

Inhibition of house dust mite-induced allergic airways disease by antagonism of microRNA-145 is comparable to glucocorticoid treatment

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Background: Glucocorticoids are used as mainstay therapy for asthma, but some patients remain resistant to therapy.

MicroRNAs (miRNAs) are important regulators of the immune system by promoting the catabolism of their target transcripts as well as attenuating their translation. The role of miRNA in regulating allergic inflammation remains largely unknown.

Blocking miRNA function may provide a new nonsteroidal anti-inflammatory approach to treatment.

Objectives: To (1) determine the role of specific miRNAs in the regulation of hallmark features of allergic airways inflammation and (2) compare the efficacy of antagonizing miRNA function with that of steroid treatment.

Methods: Mice were sensitized and then aeroallergen-challenged with house dust mite to induce allergic airways disease, and alterations in the expression of miRNAs were characterized. Next mice were treated with antagomirs that inhibited the function of specific miRNAs in the lung or treated with dexamethasone and inflammatory lesions, and airway hyperresponsiveness was measured.

Results: miR-145, miR-21, and let-7b have been implicated in airway smooth muscle function, inflammation, and airways epithelial cell function, respectively. Inhibition of miR-145, but not miR-21 or let-7b, inhibited eosinophilic inflammation, mucus hypersecretion, T_H2 cytokine production, and airway hyperresponsiveness. The anti-inflammatory effects of miR-145 antagonism were comparable to steroid treatment.

Conclusion: Our study highlights the importance of understanding the contribution of miRNAs to pathogenesis of human allergic disease and their potential as novel anti-inflammatory targets.

(*J Allergy Clin Immunol* 2011;■■■:■■■-■■■.)

Key words: miRNA, asthma, allergy, inflammation, dexamethasone, glucocorticoid, HDM, antagomir

Glucocorticoids and long-acting β -agonists¹⁻³ suppress inflammation and airway hyperresponsiveness (AHR), which are associated with the clinical expression of asthma. Indeed, the degree of inflammation and AHR often reflects the activity and severity of disease, and titration of therapy on the basis of the control of AHR may yield superior therapeutic outcomes.^{4,5} Thus, modulation of the factors that regulate the development and persistence of these clinical features are thought to be of central importance in the perpetuation of asthma.⁶ Steroid therapy is effective but primarily focuses on the management of disease and functions by suppressing a range of known and unknown components of the inflammatory response. In particular, glucocorticoids may be effective because they suppress the activation and recruitment of key inflammatory cells in the airways such as CD4⁺ T_H2 cells, eosinophils, and mast cells. These cells play central roles in pathogenesis through the release of well characterized proinflammatory factors (eg, cytokines such as IL-13 and IL-5).¹ However, steroid therapy is not always effective, it is nonspecific, and a subset of patients remain resistant to treatment.^{7,8} Long-term treatments with steroids can also have numerous adverse effects, and this is of particular concern in affected children.^{1,5} Thus, identification of more effective and specific anti-inflammatory agents for the treatment of asthma would be clinically important.^{1,5}

MicroRNAs (miRNAs) are short noncoding RNAs that function as posttranscriptional regulators of gene regulatory programs by promoting mRNA degradation or by directly blocking protein translation.⁹ Because miRNAs are not perfectly complementary to their targets, each is capable of regulating a large number of messenger RNAs, and thus miRNAs can modulate entire transcriptional programs, although key individual targets may also be critically important.¹⁰ Specific miRNAs participate in regulating a range of cellular activities (eg, differentiation, proliferation, and programmed cell death), and dysregulation of function is now implicated in some diseased states (eg, cancer).¹¹ There is also emerging evidence for an important role of miRNAs in the regulation of hematopoiesis and controlling inflammation.^{9,12-17} Thus, targeting miRNA function may provide a new way to treat diseases of the immune system and aberrant inflammation.^{12,18,19}

Currently functional data on the role of miRNA in the regulation of allergic airways inflammation is very limited.¹¹ Furthermore, whether targeting miRNA activity is as effective as steroid treatment remains unknown. In this study, we identify and characterize the function of miRNAs that are specifically expressed in the airway wall after allergen provocation of mice

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Supported by a National Health and Medical Research Council project grant (to J.M. and P.S.F.), a National Health and Medical Research Council Health Professional Research Fellowship (to J.M.), and the Cooperative Research Centre for Asthma and Airways (to J.M. and P.S.F.).

Disclosure of potential conflict of interest: J. Mattes has received a grant from the CRC for Asthma & Airways and has a patent for PCT. The rest of the authors have declared that they have no conflict of interest.

