflammation in the rat, although IL-4 does not directly accumulate the neutrophils in asthmatic rat lungs.

LPS is the endotoxin of Gram-negative bacteria, which is often used to induce lung neutrophilic inflammation. The migration mechanism of PMN to the lung

following LPS inhalation in mice involves production of inflammatory cytokines by airway cells, such as tumor necrosis factor (TNF)- α and IL-1 β , as well as CINC-1^[53]. The CD18 integrins, one ligand of which is ICAM-1, are adhesion molecules expressed on the cell surface of leukocytes and play a central role in leukocyte migration to zones of inflammation^[54]. CD18 integrins are also involved in PMN migration to the lung and were induced on endothelium cells exposed to LPS, TNF- α , CINC-1 and MIP-2^[55]. In the rat, treatment with Abs to CD11a/CD18, CD11b/CD18, as well as ICAM-1 endothelial cell inhibitor, attenuated the migration of PMNs to intrapulmonary LPS^[56]. Consistent with previous reports that the lung expression of CINC-1, -2 α , -3 and ICAM-1 was up-regulated by intratracheal administration of LPS in the normal rats^[57–61], our study further showed that the lung expression of CINC-1, -2 α , -3 and ICAM-1 remained at high levels for 6–24 h in LPS-treated normal rats. These results suggested that LPS directly stimulated the recruitment of leukocytes, especially neutrophils, within the rat airways and enhanced the airway neutrophilia by increasing expression of CINC-1, -2 α , -3 and ICAM-1.

In conclusion, our results demonstrated that IL-4 cannot directly recruit neutrophils to rat lungs, and it contributes to airway neutrophilia through up-regulation of CINC-1 and ICAM-1 expression in rat lungs. Moreover, LPS induces airway neutrophilia by directly inducing inflammatory cells, especially neutrophils to the rat lungs, and it also increases the CINC-1, -2 α , -3 and ICAM-1 expression in the rat lungs.

Conflict of Interest Statement

The authors declare that there is no conflict of interest with any financial organization or corporation or individual that can inappropriately influence this work.

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