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Effect of chemically modified IL-13 short interfering RNA on development of airway hyperresponsiveness in mice

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Background: RNA interference is an endogenous cellular mechanism in which short interfering RNAs (siRNAs) direct the sequence specific degradation of a target mRNA. siRNAs can be synthesized with chemical modifications to increase stability and reduce double-stranded RNA–induced immune responses without affecting their ability to elicit degradation of target mRNA. Objectives: This study examined the use of chemically modified siRNAs in a mouse model of allergen-induced airway hyperresponsiveness. Methods: Chemically modified siRNAs were designed and screened in a cell-based reporter assay. The most potent siRNAs were then screened in bone marrow–derived mast cells to demonstrate efficacy in primary cells. Results: A candidate siRNA was formulated and administered to sensitized mice just before airway challenge with allergen. Administration of the siRNA was shown to reduce airway resistance significantly in sensitized and challenged mice by 60%, whereas a control siRNA had no effect. Conclusion: These data demonstrate the effectiveness of introducing targeted siRNAs to prevent induction of allergen-induced airway dysfunction and suggest potential therapeutic applications. (J Allergy Clin Immunol 2008;121:88-94.)

Key words: siRNA, IL-13, AHR, eosinophilia

Asthma is a heterogeneous respiratory disease characterized by airway inflammation, airway hyperresponsiveness (AHR), and reversible obstruction. Despite the introduction and modification of new and established asthma treatments, including inhaled corticosteroids, leukotriene modifiers, and anti-IgE, a significant proportion of patients require additional therapy. As a result, there remains a continuing need for additional compounds that may function through novel mechanisms. Mouse models of allergic airway inflammation and AHR have been developed to characterize the potential cellular and molecular mediators that contribute to the establishment and maintenance of the inflammatory and hyperresponsive state. Although these models do not completely mimic the human disease state, they do provide a means by which to test the potential benefit of targeting disease mediators and exploring new therapeutic technologies.

RNA interference is a cellular mechanism in which a short interfering RNA (siRNA) elicits the sequence specific degradation of a complementary mRNA target. Chemically synthesized siRNAs designed to target a gene of interest can lead to a reduction of the target mRNA to nearly undetectable levels. Several studies have demonstrated the effectiveness of synthetic siRNAs in halting the progression of respiratory viruses including influenza,2,3 respiratory syncytial virus (RSV),4,5 and severe acute respiratory syndrome (SARS). A limited number of studies have demonstrated the effectiveness of synthetic siRNAs in nonviral conditions, including ischemia reperfusion injury and bleomycin-induced pulmonary fibrosis.4,6 The use of siRNA in the treatment of allergen-induced AHR has not been previously reported.

Chemically modified siRNAs have several advantages over their unmodified counterparts. Chemical modifications can be used to stabilize siRNAs to serum nucleases, improve duration of effect, and reduce off-target effects. However, recent publications demonstrate that chemically modified siRNAs can be used to avoid the dsRNA-induced immune response associated with some unmodified siRNA sequences.10,12 The ability of certain unmodified siRNAs to elicit the production of inflammatory mediators, including IFN-γ, TNF-α, and IL-6, is of particular concern in respiratory and immunologic studies because many of the possible end-points are sensitive to the release of such cytokines. Several studies have shown that the introduction of chemical modifications can abolish dsRNA-induced cytokine production. Recent reports demonstrate that it is possible to identify potent siRNAs bearing 2’ modifications on all, or

Abbreviations used

AHR: Airway hyperresponsiveness
BAL: Bronchoalveolar lavage
BMMC: Bone marrow–derived mast cell
dsRNA: Double-stranded RNA
GFP: Green fluorescence protein
HE: Hematoxylin-eosin
MCh: Methacholine
OVA: Ovalbumin
PAS: Periodic acid-Schiff
RL: Lung resistance
siRNA: Short interfering RNA

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nearly all, nucleotides. These modified siRNAs do not elicit a dsRNA-induced immune response, whereas unmodified siRNAs of the same sequence induce the production of IFN-γ, TNF-α and IL-6.