Received for publication October 6, 2010; revised March 24, 2011; accepted for publication April 5, 2011.

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0091-6749/\$36.00

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doi:10.1016/j.jaci.2011.04.005

Abbreviations used

AHR: Airway hyperresponsiveness
 ant: Antagomir
 HDM: House dust mite
 miR: Mature microRNA
 miRNA: MicroRNA
 qPCR: Quantitative RT-PCR
 SAL: Sterile saline

sensitized to house dust mite (HDM) and the induction of allergic airways inflammation. We also conduct a comparative study with dexamethasone to determine whether our novel antisense antagomirs designed to block specific miRNAs functionally are as effective as steroid treatment.

METHODS**Mice**

BALB/c mice were obtained from the University of Newcastle's special pathogen-free facility and housed in individually ventilated cages in approved containment facilities within the David Maddison Clinical Sciences Building, University of Newcastle (Newcastle, Australia). All experiments were approved by the Animal Ethics Committee of the University of Newcastle.

Induction of allergic airways disease

House dust mite (*Dermatophagoides pteronyssinus*) extract was obtained from Greer Laboratories (Lenoir, NC) as a lyophilized preparation of milled mites. HDM was resuspended in sterile saline (SAL) and mice were treated through the nose with 50 µg protein/50 µL (Der p 1 constituted ~10% of protein weight) or vehicle sterile endotoxin-free saline on days 1, 2, and 3 for sensitization. Sensitization was followed by daily HDM exposure (5 µg/50 µL) on days 14, 15, 16, and 17 to induce allergic airways disease (Fig 1). Nonsensitized mice (vehicle-treated) received sterile endotoxin-free saline only. Experiments were conducted on day 18, 24 hours after the last HDM exposure.

AHR measurement

Mice were anesthetized and mechanically ventilated, and AHR to nebulized methacholine (increased lung resistance) was measured as previously described.²⁰ Responses to methacholine were expressed as a percentage change over control (baseline).

Antagomirs

Target miRNA sequences were downloaded from miRBase, Wellcome Trust, Sanger Institute, Cambridge, United Kingdom (Sanger database; <http://microrna.sanger.ac.uk/sequences/>) and specific antisense antagomirs (ant) designed (chemically modified single-stranded RNA analogs, complementary to the target miR and capable of long-lasting silencing in the lung).^{12,21} Specific antagomirs and scrambled controls (ant-scrambled) nonspecific RNA VIII, blasted against the mouse genome, were synthesized by Dharmacon Inc (Lafayette, Colo).²⁰ Antagomir sequences were as follows: ant-miR-145, 'mA.*.mA.*.mG.mG.mG.mA.mA.mA.mC. mU. mG.*.mG.*.mA.*.mC.*.3'-Chl; ant-let (lethal)-7b 5'mA.*.mA.*.mC.mC.mA.mC.mA.mC.mC.mU.mA.mC.mU.mA.mC.mC.*.mU.*.mC.*.mA.*.3'-Chl; and ant-miR-21, 5'mU.*.mC.*.mA.mA.mC.mA.mU.mC.mA.mG.mU. mC.mU.mG.mA.mU.mA.mA.mG.*.mC.*.mU.*.mA.*.3'-Chl; where *m* denotes 2'-O-Methyl-modified phosphoramidites, * denotes phosphorothioate linkages, and -Chl denotes hydroxypropylol-linked cholesterol. We administered 50 µg ant-miR or ant-scrambled/50 µL sterile saline intranasally on day 13 (24 hours before the first HDM re-exposure) and then every second day until mice were killed on day 18 (Fig 1).

Administration of dexamethasone

Dexamethasone (3 mg/kg; Sigma-Aldrich, New South Wales, Australia) or vehicle control was administered by intraperitoneal injection on day 13 (24 hours before the first HDM re-exposure) and then every second day until mice were killed on day 18 (Fig 1).

Isolation of miRNA

Total RNA including miRNA was isolated with the mirVana miRNA Isolation kit (Ambion, Austin, Tex) from lower airway tissue.²² Briefly, the trachea and lungs were isolated, and using 2 pairs of forceps, the parenchyma was separated from the larger airways by blunt dissection. This facilitated effective separation of the airway wall from the parenchyma, leaving several generations of airway for analyses. The preparation consists of resident airway cells, such as epithelial cells, fibroblasts, smooth muscle cells, and the basement membrane, as well as infiltrating inflammatory cells.

miRNA microarraying

In initial studies, mice were sensitized with the administration of 50 µg HDM in 25 µL saline or 25 µL saline intratracheally. Ten days postsensitization, mice received a single intratracheal challenge with 50 µg HDM in 25 µL saline or 25 µL saline. Twenty-four hours postchallenge, mice were killed, and miRNA were isolated from the airway wall and expression levels determined by microarray. Total RNA was extracted from bluntly dissected airways by using the Ambion mirVANA kit according to the manufacturer's protocol. miRNAs were enriched with the Ambion flashPAGE system. The Ambion 1564V1 probe set was printed on microarray epoxy slides by the Australian Genome Research Facility, Parkville, Australia. Analyses of microarray data were conducted by using Genespring GX 7 software (Agilent, Santa Clara, Calif).

