

Effect of Interleukin-5 Receptor- α Short Hairpin RNA-Expressing Vector on Bone Marrow Eosinophilopoiesis in Asthmatic Mice

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ABSTRACT

Bone marrow eosinophilopoiesis induced by interleukin (IL)-5 is a major contributor to eosinophilic airway inflammation in asthma. However, research on the use of IL-5 receptor alpha (IL-5R α) as the target has seldom been reported. This study was undertaken to explore the effects of inhibition of IL-5R α expression through an IL-5R α short hairpin RNA-expressing vector on bone marrow eosinophilopoiesis and airway inflammation in an asthmatic mouse model. An effective plasmid vector was selected that could express short hairpin RNA targeted at IL-5R α (P-IL-5R α). An adenovirus vector (Ad) was then constructed that was inserted in an effective template sequence (Ad-IL-5R α). An animal model of asthma was established by sensitizing and challenging Balb/c mice with ovalbumin. Animals were treated intravenously with Ad-IL-5R α and changes in bone marrow eosinophilopoiesis and airway inflammation were detected in asthmatic mice. Investigators found that P-IL-5R α -3 targeted at the sequence of CAG CTG CCT GGT TCG TCT T markedly suppressed IL-5R α .

expression in B lymphoma cells in vitro. In addition, Ad-IL-5R α could suppress IL-5R α expression in murine bone marrow cells in vitro and in vivo, and it could significantly decrease IL-5-induced eosinophilia in cultured bone marrow cells. Additional studies indicated that intravenously injected Ad-IL-5R α not only selectively reduced the number of eosinophils in the bone marrow, peripheral blood, and bronchoalveolar lavage fluid, it also relieved airway inflammation in asthmatic mice. Results reported here show that blocking of IL-5R α expression through RNA interference can enhance effective treatment of asthma, and that bone marrow can be used as a key targeted organ in the treatment of asthmatic mice.

Keywords: asthma; airway inflammation; bone marrow; IL-5R α ; RNAi

INTRODUCTION

Infiltration of eosinophils in the lungs is a typical pathologic characteristic of asthma.¹⁻³ Eosinophils can release numerous bioactive substances, including leukotrienes, thromboxane, and major basic granule protein, that can cause damage to airway epithelial cells, as well as inflammation, hyperreactivity, and remodeling in the airway.^{4,5}

Eosinophils originate from bone marrow CD34+ cells. Although several cytokines, such as interleukin (IL)-5, IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-9, can affect the production and survival of eosinophils, increasing evidence suggests that IL-5 plays a unique role in the development and persistence of selective airway eosinophilic inflammation.⁶⁻⁸ The unique role of IL-5 in the development, activation, and localization of the eosinophil is further proved by findings that mice deficient in IL-5 are unable to produce an increase in eosinophils in response to specific antigens.⁹ Moreover, eosinophilia cannot be induced by simultaneous injection of normal mice with parasites and neutralizing antibodies of IL-5.¹⁰

By connecting with the IL-5 receptor (IL-5R), IL-5 mediates the terminal differentiation of eosinophils from eosinophil progenitors within the bone marrow. IL-5R with a high affinity consists of 2 subunits: a unique α subunit called IL-5R α , which binds the cytokine with a low affinity, and a β subunit that is shared by IL-3 and the GM-CSF receptor. This β subunit does not bind the cytokine by itself, but it acts as a signal transducer and interacts with the α chain to form a receptor with high affinity. IL-5 is an eosinophil lineage-specific factor in which expression of its receptor on eosinophils and eosinophil progenitors is restricted. This is supported by evidence showing that IL-5R α -deficient mice are unable to respond to parasitic infection with an increased number of eosinophils.¹¹ Patients with mild asthma have specific upregulation of IL-5R α subunit expression on bone marrow-derived CD34+ cells and increased eosinophil progenitor cells in the bone marrow. Therefore, IL-5 has been suggested as a specific therapeutic target for asthma, and in animal models of allergen challenge, monoclonal antibody blockade and gene deletion can effectively reduce airway eosinophilia and hyperresponsiveness.¹² Theoretically, blocking of IL-5R expression can inhibit the bioactivity of IL-5; however, there have been few reports of the use of IL-5R α as the target.

RNA interference (RNAi) is mediated by small interfering RNA (siRNA); this leads to sequence-specific gene silencing within organisms. siRNA is incorporated into a nuclease complex called the RNA-induced silencing complex, which targets and cleaves mRNA that is complementary to siRNA.^{13,14} Because of its exquisite specificity and efficiency, RNAi, as a powerful gene knockdown technique, has attracted much attention. Because siRNA synthesized *in vitro* has short-term action and low efficiency, a new method based on the expression of siRNA from DNA templates has been developed to overcome the weaknesses of siRNA.¹⁵⁻¹⁸ With use of the RNA polymerase III promoter (U6 promoter or H1 promoter), a vector that expresses short hairpin RNA (shRNA) can be constructed, and shRNA can generate siRNA after it is processed by Dicer (another dsRNA-specific Rnase-III-type endonuclease). Thus, the highly restricted experimental condition required by RNA manipulation can be avoided.

The current study was designed (1) to allow construction and selection of an effective plasmid vector that can express shRNA targeted at IL-5R α (P-IL-5R α), and then (2) to construct an adenovirus vector (Ad) that could be inserted in an effective template sequence (Ad-IL-5R α) to improve transfection efficiency¹⁹⁻²¹; investigators further explored the effects of Ad-IL-5R α on IL-5-induced bone marrow eosinophilopoiesis and airway inflammation *in vitro* and *in vivo*. (3) Finally, the specificity and effectiveness of the RNAi method were evaluated to verify its role in inhibiting IL-5R α expression in the treatment of patients with asthma.

MATERIALS AND METHODS

Culture of Cell Lines and Transfection *In Vitro*

Murine B lymphoma cells (Nanjing KeyGen Biotechnology Ltd., Nanjing, China) were cultured in RPMI 1640 medium (HyClone, South Logan, Utah, USA) supplemented with 10% heat-inactivated fetal bovine serum (HyClone), L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μ g/mL) (Gibco BRL, Gaithersburg, Md, USA). During the culture period, cells were transfected with 4 types of P-IL-5R α (Table) and control vector (Genesil Biotechnology Ltd., Wuhan, China) at a concentration of 2 μ g per 2×10^6 cells, mediated by liposome 2000 (Invitrogen, Carlsbad, Calif, USA). Cultures were harvested 48 h after transfection and were stored at -80°C .