IL-13 is a pleiotropic cytokine that is associated with several aspects of inflammation and AHR. The key role of IL-13 in airway inflammation and AHR is illustrated by experiments in which mice treated with recombinant IL-13 develop eosinophilia, mucus hypersecretion, and AHR.14,15 In contrast, interference with IL-13 or genetic elimination of IL-13 prevents development of AHR and eosinophilic inflammation.16,17 IL-13 acts through several mechanisms. The proinflammatory role of IL-13 is mediated through the induction of vascular cell adhesion molecule-1 and numerous chemokines (eotaxin, macrophage inflammatory protein-1α, thymus and activation-regulated chemokine, and monocyte chemoattractant proteins) that serve to recruit and activate inflammatory cells.18 However, some of the effects of IL-13 appear to be independent of inflammatory cell recruitment. Several studies suggest that IL-13 can induce AHR in the absence of inflammatory cells,18-20 potentially through a direct effect on airway smooth muscle.21 In addition to airway inflammation, IL-13 is involved in mucus secretion and therefore may play a role in airway remodeling in chronic asthma.22

The current study examined the consequences of administering a stabilized IL-13 siRNA to sensitized and challenged mice and showed the effectiveness of this approach in preventing the development of allergen-induced AHR.

METHODS
siRNA sequence and preparation
siRNAs were synthesized by Sima Therapeutics (Boulder, Colo) using standard synthesis techniques.23 Duplexed siRNAs were prepared by mixing the complementary strands in equimolar concentrations in buffer containing 10 mmol/L HEPES pH 7.5, 100 mmol/L potassium acetate, and 2 mmol/L magnesium acetate. The sequence of the IL-13 siRNA used in vivo is shown in Fig. 1A. Two chemically modified control siRNAs were also used in these studies. An siRNA targeting hepatitis B virus was used as a control for in vitro studies (sense strand 5′-iB GeAcuGuAcuAccuGTTiB, antisense strand 5′-CAGGGuGAAGcGAAGGuGaTT, modifications defined as in Fig 1, A). A control siRNA targeting enhanced green fluorescent protein was used for in vivo studies (sense strand 5′-iB AGAAcGGcAccAGGcGAATTTiB, antisense strand 5′-UCCAuGuAcuGuAucuTT).

Formulation of siRNAs for in vivo studies
siRNAs were annealed in sterile 5% dextrose and formulated in in vivo jetPEI (Qiogene, Irvine, Calif) to an N/P ratio (number of nitrogen residues of jetPEI per RNA phosphate) of 5 according to the manufacturer’s instructions. siRNAs were formulated immediately before injection.

Luciferase-reporter fusions and siRNA functional assays
The rat IL-13 sequence was chemically synthesized (Integrated DNA Technologies, Coralville, Iowa) with Xhol and PvuII sites at the 5′ and 3′ ends, respectively. The sequence was cloned into the same sites in the psiCHECK-2 reporter plasmid (Promega, Madison, Wis) to create a dual luciferase reporter plasmid in which the target of interest is in the 3′ untranslated region of renilla luciferase. Luciferase-reporter assays were performed in 96-well black plates. Fifteen thousand HeLa S3 cells (ATCC, Manassas, Va) were plated in 75 μL Iscove’s modified Dulbecco’s medium (Life Technologies, Grand Island, NY) supplemented with 10% BCS. Plates were incubated overnight (37°C, 5% CO2) before transfection. Each well received 50 μL of a mixture containing 0.5 μL Lipofectamine 2000, 100 ng reporter plasmid, and siRNA diluted in OptiMEM (Invitrogen, Carlsbad, Calif). This mixture was incubated at room temperature for 20 minutes before addition to cells. Cells were incubated as above for 17 to 24 hours after transfection. Renilla and firefly luciferase activities were determined using the Dual Glo luciferase reagents (Promega, Madison, Wis) according to the manufacturer’s instructions. The ratio of renilla to firefly luciferase activity was normalized to that observed for each reporter plasmid after transfection with a control siRNA of the same stabilization motif. All transfections were performed in triplicate, and data are reported as means ± SDs.