Quantitative RT-PCR

Quantitative RT-PCRs (qPCR) were performed with the TaqMan Gene Expression Assays for the respective miRNA (Applied Biosystems, Foster City, Calif). miRNA expression was normalized to 18S RNA.

Airway morphology studies

Lung tissue was stained. Cells were identified by morphologic criteria and quantitated by counting 10 high-power fields in each slide.²⁰

Cytokine analysis

Peribronchial lymph node cells were excised, filtered, and cultured in the presence or absence of 50 µg/mL HDM (optimal concentration) for 6 days. The concentration of IL-13, IL-5, and IFN-γ was determined by ELISA (BD Biosciences PharMingen, San Jose, Calif).

Statistics

Data are expressed as mean ± SEM. Graphpad Prism 4 (La Jolla, Calif) was used to determine the significance of differences between groups by using the Student *t* test or the Mann-Whitney test as appropriate. Comparison of airways resistance between groups was performed by using ANOVA, and significance was assessed for the entire dose-response curve.

RESULTS**HDM increases the expression of miR-145, miR-21, and let-7b in the airways**

Twenty-four hours after the first re-exposure to HDM, miRNAs were isolated from the airway wall and expression levels determined by microarray. Of the miRNAs expressed, miR-145, miR-21, and let-7b were increased greater than 5-fold (*P* < .05; *n* = 4-6 mice) in contrast with control vehicle, and their

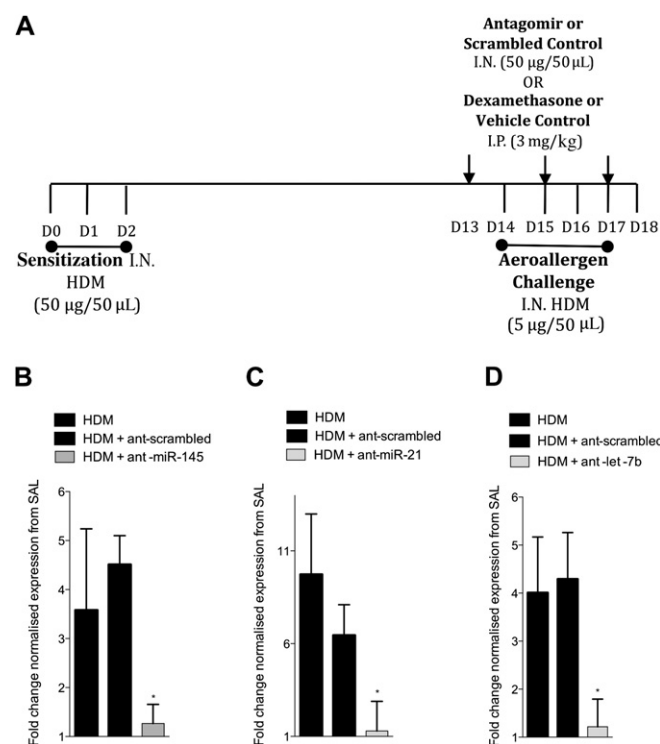


FIG 1. miRNA expression is increased in the airway wall of HDM-sensitized and aeroallergen-challenged mice, and tissue levels are decreased by specific antagomirs. Mice were sensitized to HDM. Twelve days later they were exposed to 4 consecutive low-dose HDM challenges and killed 24 hours after the final challenge for analysis. Mice were treated with either antagomir, dexamethasone, or control prior to and during the HDM challenges as shown (A). Control nonsensitized mice received SAL. Mice were also treated with antagomirs (*ant-miR*) intranasally (50 µg/50 µL saline) or scrambled control (*ant-scrambled*) or dexamethasone (3 mg/kg) or vehicle control intraperitoneally (A). miRNA-specific qPCR for miR-145, (B) miR-21, (C) and let-7b (D) was conducted on RNA isolated from the airways and normalized to the expression of ribosomal RNA 18S. Data are expressed as fold change from expression levels of SAL controls $\pm$ SEM (n = 4-8). * $P \leq .05$. D, Day; I.N., intranasal; I.P., intraperitoneal.

increased expression could be confirmed by qPCR (not shown). Furthermore, miR-145, miR-21, and let-7b have been implicated in airway smooth muscle function, allergic inflammation, and airways epithelial cell function, respectively, suggesting that these miRNAs may regulate aspects of the host response to HDM. The levels of expression of miR-145, miR-21, and let-7b were not altered by exposure to saline. The increased expression of these miRNAs was also validated by qPCR on day 18 after repeated re-exposure to HDM (Fig 1, B) and the induction of hallmark features of allergic inflammation and AHR (Figs 2-4).