Sequences Used for Constructing a Plasmid Vector	
Plasmid Vector	Sequence
P-IL-5R α -1	GGC ATG TTG ATC TAG AGT A
P-IL-5R α -2	CTG ATC GCC AAT AAG TTC A
P-IL-5R α -3	CAG CTG CCT GGT TCG TCT T
P-IL-5R α -4	GAC TTC ATA AGG CGC ATG C

Experimental Animals

Eight-wk-old male Balb/c mice (~23.5 g) were purchased from the Experimental Animal Center of West China Center of Medical Sciences at Sichuan University and were housed in a conventional animal facility. All methods used in this experiment were evaluated and approved by the Animal Ethics Committee at the University.

Preparation and Culture of Bone Marrow Cells

Twenty healthy mice were anesthetized with sodium pentobarbital (70 mg/kg) and were exsanguinated and soaked in 75% alcohol for 10 min. They were then restrained in the supine position on an operating table. The femora were stripped off and both ends of the femora were cut off; bone marrow cells were removed by perfusion of the femora with 3 mL of heparinized Hanks solution. Bone marrow cells were whipped into a cell suspension with a syringe and were washed 3 times in the Hanks solution. The cell suspension was diluted with fresh medium so that a cell density of 10^6 /mL was maintained, and it was cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), streptomycin (100 μ g/mL), and recombinant murine IL-5 (2.5 ng/mL) (Sigma, St. Louis, Mo, USA). Cultures were divided randomly into 6 groups (6 flasks per group) and were transfected by Ad-IL-5R α (Genesil Biotechnology Ltd.). Bone marrow cells were then harvested at 0, 24, 48, 72, 96, and 120 h after transfection. At 2 h before harvest, 200 μ g of 5-bromo-2'-deoxyuridine (BrdU; Roche, Mannheim, Germany) was added to each flask for further examination.

Sensitization and Allergen Exposure Protocol

Fifty Balb/c mice were randomly divided into 5 groups of 10 mice each. All mice were sensitized and challenged with ovalbumin (OVA) according to the modified protocol, as reported previously.²² Mice were immunized at 1 and 8 d (7 d apart) through a peritoneal injection of 0.5 mL sterile saline containing 100 μ g of OVA (Sigma) and 4 mg of aluminum hydroxide. Fourteen days after the first sensitization, mice were challenged 3 times (once every 24 h) by inhalation of 1% OVA through an ultrasound aerification inhaler for 20 min. Four groups of mice were given Ad-IL-5R α via the tail vein 30 min before the last challenge, and 1 of these groups was killed at 24, 48, 72, and 96 h after the last challenge; another group of mice, which acted as a control group, was given sterile saline via the tail vein 30 min before the last challenge and was killed 24 h after the last challenge.

Cell Collection and Sample Processes

All samples were collected at different time points. Mice were anesthetized with sodium pentobarbital (70 mg/kg) and were restrained in the supine position on the operating table. The heart was punctured so that peripheral blood could be collected. After the tracheostomy, the left lung was held with forceps. Bronchoalveolar lavage was performed through instillation of 0.4 mL of sterile saline 3 times through the tracheal cannula; 1.0 mL of bronchoalveolar lavage fluid (BALF) was collected in a 1.5-mL heparinized tube and was stored at 4°C to enhance detection. Femora were removed and bone marrow cell suspension was prepared according to the protocol described earlier. CD34+ cells from 0.5 mL of bone marrow were enumerated through

flow cytometry (PE Labeled Rat–Anti-Mice CD34 monoclonal antibody; MultiScience Biological Co., Suzhou, China); this was expressed as the ratio of CD34+ cells to total nucleate cells. With the cytospin machine, BALF and a portion of the bone marrow cells were prepared on aminopropyltriethoxysilane-coated slides, were fixed in 4% paraformaldehyde for 20 min, and then were stored at –80°C. Remaining bone marrow cells were also stored at –80°C for further use. The right lung was removed, fixed in 10% formalin, and embedded in paraffin for hematoxylin and eosin staining so that airway inflammation could be evaluated.

Total Cell and Leukocyte Differential Counts

BALF, blood, and bone marrow samples were stained with May–Grünwald–Giemsa for the differential leukocyte count. Staining properties and cytoplasmic granulation were similar to those previously described.²³ The differential count was determined through a standard hematologic procedure in which 400 cells were counted under a light microscope. Cells were identified by standard morphologic criteria and were expressed as the relative numbers of leukocytes.

Evaluation of Eosinophilopoiesis

Investigators labeled the newly produced eosinophils with BrdU and Luxol fast blue. BrdU could be incorporated into DNA during the S phase of the cell cycle. The procedure was referenced to a method reported previously.²⁴ Bone marrow cells were fixed in alcohol, and BrdU immunostaining was performed with the specific antibody, according to the manufacturer's protocol (Roche). BrdU-labeled cells were detected with biotinylated rat anti-BrdU monoclonal antibody, and then with streptavidin-biotin peroxidase. Subsequently, cells were visualized with 3,3'-diaminobenzidine. Slides were stained again for eosinophils with Luxol fast blue for 30 min, followed by counterstaining with Mayer's hematoxylin for 30 s. All slides were evaluated in random fields through light microscopy. Cells with nuclear brown staining and green Luxol staining in the cytoplasm were counted as BrdU+ eosinophils; these were expressed as the ratio between BrdU+ eosinophils and eosinophils.

In Situ Hybridization and Immunocytochemistry

To confirm the association of the membrane-bound form of IL-5R α mRNA with CD34+ progenitor cells, in situ hybridization and immunocytochemistry were performed on the same slide; methods were modified as needed.²⁵ The hybridization signal of IL-5R α mRNA was detected by the streptavidin–biotin/alkaline phosphatase complex technique (IL-5R α In Situ Hybridization Kits; Boster, Wuhan, China), and CD34+ immunoreactivity was detected with the use of polyclonal antibodies (rabbit-anti-mouse CD34 antibody; Biotechnology, Inc., Santa Cruz, Calif, USA), which were directed at cell surface markers specific for progenitor cells by the streptavidin–biotin/peroxidase complex (streptavidin–biotin/peroxidase complex Kits; Boster). Positive staining of bone marrow cells for CD34 and IL-5R α mRNA revealed that CD34/IL-5R α mRNA-positive (CD34+/IL-5R α mRNA+) cells exhibited a brown cell membrane and purplish blue cytoplasm. The cell count on these slides was performed by 2 separate investigators in a blinded manner. A total of at least 1000 cells were counted on each slide. Results were expressed as the mean ratio of CD34+/IL-5R α mRNA+ cells to the total number of CD34+ cells.