Animals
Pathogen-free, female BALB/c BYJ mice 8 to 12 weeks old were obtained from the Jackson Laboratory (Bar Harbor, Me) and maintained on an ovalbumin (OVA)-free diet. All experimental animals used in this study were under a protocol approved by the institutional animal care and use committee of National Jewish Medical and Research Center.

Sensitization and challenge
Sensitization to OVA was conducted with 2 intraperitoneal injections of 20 μg of OVA (Grade V, Sigma-Aldrich, St Louis, Mo) emulsified in 2.25 mg alum hydroxide (AlumImuject; Pierce, Rockford, Ill) in a total volume of 100 μL conducted 14 days apart. Two weeks after the last OVA sensitization, aerosol challenges were conducted for 20 minutes for 3 consecutive days (days 28, 29, and 30) with 1% OVA in PBS by using an ultrasonic nebulizer (AeroSonic ultrasonic nebulizer; DeVilbiss, Sommerset, Pa). Control mice received aerosol OVA challenges only. On day 32, airway function was measured as described, followed by collection of bronchoalveolar lavage (BAL) fluid for further analyses. For siRNA treatments, mice were injected intravenously via the tail vein with 60 μg siRNA complexed with in vivo jetPEI (Qiogene, Irvine, Calif) in a volume of 100 μL 24 and 3 hours before the first nebultzed OVA challenge at 28 days and 3 hours before the challenges at 29 and 30 days. Each treatment group included 8 animals.

Determination of airway function
Airway function was assessed as previously described by measuring changes in lung resistance (RL) in response to increasing doses of inhaled methacholine (MCh). Data are expressed as percentage change from baseline RL values obtained after inhalation of PBS. Percent changes for animals in each group (n = 8) are presented as means ± SEMs.

Determination of cell numbers in BAL fluid
Immediately after the assessment of AHR, lungs were lavaged via tracheal tube with PBS (1 mL/mouse). Cells in BAL fluid were pelleted, and supernatants for determination of cytokine levels were frozen at −80°C. Total leukocyte numbers were determined using a Coulter Counter (Coulter Corporation, Hialeah, Fla). Cells were immobilized using a Cytospin 2 (Shandon Ltd, Runcorn, United Kingdom) and stained with Leukostat (Fisher Diagnostics, Pittsburgh, Pa). Differential cell populations were determined by counting at least 200 cells.

Histologic studies
Lungs were harvested after isolation of BAL fluid and stained as previously described.17 Briefly, lungs were fixed by inflation with 1 mL 10% formalin via the tracheal tube and immersed in 10% formalin. Sections of tissue were prepared and stained by a morphology core facility. Individual sections were
stained by using hematoxylin-eosin (HE) for characterization of inflammatory cell infiltrate or periodic acid-Schiff (PAS) with HE counter stain to identify mucus-containing cells.

Bone marrow–derived mast cells
Mast cells were derived from femur bone marrow of female BALB/cBYJ mice (Jackson Laboratory) as previously described. Bone marrow was cultured in Iscove’s modified Dulbecco’s medium supplemented with 10% FBS (Summit Biotechnology, Fort Collins, Colo), 50 μmol/L 2-β-mercaptoethanol (Life Technologies), 2 mmol/L glutamine, 100 μg/mL streptomycin, 100 U/mL penicillin, 1% IL-3 conditioned medium, and 2% Kit ligand-conditioned medium.

siRNA transfection
Bone marrow–derived mast cells (BMMCs) (6 × 10⁷ in 0.3 mL) were mixed with 100 nmol/L siRNA in Iscove’s modified Dulbecco’s medium, placed in an electroporation chamber, and incubated for 15 minutes at room temperature. Conditions for electroporation were 350 V, 1180 μF, high V, and fast charge. After 30 minutes of recovery at room temperature, cells were returned to normal growth conditions. Passive sensitization and stimulation were begun 24 hours after transfection.

BMMC passive sensitization
Bone marrow–derived mast cells were passively sensitized with 500 ng/mL of anti-OVA IgE antibodies overnight at 37°C and washed twice with prewarmed completed medium. Cells were then challenged with OVA (10 μg/mL) for 15 minutes, washed with complete media, and incubated at 37°C. For mRNA analysis, a portion of the BMMCs were harvested by centrifugation at 500 g for 5 minutes at 4°C 1 hour after OVA addition. The remaining BMMCs were incubated 24 hours before harvesting media for IL-13 ELISA assays.