Effect of specific antagomirs on miRNA levels after HDM exposure

Next we designed antagomirs to block the function of miR-145, miR-21, or let-7b or scrambled controls. Antagomirs or scrambled controls were administered to HDM-sensitized mice intranasally 24 hours before to re-exposure and then given every 48 hours (Fig 1, A). Expression of target miRNAs was then quantified 24 hours after the last re-exposure to HDM (day 18) by qPCR (Fig 1, B-D). HDM exposure significantly increased the expression of miR-145, miR-21, and let-7b, and levels of transcripts were not

affected in the presence of scrambled controls (Fig 1, B-D). By contrast, tissue levels of these miRNAs after HDM exposure were significantly decreased by treatment with the respective antagomir (Fig 1, B; antagomirs were highly specific and did not alter the expression of nontarget miRNAs; see this article's Fig E1 in the Online Repository at www.jacionline.org).

Inhibition of the function of miR-145 but not miR-21 or let-7b suppresses HDM-induced mucus hypersecretion in airway epithelial cells and eosinophilic inflammation as effectively as dexamethasone treatment

Next we determined the regulatory role for miR-145, miR-21, or let-7b in the development of phenotypic features of allergic asthma and compared effects with those of dexamethasone. Disease severity in HDM-sensitized and rechallenged mice was determined on day 18 when cellular levels of miR-145, miR-21, or let-7b were shown to be significantly reduced by antagomir treatment (Fig 1, B). HDM induced significant mucus hypersecretion in airway epithelial cells and promoted the recruitment of eosinophils to the airways, and these inflammatory responses were not attenuated by treatment with scrambled controls (Fig 2). Notably, ant-miR-145 significantly reduced the number of both mucus-producing cells and eosinophils present in the airways of HDM-challenged mice (Fig 2, A, D, G). By contrast, although ant-miR-21 and ant-let-7b both effectively reduced the levels of their target miRNA to prechallenge levels, treatment did not inhibit mucus hypersecretion or the accumulation of eosinophils in airway tissue after HDM exposures (Fig 2, B and E). Notably, the suppressive effects of ant-miR-145 were comparable to the anti-inflammatory effects of dexamethasone (Fig 2, C and F) with an approximately 50% reduction in the number of mucus-secreting cells and eosinophils in airway tissue (Fig 2, A and D, ant-miR-145; C and F, dexamethasone; G, tissue histology). These trends were also mirrored in the bronchoalveolar lavage fluid, where ant-miR-145 and dexamethasone-treated mice showed significantly reduced eosinophil numbers (see this article's Fig E2 in the Online Repository at www.jacionline.org), whereas numbers of eosinophils in ant-miR-21 and ant-let-7b-treated mice were equivalent to those treated with scramble controls (data not shown).

Effect of antagomir or dexamethasone treatment on the development of AHR

Next we determined the effect of blocking miR-145, miR-21, or let-7b on the development of HDM-induced AHR (Fig 3). AHR in HDM-sensitized and rechallenged mice was determined on day 18 after treatment with antagomirs, scrambled controls, or dexamethasone. HDM challenge induced significant AHR (Fig 3) in mice treated with scrambled controls, and this correlated with the development of the allergic inflammatory lesions (Fig 2). Notably, treatment with ant-miR-145 or dexamethasone, but not with ant-miR-21 and ant-let-7b, resulted in marked attenuation of bronchial reactivity to methacholine (Fig 3). Inhibition of AHR by ant-miR-145 or dexamethasone treatments was directly associated with a reduction in eosinophil infiltrates and suppression of mucus production in the airways (Fig 2). We administered a single dose of ant-miR-145 or dexamethasone during established inflammation 12 hours before final HDM exposure. Inhibition of miR-145 significantly inhibited AHR, whereas DEX