Analysis of IL-5R α mRNA by Real-Time Reverse Transcriptase Polymerase Chain Reaction

Total RNA was extracted from bone marrow cells with the use of Trizol (Gibco BRL). Reverse transcription was performed according to the manufacturer's instructions (Life Technologies, Inc., Gaithersburg, Md, USA). Real-time analysis to quantify the expression of IL-5R α and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was performed on the Cycle (Bio-Rad, Munich, Germany) with the use of SYBR Green (TaKaRa, Dalian, China). Polymerase chain reaction (PCR) amplification was performed during the following procedures: initial denaturation at 94°C for 5 min, addition of Taq polymerase at 94°C for 30 s, annealation at 58°C for 60 s, extension at 72°C for 60 s for 35 cycles, and extension at 72°C for 7 min (at the end). Specific primers used and expected fragment sizes were as follows: IL-5R α forward 5'-CCT ATA CTA CAG GTT TGG TGT-3', reverse 5'-GCT TGC TTG AGC CAT TAA TGT-3' (152 bp); and GAPDH forward 5'-CCT CAA GAT TGT CAG CAA T-3', reverse 5'-CCA TCC ACA GTC TTC TGA GT-3' (141 bp). A standard curve was generated with a dilution series of a reference complementary DNA sample. This curve was used to calculate relative amounts of target gene expression in tested samples. Quantities of target gene expression in tested samples were normalized to the corresponding GAPDH mRNA transcript in examined cells.

Western Blot Analysis

Total protein was extracted from bone marrow cells for analysis upon expression of IL-5R α , IL-3R α , and GM-CSFR α in the samples. Protein samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis and were transferred to polyvinylidene difluoride membranes. Protein expression degree was determined through the use of enhanced chemiluminescence reagents (Biotechnology, Inc.). The primary antibodies used in this procedure consisted of rabbit-anti-mouse IL-5R α polyclonal antibodies (Biotechnology, Inc.), rabbit-anti-mouse IL-3R α , and rabbit-anti-mouse GM-CSFR α polyclonal antibodies (Abcam Biotechnology, Cambridge, UK).

Statistical Analysis

All data were presented as means $\pm$ standard deviation and were analyzed with the use of the Statistical Package for the Social Sciences, version 10.0 (SPSS Inc., Chicago, Ill, USA). Statistical analysis was conducted through one-way analysis of variance so that variance among more than 2 groups could be evaluated; Student *t* test was used to evaluate variance between 2 groups. *P*<.05 was considered statistically significant.

RESULTS

Selection of Effective P-IL-5R α

To identify an effective P-IL-5R α that was able to block the biological function of IL-5, we designed and constructed 4 types of P-IL-5R α (Table) to transfect B lymphoma cells that had been known to express IL-5R α chain mRNA and protein in vitro. As shown in Figure 1, P-IL-5R α -1, P-IL-5R α -2, and P-IL-5R α -3 were all found

to decrease the expression of IL-5R α mRNA and protein as measured by the real-time reverse transcriptase polymerase chain reaction (RT-PCR) and Western blot, but the effects of P-IL-5R α -1 and P-IL-5R α -2 were weaker than those of P-IL-5R α -3, which decreased the expression of IL-5R α protein by 80% and could be used in additional studies.

Effects of Ad-IL-5R α on IL-5R α Expression of Bone Marrow Cells In Vitro

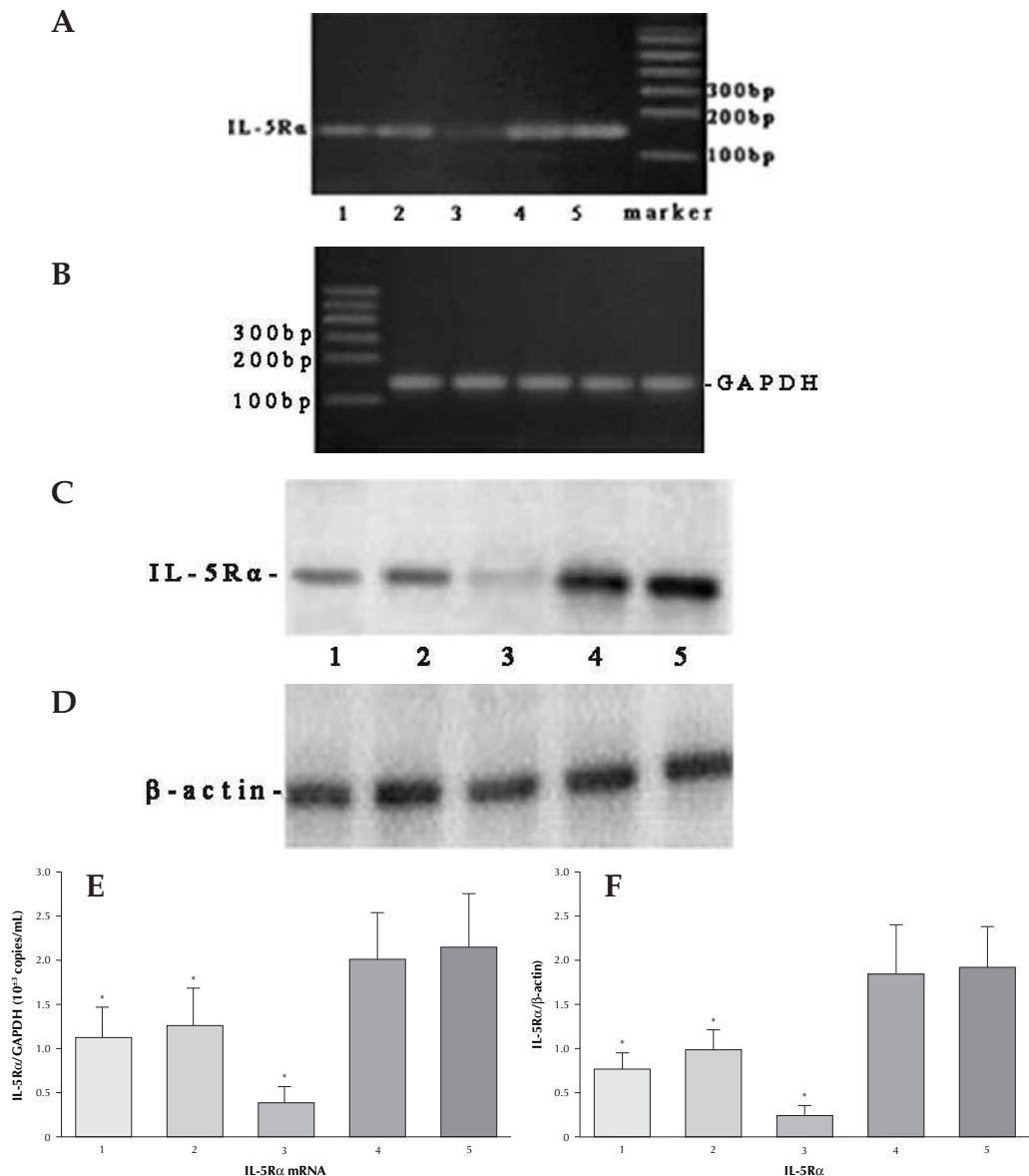
Results obtained so far clearly show that P-IL-5R α -3 was able to markedly down-regulate the expression of IL-5R α mRNA and protein in B lymphoma cells. Therefore, the next important study should be designed to determine whether the RNAi method could also inhibit IL-5-mediated effects on normal bone marrow hematopoietic progenitor cells. To answer this question, investigators co-cultured murine bone marrow cells with Ad-IL-5R α and detected IL-5R α mRNA and protein expression through real-time RT-PCR and Western blot (Figs 2A and 2B). Investigators found that Ad-IL-5R α began to significantly inhibit the expression of IL-5R α mRNA in bone marrow cells at 24 h after culture ($P<.05$), and that this inhibition continued up until 96 h later; after that time, cells started to re-express a new mRNA for this cytokine receptor. As expected, the maximum inhibition level of IL-5R α protein expression was reached at 48 h after culture; this revealed a similar inhibition profile with a mild delay.