Real-time PCR
mRNA was isolated from BMMCs using an RNeasy Mini Kit (Qiagen, Chatsworth, Calif), according to the manufacturer’s directions. For cDNA synthesis, 10 μL RNA was linearized at 68°C for 10 minutes before reverse transcription in 20 μL with the addition of MMLV Reverse Transcriptase and 1X buffer (Invitrogen) with RNase inhibitor mixture (Promega, Madison, Wis) at 42°C for 1 hour followed by denaturation at 94°C for 5 minutes. Real-time PCR (TaqlMan-PCR, ABI Prism 7000 Sequence Detection System; Perkin Elmer Applied Biosystems, Foster City, Calif) with glyceraldehyde-3-phosphate dehydrogenase and IL-13 probes and primers were performed according to the manufacturer’s directions. Ratios of analyte mRNA (IL-4 or IL-13) to GAPDH were normalized to that of the control siRNA-treated cells. Reported values are means ± SE for 3 independent experiments, each of which was conducted in duplicate.

ELISA
Media from BMMCs was harvested and the levels of IL-4 and IL-13 were determined by ELISA according to manufacturers protocol (R&D Systems, Minneapolis, Minn). Levels of each cytokine were normalized to those observed after treatment with a control siRNA targeting hepatitis B virus and are reported as normalized IL-13. Reported values are means ± SE for values obtained in 3 independent experiments. Cytokine levels in BAL fluid were similarly determined.

Statistical analysis
Values for all measurements were expressed as the means ± SE, except for the siRNA functional assay using luciferase-reporter fusions, where data were reported as means ± SDs as described. Data were compared by using the t-test: 2-sample assuming unequal variances. A P value of <.05 was considered statistically significant.
RESULTS

Chemical modification of the siRNAs

The siRNAs used in this study incorporated the chemical modification motif shown in Fig 1, A, which served to stabilize the siRNA against nucleases and evade a dsRNA-induced immune response. In this motif, all but 3 of the 2′ hydroxyls are replaced with 2′F, 2′O-methyl or 2′H moieties. The siRNA was further stabilized against exonuclease digestion by the addition of inverted abasic residues at both ends of the siRNA sense (passenger) strand. This pattern of modifications is closely related to that previously described by Morrissey et al12,13; the 2 differences are that the motif in Fig 1, A, lacks a 3′-terminal phosphorothioate linkage on the antisense strand and places 3 unmodified nucleotides at the 5′-end of the antisense strand regardless of sequence (versus unmodified purine nucleotides and modified pyrimidine nucleotides at these positions in the previous work). An siRNA sequence synthesized with this pattern of modifications will differ from that described by Morrissey et al12,13 at 1 to 4 of 44 positions (depending on sequence), and in vitro siRNA screens using either system of modifications yield a significant number of active siRNAs.

Screening of the siRNAs for IL-13 inhibitory activity

Chemically modified anti-IL-13 siRNAs were screened for activity by using a cell-based dual-luciferase reporter assay. In this assay, candidate siRNAs were cotransfected into HeLa cells with a plasmid coding for both renilla and firefly luciferase with the gene target inserted into the 3′UTR of the renilla luciferase gene. RNA interference activity directed against the target sequence resulted in a reduction of renilla luciferase activity relative to that of firefly luciferase, which served as a control for transfection efficiency. A series of 32 siRNAs was designed and synthesized with the modification motif shown in Fig 1, A. All of the siRNAs were designed to target rat IL-13, and 12 of the 32 siRNAs target sequences that are found in both mouse and rat IL-13. In a preliminary screen for activity, the siRNAs (12 nmol/L) were screened against a rat IL-13 reporter. Ten of the 32 siRNAs tested, including 7 of the sites that target both mouse and rat IL-13, elicited a >75% reduction in target reporter activity. The potency of the 6 most active siRNAs that targeted both mouse and rat IL-13 was subsequently assessed in dose-response curves using amounts in the range of 20 to 5000 pmol/L. The results shown in Fig 1, B, demonstrate that the chemically modified siRNAs have inhibitory concentrations 50% (IC50s) between 0.3 and 1.2 nmol/L. The site 2–specific siRNA showed an effective dose-dependent reduction in target reporter activity and was chosen for further study.