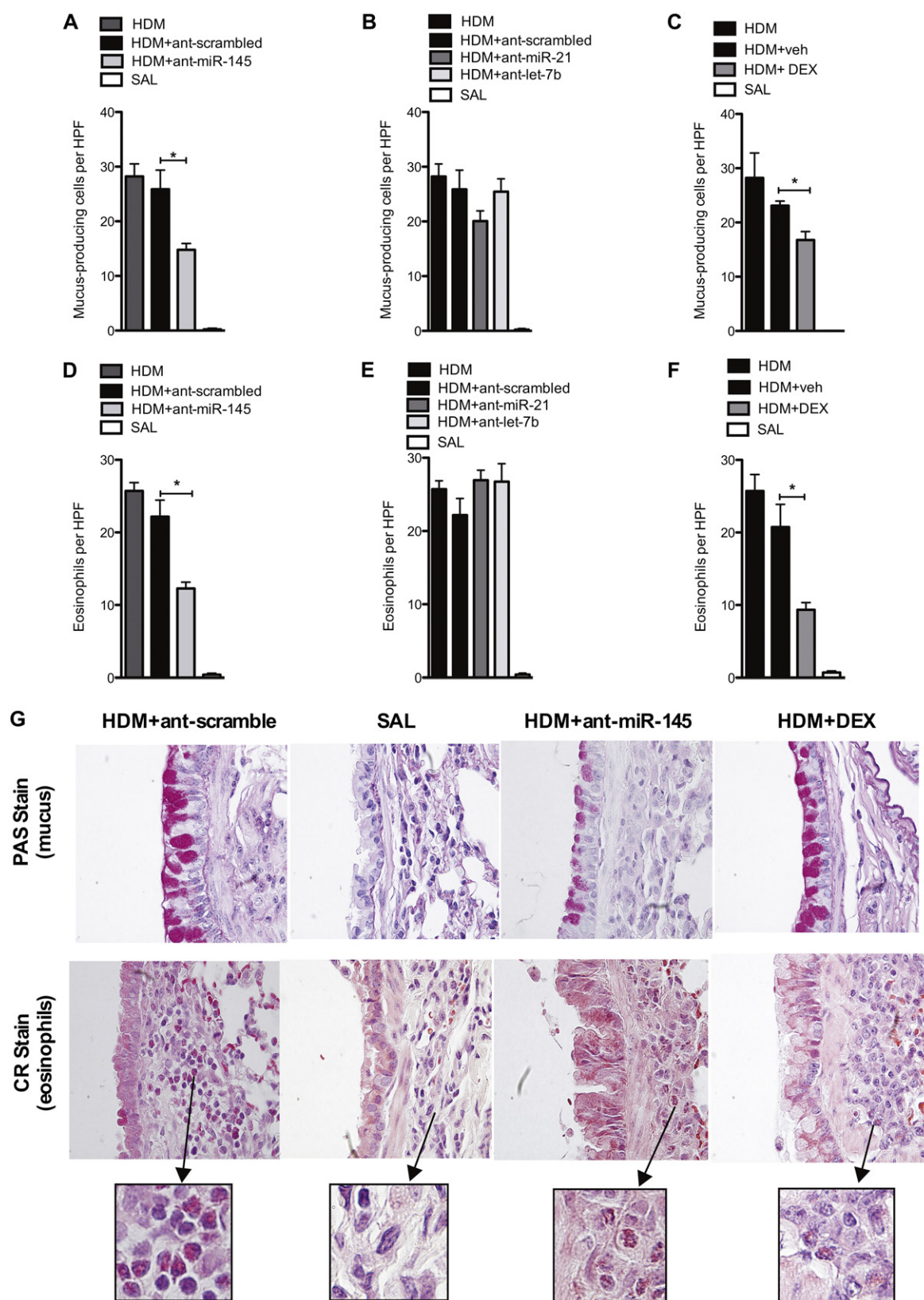


FIG 2. Inhibition of miR-145 function by antagomir reduced inflammation in the airways in a manner comparable to dexamethasone treatment. Paraffin-embedded airway tissues from mice treated with specific antagomirs (*ant-miR*), scrambled control sequence (*ant-scrambled*), dexamethasone (*DEX*), or vehicle control (*veh*) were stained with periodic acid-Schiff (*PAS*) or Congo Red (*CR*) for the detection of mucus-producing cells (**A-C**) or peribronchial eosinophils (**D-F**), respectively. Cells were identified