To further evaluate the specificity of Ad-IL-5R α , protein expressions of IL-3R α and GM-CSFR α were detected by Western blot (Fig 3). Results revealed no noticeable change in protein expression of IL-3R α or GM-CSFR α among Ad-IL-5R α -treated bone marrow cells at 48 h, when compared with the corresponding index of the control group ($P>.05$).

Effects of Ad-IL-5R α on Eosinophilopoiesis of Murine Bone Marrow In Vitro

For further analysis of the inhibitory effects of Ad-IL-5R α on eosinophilopoiesis in vitro, bone marrow cells transfected by Ad-IL-5R α were analyzed for eosinophils and newly produced eosinophils (BrdU+ EOS) at various time points for 120 h. Numbers of eosinophils and BrdU+ EOS were markedly decreased in cultures from 48 h to 120 h (Fig 4; see also 5A and 5B) compared with the corresponding index of the 0 h group (control group) ($P<.05$). Results attained by flow cytometry showed that Ad-IL-5R α did not significantly change the ratio of CD34+ to nuclear cells at various time points (Fig 5A). Staining of bone marrow cells for CD34 and IL-5R α mRNA revealed that CD34+/IL-5R α mRNA+ cells exhibited a brown cell membrane and purplish blue cytoplasm, and that their count was markedly decreased from 48 h to 96 h after transfection (Figs 5A and 6) ($P<.05$).

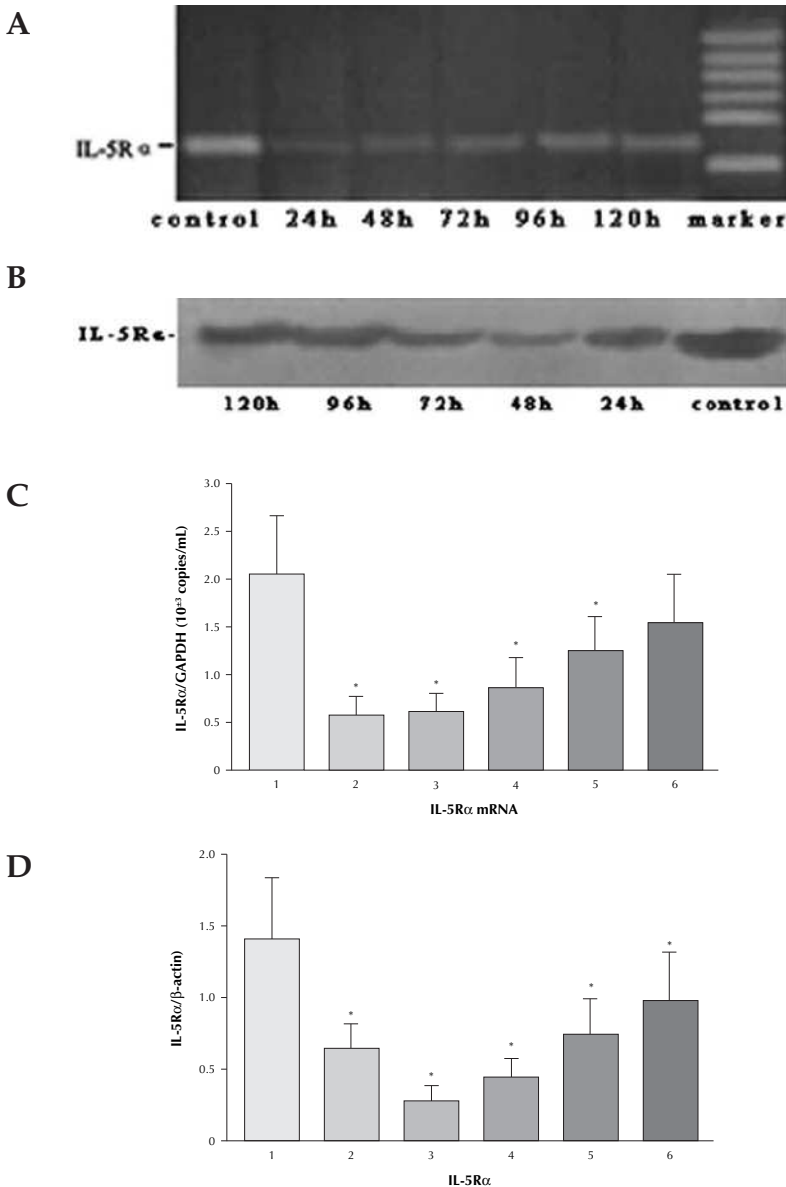
Fig 1. Analysis of IL-5R α mRNA (A) and protein (C) expression in B lymphoma cells that were transfected by P-IL-5R α -1 (lane 1), P-IL-5R α -2 (lane 2), P-IL-5R α -3 (lane 3), P-IL-5R α -4 (lane 4), and a control vector (lane 5).