Additional experiments were conducted in bone marrow–derived mast cells to demonstrate that the active siRNA identified in the reporter-based assay was active in cells derived from the BALB/c mice used in the model of allergen-induced AHR. The data shown in Fig 2 demonstrate that treatment with IL-13 siRNA reduced the levels of IL-13 mRNA (Fig 2, A) and protein (Fig 2, B) formed on activation with anti-OVA IgE and OVA. IL-4 expression was relatively unchanged in response to siRNA treatment, and a control siRNA targeting a sequence in hepatitis B virus had little discernible effect. These results demonstrate that the selected siRNA sequence elicited a sequence-specific effect in primary cells from BALB/c mice and suggest that the siRNA may also function in vivo.

siRNA administration attenuates development of AHR

The site 2 siRNA described was formulated and administered to sensitized mice before challenge by using the protocol outlined in Fig 3, A. That is, mice were sensitized on days 1 and 14 by intraperitoneal injection with OVA and subsequently challenged via the airways by nebulization with 1% OVA on days 28, 29, and 30. Mice were injected intravenously via the tail vein with control or IL-13 siRNA at 24 and 3 hours before the first nebulized OVA challenge at 28 days and 3 hours before the challenges at 29 and 30 days. RL was measured 48 hours after the last OVA nebulization. The siRNAs were formulated in linear polyethyleneimine. This formulation has been reported to facilitate efficient delivery of oligonucleotides to lung and was previously used to deliver siRNAs in a study that demonstrated siRNA-based restriction of influenza replication.

The data in Fig 3, B, demonstrate that treatment with IL-13 siRNA significantly reduced RL (P < .05) at both higher doses of MCh when assayed 48 hours after the last airway challenge. A control siRNA of the same chemical stabilization motif that targets green fluorescent protein (GFP) had no effect on RL. This suggests that the reduction in RL is sequence-specific and that the effect may be related to IL-13 target reduction. A significant shift (P < .05) in airway responsiveness induced by IL-13–specific siRNAs was also illustrated by calculation of PC200 values for each condition. The value for sensitized and challenged animals treated with IL-13 siRNA was 6.009 ± 0.583 (mg/mL MCh) whereas the values for sensitized and challenged animals and sensitized and challenged animals treated with the control siRNA were 3.527 ± 0.454 and 3.585 ± 0.343, respectively.

Immediately after AHR measurements were performed, the lungs were lavaged via the tracheal tube to collect samples for cell counts and lung cytokine profiles by ELISA assays. Total cell count numbers were unchanged between sensitized and challenged mice compared with mice treated with siRNA. Also, differential cell counts performed by cell staining did not show statistically significant differences with IL-13 siRNA treatment compared with sensitized and challenged mice (data not shown). In addition to determining IL-13 levels, levels of several Th1 (IL-10, IL-12) and Th2 (IL-4, IL-5) cytokines were measured and were also found to show no statistically significant differences between siRNA-treated and siRNA-un-treated sensitized and challenged mice, at least at this single time point examined (data not shown). Lung histochernistry was also performed. As shown in Fig 4, no substantial changes in lung inflammation by HE staining or changes in goblet cell metaplasia as performed by PAS staining were observed at the single time point.

DISCUSSION

RNA interference is a powerful new tool that can be used to explore the contribution of specific genes to a biological process or disease state. The current study demonstrates the utility of synthetic siRNAs in a well characterized mouse model of allergen-induced AHR. In this proof-of-concept study, we chose to target IL-13, a well characterized mediator involved in the development of AHR. Previous studies have demonstrated that neutralization of IL-13 during the challenge phase of a sensitization and challenge protocol reduces AHR. Neutralization of IL-13 or genetic deletion of IL-13 also affected AHR in a murine model of established allergic airway disease.
FIG 2. Reduction of IL-13 mRNA (A) and cytokine (B) by siRNA in BMMCs. BMMCs were electroporated with 100 nmol/L siRNA before stimulation with anti-OVA IgE and OVA. For A, levels of IL-13 and IL-4 mRNA measured by RT-PCR were normalized to GAPDH. For B, cell media was harvested 24 hours after activation, and IL-13 cytokine levels were measured by ELISA and normalized to control siRNA-treated samples. The results are from 3 independent experiments. *P < .05.