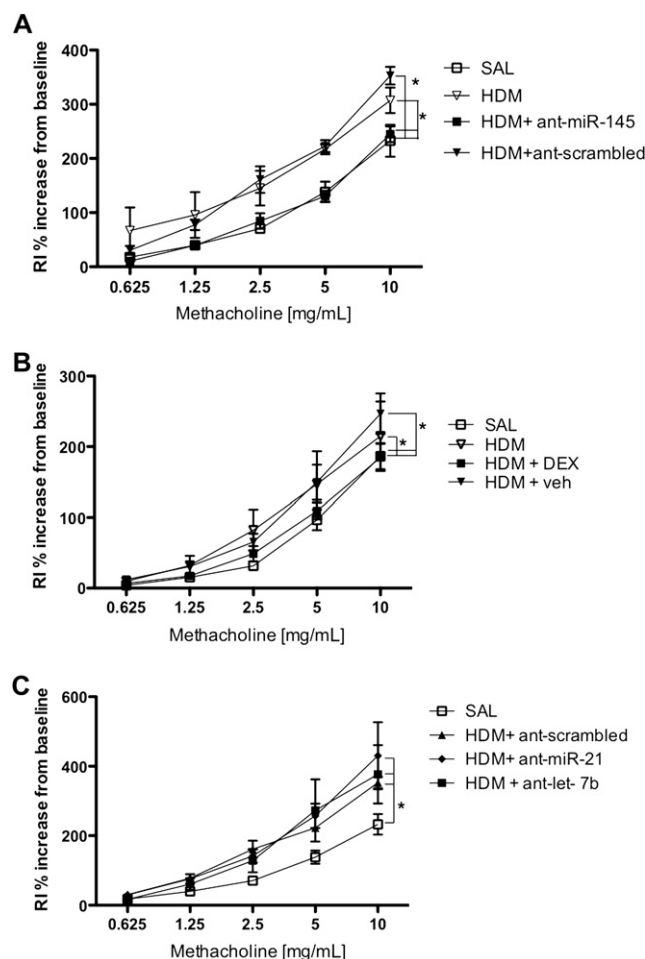


FIG 3. Inhibition of miR-145 function by antagomir reduced AHR in a magnitude comparable to dexamethasone treatment. **A-C**, Lung resistance is presented as a percentage change over baseline measurement (SAL) in response to inhaled methacholine. Mice were treated with specific antagomirs (*ant-miR*), scrambled control sequence (*ant-scrambled*), or dexamethasone (DEX) or vehicle control (*veh*). Resistance (RI) results are mean $\pm$ SEM ($n = 6-10$). * $P \leq .05$ between dose response curves.

treatment was not effective. Ant-miR-145 and dexamethasone both reduced mucus production. However, there was no effect on the accumulation of eosinophils into the BALF or tissues because their influx had already peaked (see this article's Fig E3 in the Online Repository at www.jacionline.org).

Effect of antagomir or dexamethasone treatment on the production of T_H2 -cell cytokines

We and others have previously shown that T_H2 cells through the production of IL-5 and IL-13 play pivotal roles in the induction of hallmark features of allergic airways disease such as eosinophil recruitment, mucus hypersecretion, and the development of AHR.²³⁻²⁶ Thus, we next investigated whether the secretion of these key cytokines was suppressed by ant-miR-145 treatment

and compared responses to dexamethasone treatment after HDM challenge. HDM-sensitized and challenged mice were treated with antagomirs, scrambled controls, or dexamethasone, and on day 18, T_H2 cells from the draining lymph nodes were cultured and stimulated with HDM or without HDM (saline vehicle control) and the levels of IL-5 and IL-13 measured in supernatants (Fig 4). Ant-miR-145 and dexamethasone treatments significantly reduced the production of IL-5 and IL-13 from antigen-specific T_H2 cells. The effect of dexamethasone on T_H2 -cell cytokine production appeared to be more potent because the levels of IL-5 and IL-13 were reduced to almost nonstimulated levels in these cultures (Fig 4, B). Notably, inhibition of cytokine production by ant-miR-145 or dexamethasone correlated with suppression of hallmark features of allergic airways disease (Figs 2 and 3). By contrast, antagonism of miR-21 or let-7b did not significantly inhibit T_H2 -cell cytokine production (Fig 4, C) or mucus production, eosinophil infiltrates, or AHR (Figs 2 and 3).