GAPDH (B) and β -actin (D) were used as internal controls for quantitative RT-PCR and Western blot, respectively. The IL-5R α mRNA level (E) was expressed as the ratio of relative copies between IL-5R α and GAPDH mRNA; the IL-5R α protein level (F) was expressed as the ratio of optical density between IL-5R α and its corresponding β -actin expression.

* $P < .05$, compared with the control vector group.

Fig 2. Expression of IL-5R α mRNA (A) and protein (B) in cultured bone marrow cells at various time points after Ad-IL-5R α transfection.

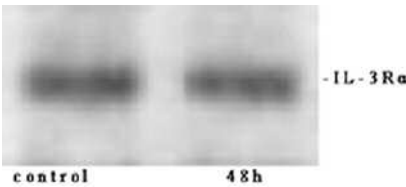


The IL-5R α mRNA level (C) was expressed as the ratio of relative copies between IL-5R α and GAPDH mRNA. The IL-5R α protein level (D) was expressed as the ratio of optical density between IL-5R α and its corresponding β -actin expression.

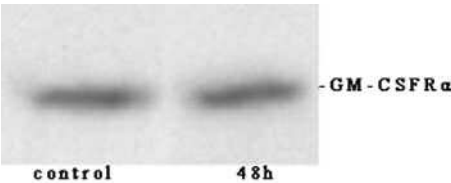
* P <.05, compared with the control group.

Fig 3. Western blot of IL-3R α (A) and GM-CSFR α (B).

A

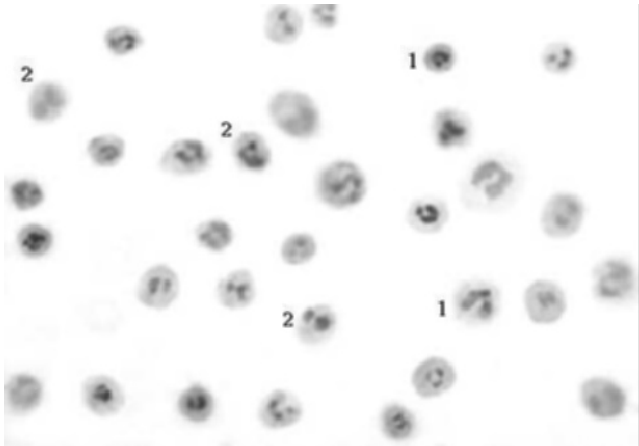


B



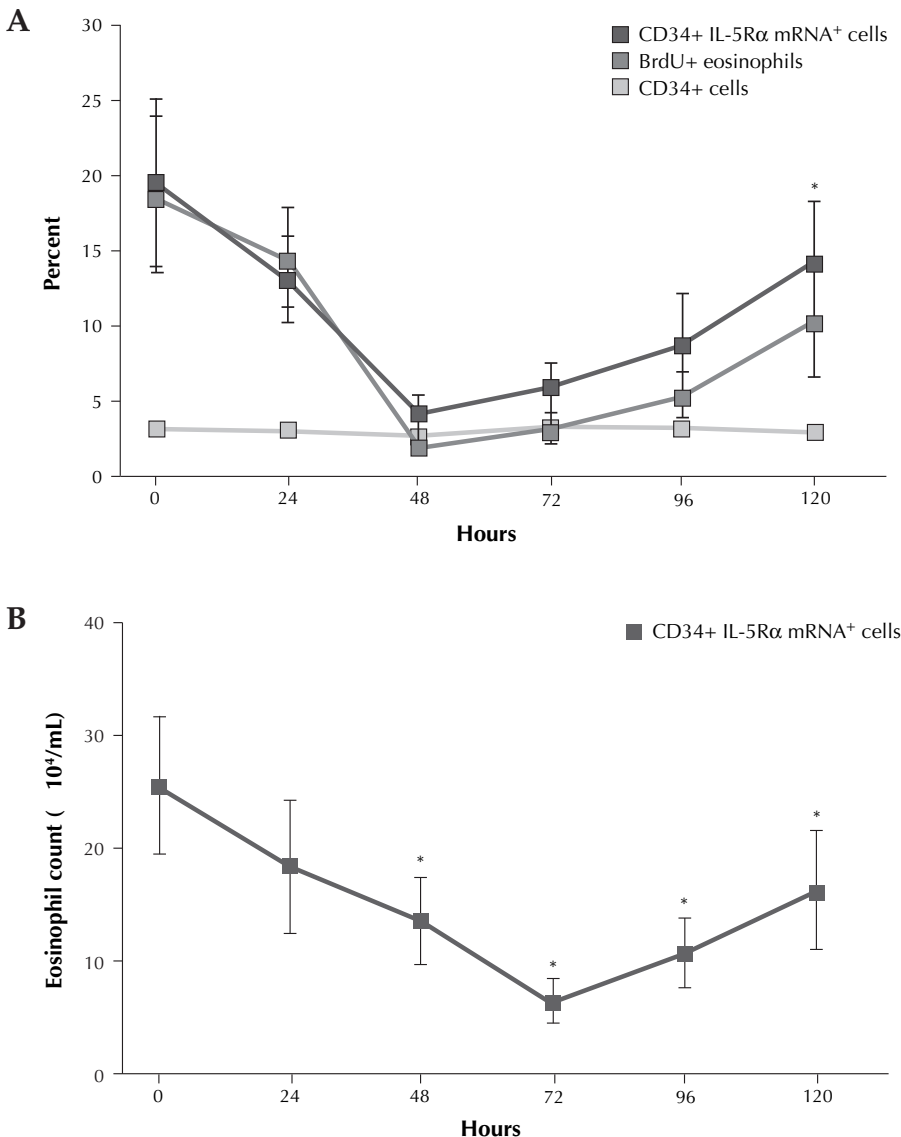
No changes were noted in expression of IL-3R α and GM-CSFR α in cultured bone marrow cells at 48 h after Ad-IL-5R α transfection when a comparison with the corresponding index of the control group was undertaken.

Fig 4. Bone marrow cells with double staining for BrdU and eosinophils in an asthmatic mouse model (original magnification, $\times 400$).