FIG 3. Reduction of airway resistance in sensitized and challenged mice after treatment with IL-13 siRNA. A, Schematic diagram of allergen sensitization and challenge and siRNA administration protocol. B, IL-13 siRNA decreased airway resistance in sensitized and challenged mice. With increasing MCh challenges, sensitized and challenged mice exhibited increases in airway resistance compared with challenged only mice. Treatment with IL-13 siRNA decreased airway resistance in the mice. n = 8, *P < .05. i.p., Intraperitoneal.
The current study used a chemically modified siRNA screened for potency in a cell-based reporter gene assay and for activity in BMMCs. The chemical modifications in this series of siRNAs were used to stabilize the compound to nucleases, extend the duration of effects, and eliminate dsRNA-induced inflammatory responses. A duplex that had a significant effect on IL-13 mRNA and protein levels in IgE-antigen–stimulated mast cells was identified. Sequence specificity was apparent from the fact that the IL-13 siRNA had no effect on IL-4 mRNA (Fig 2, A).

The site 2 siRNA that was identified and characterized in vitro elicited a significant reduction in MCh-induced lung resistance after allergen challenge of sensitized mice. The sequence dependence of this effect was demonstrated by a control siRNA that had no effect on lung resistance. The control siRNA carried the same pattern of chemical modifications as the IL-13 siRNA and effectively reduced the levels of its GFP target in vitro. To the best of our knowledge, this is the first demonstration that synthetic siRNAs are effective in an in vivo model of allergen-induced AHR and one of the first studies to show siRNA activity in a nonviral respiratory model.

Reduced lung resistance was observed in mice that were treated with 4 intravenous injections containing 60 μg siRNA formulated in polyethylenimine. The polyethylenimine formulation has been shown to facilitate delivery of oligonucleotides to the lung and was previously used in a study that demonstrated the ability of siRNAs to restrict influenza virus replication in mice. This dose corresponds to approximately 3 mg/kg, which is relatively low compared with doses used in other studies demonstrating siRNA efficacy in vitro. Nevertheless, it may be possible to observe an effect in vivo by using a lower dose delivered directly to the lungs. Previous studies suggest that chemically synthesized siRNAs reduced viral replication after intranasal delivery in mice. More recent work has shown that siRNAs formulated in lipid nanoparticles show extensive lung exposure (half-life >9.0 hours) and are broadly distributed in mouse lungs after intratracheal instillation.

Although siRNA treatment leads to a reduction in lung resistance in vivo, there are significant questions to be addressed in future studies. Primary among these is the identification of the site or timing of siRNA action and an associated reduction in IL-13 mRNA and protein levels. Although the sequence specificity of the IL-13 siRNA was established in vitro and the siRNA effect on lung resistance was clearly sequence-dependent, a reduction in IL-13 was not observed in BAL immediately after lung function studies. This may reflect a more localized reduction in IL-13, potentially in or in close vicinity to the airway smooth muscle, which has recently been proposed to respond directly to IL-13. IL-13 is also an essential contributor to the development of goblet cell metaplasia and mucus hyperproduction. The administration of the IL-13 siRNA did not affect these parameters. AHR and goblet cell metaplasia have been dissociated, and it is also possible that the amounts of IL-13 required for goblet cell metaplasia are lower than required in the development of AHR. Alternatively, there may be a temporary reduction in IL-13 that is critical for AHR but is later masked by the influx of inflammatory cells to the airways, which are also sources of IL-13. Because the number of infiltrating inflammatory cells may be a secondary response or dissociated from development of the AHR response, they proceed unimpeded in face of IL-13 siRNA treatment.