DISCUSSION

In this investigation, we have used a well characterized model of HDM-induced allergic airways disease that mimics hallmark features of allergic asthma to demonstrate that the expression of specific miRNAs are induced in the airways in response to allergen provocation. The expressions of miR-145, miR-21, and let-7b were significantly increased in the airway wall, and upregulation was directly correlated with the development of inflammation and AHR. However, of the miRNAs upregulated, only miR-145 played a proinflammatory role for the onset of allergic airways disease. Expression of miR-145 is regulated by a highly conserved genomic sequence 3' to the pre-miRNA.²⁷ miR-145 is one of the most highly expressed miRNAs within the human airway and trachea.²⁸ Furthermore, miR-145 regulates smooth muscle development and the differentiation of fibroblasts into smooth muscle cells.²⁹ miR-145 also functions as a tumor suppressor, where it targets transcripts for sex determining region Y-related high mobility group-box 2, octamer-binding transcription factor 4, krueppel-like factor 4, mucin 1, junctional adhesion molecule A, and fascin as well as insulin receptor substrate 1.³⁰⁻³⁴ The role of miR-145 in immune responses and inflammation has not yet been described. In this study, selective inhibition of miR-145 function but not that of miR-21 or let-7b with antagomirs inhibited the production IL-5 and IL-13 by T_H2 cells, the recruitment of eosinophils to the airways, mucus hypersecretion, and the development of AHR. Notably, the anti-inflammatory effects of blocking the specific function of miR-145 were equivalent to those of steroid treatment with dexamethasone.

T_H2 cells through the secretion of cytokines are thought to play key roles in the pathogenesis of allergic asthma, and glucocorticoid therapy may be effective, in part, through suppression of T_H2 cells. In models of allergic asthma, IL-5 and IL-13 derived from activated T_H2 cells have been shown to play central roles in the induction of allergic inflammatory lesions and AHR. IL-5 regulates the induction of eosinophilia and the recruitment of this granulocyte into tissues.^{25,35} IL-13, by activating the IL-4

morphologically and enumerated from 10 high-power fields (HPFs) at $\times 1000$ magnification. **G**, Representative photographs of cells stained for mucus expression (PAS) and eosinophil infiltrates (CR) in each group with amplified sections showing eosinophil morphology. Results are mean $\pm$ SEM ($n = 4-8$). * $P \leq .05$.