Cells with nuclear brown staining and green Luxol staining in the cytoplasm were counted as BrdU+ eosinophils (BrdU+ eosinophil, 1; BrdU- eosinophil, 2). Data were expressed as the ratio between BrdU+ eosinophils and eosinophils.

Fig 5. Changes in the cytology of cultured bone marrow cells at different time points after Ad-IL-5R α transfection.

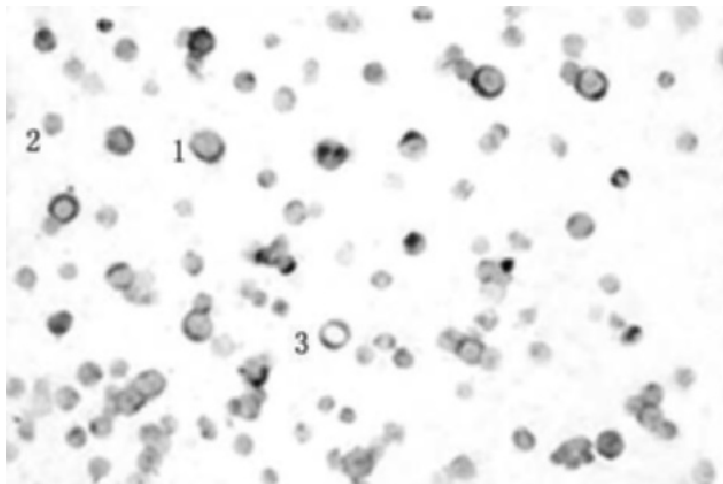


Cells were counted through different methods, as described in the "Materials and Methods" section. Data shown consist of means $\pm$ standard deviation ($n=6$, per time point). Ad-IL-5R α reduced the numbers of CD34⁺/IL-5R α mRNA⁺ cells and BrdU⁺ eosinophils (A); it also decreased eosinophil numbers (B) in cultured bone marrow cells.

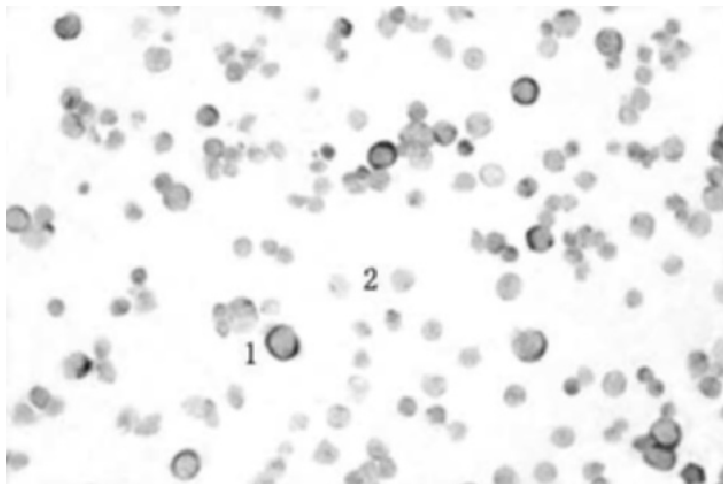
* $P<.05$, compared with the control group.

Fig 6. In situ hybridization and immunocytochemistry staining for CD34+ IL-5R α mRNA+ cells in cultured bone marrow cells (original magnification, $\times 400$).

A



B

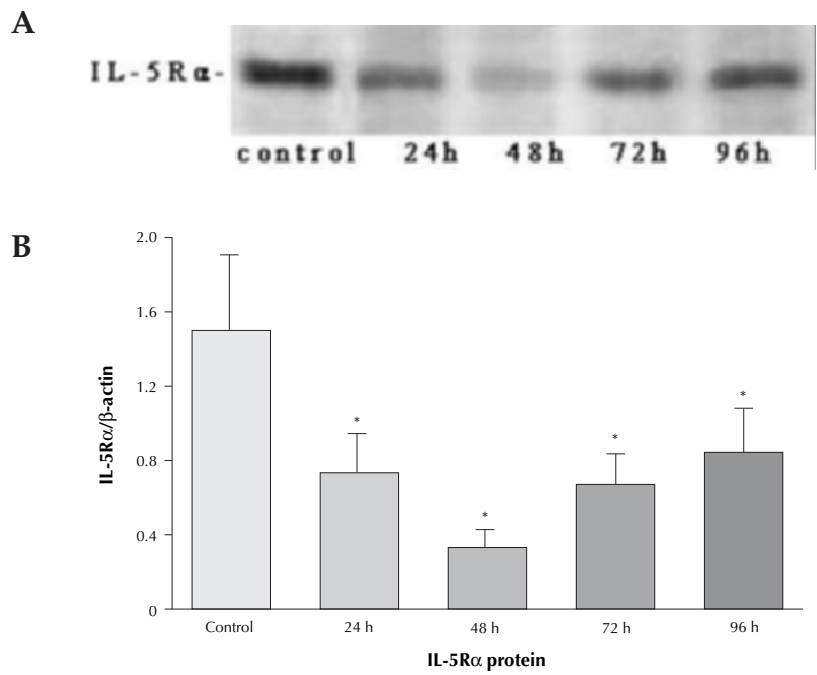


Detailed staining methods are described in the “Materials and Methods” section. CD34+/IL-5R α mRNA+ cells exhibited brown cell membrane together with purplish blue cytoplasm (CD34+/IL-5R α mRNA+ cells, 1; CD34+ cells, 2; IL-5R α mRNA+ cells, 3). Representative photomicrographs show double staining of bone marrow cells in the control group (A) and in the 48-h group (B).