The goal of the study was to determine the feasibility of interfering with allergen-induced AHR by using an siRNA that targeted a critical mediator of the lung allergic response. Clearly, IL-13 is just one of several critical mediators of AHR, and...
more widespread effects may be observed when targeting more than a single mediator. The data support the notion that siRNA-mediated reduction of IL-13 can alter the course of development of alterations in airway function. Although further work is needed to determine the cells targeted by the IL-13 siRNA, we demonstrated an IL-13 sequence-dependent reduction in AHR in this model. With the development of better delivery and perhaps distribution (ie, to the lungs), siRNA treatment may be a promising new therapeutic approach for the treatment of allergen-induced airway dysfunction.

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Clinical implications: The use of siRNA to target IL-13 is a novel approach to reducing altered airway function after allergen exposure and could provide the basis for new asthma treatments.

REFERENCES

1. Takeda K, Dakhama A, Gelfand EW. Allergic asthma: what have we learned from the mouse model? Allergyology Intl 2005;54:263-71.
2. Ge Q, Filip L, Bai A, Nguyen T, Eisen NH, Chen J. Inhibition of influenza virus production in virus-infected mice by RNA interference. Proc Natl Acad Sci U S A 2004;101:8076-81.
3. Tompkins SM, Lo CY, Tunpey TM, Epstein SL. Protection against lethal influenza virus challenge by RNA interference in vivo. Proc Natl Acad Sci U S A 2004;101:8682-6.
4. Zhang W, Yang H, Kong X, Mohapatra S, San Juan-Vergara H, Hellermann G, et al. Inhibition of respiratory syncytial virus infection with intranasal siRNA nanoparticles targeting the viral NS1 gene. Nature Med 2005;11:56-62.
5. Bitko V, Masijenko A, Shiuayava O, Barik S. Inhibition of respiratory viruses by nasally administered siRNA. Nat Med 2005;11:50-5.
6. Li BJ, Tang Q, Cheng D, Qin C, Xie FY, Wei Q, et al. Using siRNA in prophylactic and therapeutic regimens against SARS coronavirus in Rhesus macaque. Nat Med 2005;11:944-51.
7. Zhang X, Shan P, Jiang D, Noble PW, Abrahm NG, Kappas A, et al. Small specific chemical modification of siRNAs reduces “off-target” transcript silencing. RNA 2006;12:1197-205.
8. Morrissey DV, Lockridge JA, Shaw L, Blanchard K, Jensen K, Breen W, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nature Biotechnol 2005;23:1002-7.
9. Morrissey DV, Blanchard K, Shaw L, Jensen K, Lockridge JA, Dickinson B, et al. Activity of stabilized short interfering RNA in a mouse model of hepatitis B virus replication. Hepatology 2005;41:1349-56.
10. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Remnick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998;282:2261-3.