Effects of Ad-IL-5R α on Eosinophilopoiesis and Airway Inflammation of Asthmatic Mice In Vivo

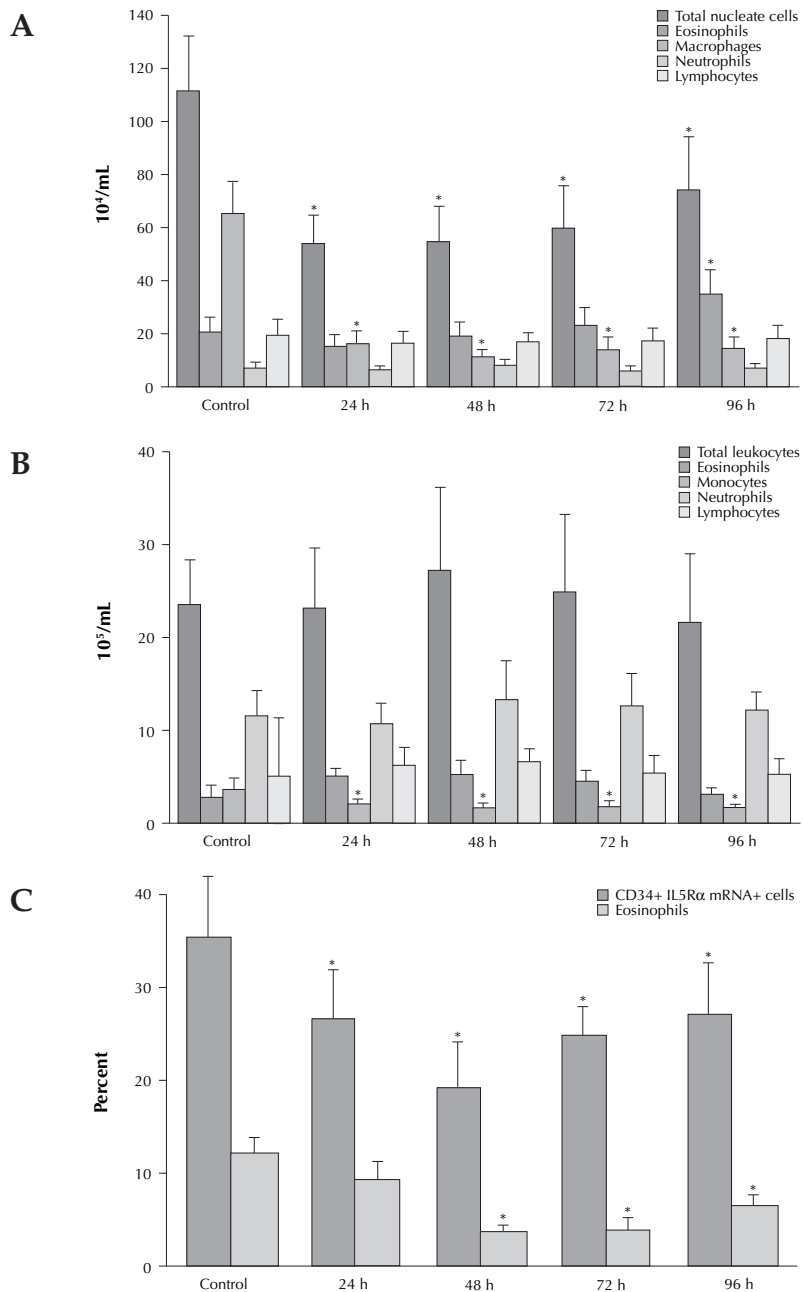
To determine the effectiveness of Ad-IL-5R α on bone marrow eosinophilopoiesis and airway inflammation in vivo, asthmatic mice were injected intravenously with Ad-IL-5R α via the tail vein and were evaluated for 96 h. Results showed that Ad-IL-5R α similarly inhibited the expression of IL-5R α protein within the bone marrow of asthmatic mice in vivo (Fig 7). The results shown in Figure 8 indicate that, compared with the index of the control group, eosinophils in the blood and bone marrow (Figs 8B and 8C) began to decrease in mice in the 24-h group and reached the lowest degree in the 48-h group ($P<.05$). The reduction lasted for 96 h, and eosinophils in BALF (Fig 8A) began to significantly decrease in mice in the 48-h group ($P<.05$); this continued up to 96 h. CD34+/IL-5R α mRNA+ cells in the bone marrow decreased from 24 h to 96 h after Ad-IL-5R α treatment. Moreover, investigators did not find that Ad-IL-5R α treatment had a remarkable effect on leukocyte differential count in BALF and the peripheral blood, except in eosinophils. In addition, hematoxylin and eosin staining of the lungs revealed that, to some extent, Ad-IL-5R α treatment relieved airway inflammation in asthmatic mice (Fig 9).

Fig 7. Western blot analysis showed the effects of Ad-IL-5R α on IL-5R α expression in the bone marrow cells of asthmatic mice.



The IL-5R α protein level was expressed as the ratio of optical density between IL-5R α and its corresponding β -actin expression. Data presented consist of means $\pm$ standard deviation (n=10).
* $P<.05$, compared with the control group.

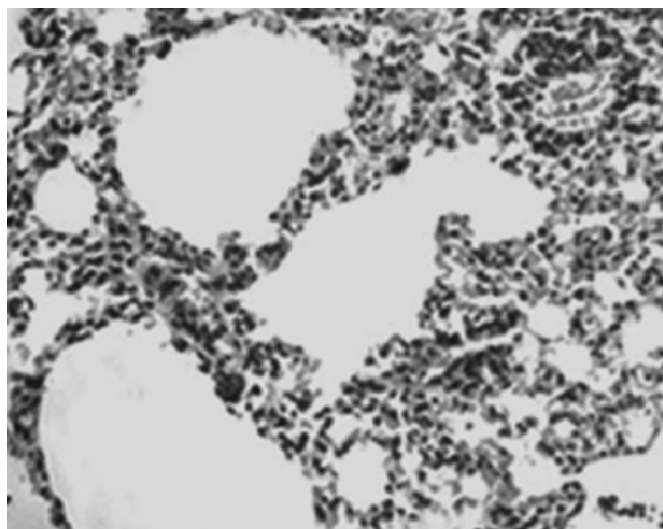
Fig 8. Effects of Ad-IL-5R α on the enumeration of inflammatory cells in the BALF (A), peripheral blood (B), and bone marrow (C) of asthmatic mice.



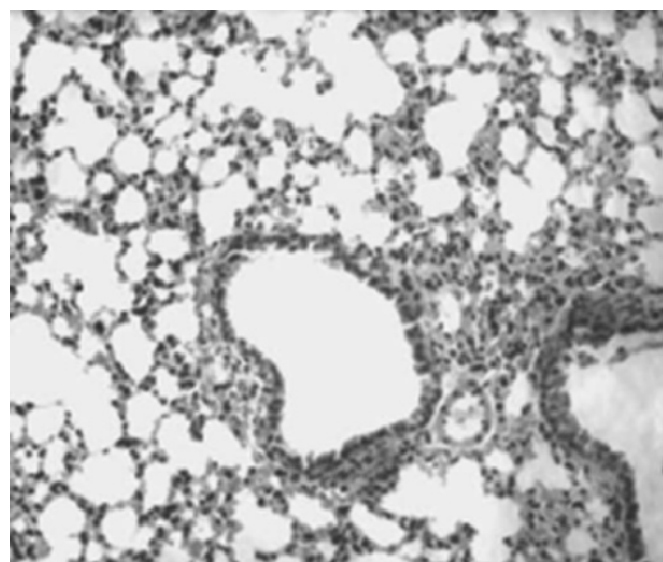
Cells were counted through different methods, as described in the “Materials and Methods” section. Data shown consist of means $\pm$ standard deviation (n=10).
 * $P < .05$, compared with the control group.