11. Jackson AL, Burchard J, Leake D, Reynolds A, Schelter J, Guo J, et al. Position-specific chemical modification of siRNAs reduces “off-target” transcript silencing. RNA 2006;12:1197-205.
12. Morrissey DV, Lockridge JA, Shaw L, Blanchard K, Jensen K, Breen W, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nature Biotechnol 2005;23:1002-7.
13. Morrissey DV, Blanchard K, Shaw L, Jensen K, Lockridge JA, Dickinson B, et al. Activity of stabilized short interfering RNA in a mouse model of hepatitis B virus replication. Hepatology 2005;41:1349-56.
14. Grunig G, Warnock M, Wakil AE, Venkayya R, Brombacher F, Remnick DM, et al. Requirement for IL-13 independently of IL-4 in experimental asthma. Science 1998;282:2261-3.
15. Wills-Karp M, Luyimbazi J, Xu X, Schotfield B, Neben TY, Karp CL, et al. Interleukin-13: central mediator of allergic asthma. Science 1998;282:2258-61.
16. Taube C, Duec C, Cui ZH, Takeda K, Rha YH, Park JW, et al. The role of IL-13 in established allergic airway disease. J Immunol 2002;169:6482-9.
17. Taube C, Wei X, Swasey CH, Joetham A, Zarnice S, Lively T, et al. Mast cells, FceRI, and IL-13 are required for development of airway hyperresponsiveness after aerosolized allergen exposure in the absence of adjuvant. J Immunol 2004;172:3968-400.
18. Wills-Karp M. Interleukin-13 in asthma pathogenesis. Immunol Rev 2004;202:75-90.
19. Yang M, Hogan SP, Henry PJ, Matheai KL, McKenzie AN, Young IG, et al. Interleukin-13 mediates airways hyperreactivity through the IL-4 receptor-alpha chain and STAT-6 independently of IL-5 and eotaxin. Am J Respir Cell Mol Biol 2001;25:522-30.
20. Townley RG, Horiba M. Airway hyperresponsiveness: a story of mice and men and cytokines. Clin Rev Allergy Immunol 2003;24:85-110.
21. Lepore JT, Moore PE, Baraldo S, Jouvin MH, Church TL, Schwartzman IN, et al. Direct effects of interleukin-13 on signaling pathways for physiological responses in cultured human airway smooth muscle cells. Am J Respir Crit Care Med 2001;164:141-8.
22. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, et al. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. J Clin Invest 1999;103:779-88.
23. Wincott F, DiRenzo A, Shaffer C, Grims S, Tracz D, Workman C, et al. Synthesis, deprotection, analysis and purification of RNA and ribozymes. Nucleic Acids Res 1995;23:2677-84.
24. Takeda K, Hamelmann E, Joetham A, Shultz L, Larsen GL, Irvin CG, et al. Development of eosinophilic airway inflammation and airway hyperresponsiveness in mast cell-deficient mice. J Exp Med 1997;186:449-54.
25. Ishizuka T, Chayama K, Takeda K, Hamelmann E, Terada N, Keller GM, et al. Mitogen-activated protein kinase activation through FcεRI receptor I and stem cell factor receptor is differentially regulated by phosphatidylinositol 3-kinase and calcineurin in mouse bone marrow-derived mast cells. J Immunol 1999;162:2087-94.
26. Karasuayama H, Melchers F. Establishment of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5, using modified cDNA expression vectors. Eur J Immunol 1988;18:97-104.
27. Li G, Lucas JJ, Gelfand EW. Protein kinase Cβ, βI, and βII isoforms regulate cytokine production in mast cells through MEKK2/ERK5-dependent and -independent pathways. Cell Immunol 2005;238:10-8.
28. Dragooni A, Boletta A, Biffi A, Muggia A, Sersale G, Cheng SH, et al. Comparison between cationic polymers and lipids in mediating systemic gene delivery to the lungs. Gene Ther 1999;6:1995-2004.
29. Walter DM, McIntire JJ, Berry G, McKenzie AN, Donaldson DD, DeKruyff RH, et al. Critical role for IL-13 in the development of allergen-induced airway hyperreactivity. J Immunol 2001;167:4668-75.
30. Soutysek J, Aikne A, Bramlage B, Charrise K, Constien R, Donoghue M, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 2004;432:173-8.
31. Hartsough KC, Dickinson BA, Gomez AM, Jamison SF, Shadel TR, Lockridge JA, et al. Chemical modification and formulation of short interfering RNA (siRNA) for local delivery to the lungs. Proc Am Thor Soc 2006;3:A584.
32. Tomkinson A, Duec C, Lahm M, Gelfand EW. Adoptive transfer of T cells induces airway hyperresponsiveness independently of airway eosinophilia but in a signal transducer and activator of transcription 6-dependent manner. J Allergy Clin Immunol 2002;109:810-6.
33. Miyahara N, Swanson BJ, Takeda K, Taube C, Miyahara S, Kodama T, et al. Effector CD8+ T cells mediate inflammation and airway hyper-responsiveness. Nature Med 2004;10:865-9.
34. Corry DB, Folkesson HG, Warnock ML, Erle DJ, Matthay MA, Wiener-Kronish JP, et al. Interleukin 4 but not interleukin 5 or eosinophils, is required in a murine model of acute airway hyperresponsiveness. J Exp Med 1996;183:109-17.
35. Henderson WR Jr, Lewis DB, Albert RK, Zhang Y, Lamm WJ, Chiang GK, et al. The importance of leukotrienes in airway inflammation in a mouse model of asthma. J Exp Med 1996;184:1483-94.