Fig 9. Hematoxylin and eosin staining of murine lung tissue (original magnification, $\times 200$).

A



B



Remarkable airway inflammation in a representative untreated asthmatic mouse (**A**). After Ad-IL-5R α treatment was provided, airway inflammation was relieved in a representative asthmatic mouse (**B**).

DISCUSSION

RNAi is an important technique by which RNA is used to suppress gene expression. Because of its exquisite specificity and efficiency, the RNAi technique has gained much attention in investigations of allergy and asthma treatment. The first report on the presumable benefit of RNAi in allergic disease revealed that Bruton's tyrosine kinase (Btk) protein, a nonreceptor tyrosine kinase, played a vital modulatory role in many cellular processes, and that it could be transiently depleted after transfection of Btk siRNA duplexes. Histamine release in mast cells was reduced by 20% to 25% when Btk downregulation occurred.²⁶

Many studies have confirmed that IL-5 can especially induce eosinophil differentiation from CD34+ hematopoietic cells within the bone marrow, and researchers have deduced that the IL-5R α chain may be a characteristic marker of eosinophil lineage-committed progenitors; thus, CD34+ progenitors that express the IL-5R α chain may be the eosinophil progenitors.²⁷⁻²⁹ Previous research has also indicated that the bone marrow response makes a major contribution to eosinophilic airway inflammation.^{30,31} Therefore, IL-5R α may be an important target in the control of bone marrow to produce a greater number of eosinophils, as well as in the reduction of eosinophil trafficking into inflamed tissues.

In the present study, investigators first selected an effective P-IL-5R α by constructing and testing 4 different types of P-IL-5R α while targeting different sequences of IL-5R α mRNA. P-IL-5R α was found to effectively transfect B lymphoma cells. Among these types, P-IL-5R α -3 showed the strongest inhibition of expression of IL-5R α (reduced by 80%); therefore, its template sequence can be used for further study. The present study has shown that the shRNA method can be used successfully. Stat6 is a key transcription factor that can play a role in the development of Th2 cells. Tahvanainen et al³² reported that introduction of an shRNA targeting Stat6 resulted in impaired Th2 cell differentiation. These results have further proved that the RNAi technique is an efficient method for suppression of gene expression.

However, plasmid-based shRNA expression has limitations in cases in which transfection efficiency is low. To overcome this problem, viral vectors were used to deliver shRNA expression.^{33,34} Ad is commonly used for in vitro gene transfer and in vivo gene therapy.^{20,35} Investigators further constructed Ad-IL-5R α according to the targeting sequence of P-IL-5R α -3. To verify the effectiveness of P-IL-5R α -3 in healthy mammalian cells, investigators co-cultured murine bone marrow cells with Ad-IL-5R α in vitro. Results showed that Ad-IL-5R α could selectively suppress IL-5R α mRNA and protein expression for 5 d consecutively and could markedly inhibit IL-5-induced eosinophilopoiesis. In that IL-5, IL-3, and GM-CSF all participate in the differential regulation of bone marrow progenitor cells, their receptors contain the common β chain and specific α chain. The effectiveness of Ad-IL-5R α in the expression of IL-3R α and GM-CSFR α was also explored; investigators found that closely related receptors, including IL-3R α and GM-CSFR α , were not markedly changed in cultures after treatment with Ad-IL-5R α . These findings further confirmed the specific effects of Ad-IL-5R α and suggested that inhibition of IL-5R α expression may be critical in controlling the bone marrow to increase production of eosinophils. Because eosinophils play a key role in the chronic airway inflammation of asthma, investigators presumed that airway inflammation could be relieved by blocking IL-5R α expression. Moreover, the high specificity and efficiency of the RNAi technique ensured that the method had good prospects for future use.

To prove the above-mentioned hypothesis, researchers intravenously injected Ad-IL-5R α into the caudal veins of asthmatic mice and found that Ad-IL-5R α treatment markedly inhibited IL-5R α expression in the bone marrow, and that eosinophils were decreased in the BALF, peripheral blood, and bone marrow of experimental mice, resulting in relieved airway inflammation in asthmatic mice. This result corresponds well with that obtained in the previous study, that is, that IL-5R α antisense oligonucleotides can suppress the development of eosinophilia in mice with ragweed-induced allergic peritonitis³⁶; however, the study cited here did not further explore the bone marrow response. These findings are consistent with the idea that IL-5 is most important in the production and migration of eosinophils. After observing mice treated with Ad-IL-5R α , the investigators concluded that the decreased eosinophil count in the peripheral blood and bone marrow occurred earlier than in BALF. It was presumed that the bone marrow might be the major targeted organ of Ad-IL-5R α that suppresses eosinophilopoiesis in the bone marrow, causing decreased infiltration of eosinophils into the lungs. Previous studies revealed that IL-5R α could not be detected in the lung tissue of allergen-exposed mice examined by immunohistochemistry; however, staining was prominent in samples from the bone marrow.²³ Thus, the expression of IL-5R α is more prominent in the bone marrow than in BALF, which suggests that the main target tissue of Ad-IL-5R α may be the bone marrow; otherwise, Ad-IL-5R α did not influence the differential count of leukocytes, except eosinophils in the BALF and blood. This result suggested that treatment targeted at IL-5R α was highly specific, but that the safety and long-term curative effects attained by blocking IL-5R α expression require further study.

CONCLUSIONS

Because IL-5R α can be highly expressed in the bone marrow, and because bone marrow eosinophilia has a prominent effect on asthmatic airway inflammation, IL-5R α may be an effective target for blocking IL-5-induced bone marrow eosinophilopoiesis. The RNAi technique can (1) suppress the expression of IL-5R α in vitro and in vivo, (2) decrease the production of eosinophils from the bone marrow, and (3) relieve airway inflammation in asthmatic mice. Therefore, if the safety and long-term therapeutic effects of the RNAi technique can be confirmed, this technique will provide an alternative approach for controlling IL-5-mediated disease, including asthma.

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