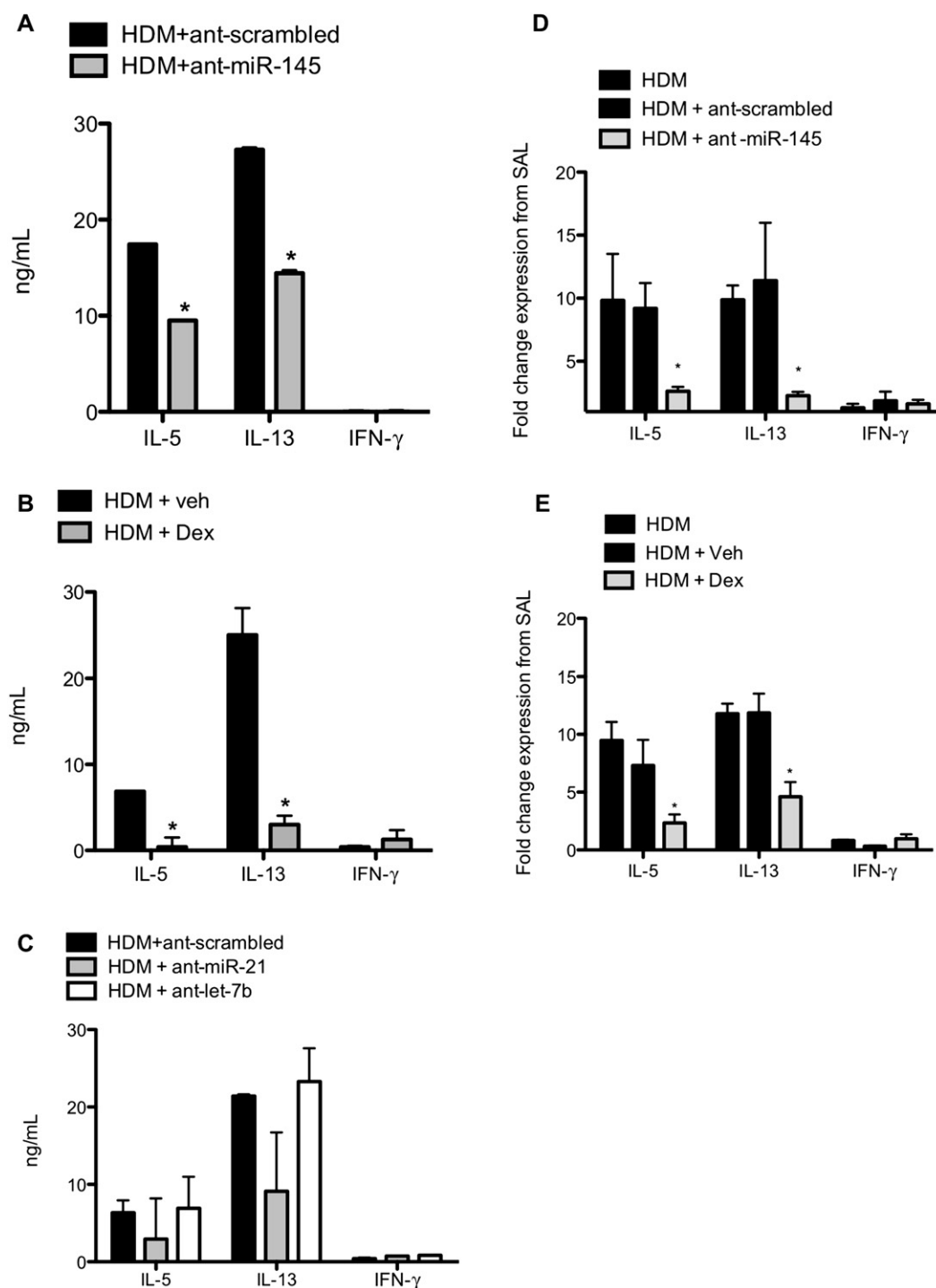


FIG 4. Inhibition of miR-145 function by antagomir reduced inflammatory cytokine production within the draining lymph nodes to a level comparable to dexamethasone treatment (**A-C**). Peribronchial lymph nodes of the lung were collected from mice treated with specific antagomirs (*ant-miR*), scrambled control sequence (*ant-scrambled*) (**A** and **C**), dexamethasone (*Dex*), or vehicle control (*veh*) (**B**). Cells were cultured for 6 days before to supernatant collection. ELISA was conducted for IL-5, IL-13, and IFN- γ , and levels are shown from *in vitro* HDM-stimulated peribronchial lymph node supernatants above unstimulated levels. Cytokine-specific qPCR was performed in whole lung lysates and is shown for allergic mice treated with antagomirs (**D**) or dexamethasone (**E**). Results are mean $\pm$ SEM ($n = 6-10$). * $P \leq .05$.

receptor type-2 complex and signal transducer and activator of transcription 6 pathway, induces mucus hypersecretion, tissue accumulation of eosinophils, and development of AHR.^{26,36,37} Notably,

targeting miR-145 suppressed antigen-induced production of IL-5 and IL-13 from TH2 cells, which correlated with attenuated eosinophil infiltration into the airways, reduced mucus hypersecretion, and

abrogated AHR in response to methacholine inhalation. Thus, miR-145 acts a critical proinflammatory molecule in the regulation of allergic inflammation of the airways. Furthermore, antagomirs enter all cells, suggesting that a global effect of suppressing miR-145 function may have contributed to the anti-inflammatory effects. Although we do not know the precise molecular targets of miR-145, it is emerging that the primary function of miRNAs is to inhibit translation of specific mRNA transcripts to alter cellular function, and in the immune system, the targets are often factors that regulate key transcription axis.^{9,38} Because miR-145 expression was highly induced by HDM exposure, it is thus likely to function by inhibiting mRNA transcripts that are negative regulators of inflammatory pathways and provide a proinflammatory transcriptional environment. Blockade of miR-145 function with antagomirs prevents subsequent activation of these proinflammatory transcriptional circuits. Interestingly, miR-145 is expressed in the human respiratory tract²⁸ and has also been linked to regulation of innate host defense signaling by inhibiting IFN- β production.³⁹ Recently, miR-126 was also shown to exert proinflammatory effects by altering the levels of mRNA transcripts for factors that negatively regulate the production of the T_H2 differentiation factor GATA3.¹² Inhibition of miR-126 significantly resulted in attenuation of HDM-induced allergic airways inflammation and AHR.¹² Our studies provide further proof-of-principle data that targeting specific miRNA may provide a new approach to inhibiting allergic inflammation.

Glucocorticoids are used as mainstay therapy for asthma; however, treatment is not always effective, and there are side effects.^{40,41} Thus, the development of more targeted anti-inflammatory therapies may be beneficial for the treatment of asthma. Although modes of delivery of our interventions are not directly comparable and dexamethasone is currently the most relevant approach to therapy, our study provides proof-of-principle data that targeting miRNA has the potential to suppress allergic inflammation. Here we demonstrate that antagonism of miR-145 function was as effective as glucocorticoid therapy. In a side-by-side analysis, mice treated with ant-miR-145 or dexamethasone displayed significant reduction in the severity of the inflammatory lesions induced by HDM challenge. Dexamethasone has previously been shown to inhibit inflammation in ovalbumin and chronic HDM exposure models of allergic asthma.⁴² Here we confirm the anti-inflammatory actions of dexamethasone after acute exposure to HDM. Dexamethasone suppressed eosinophil recruitment, mucus hypersecretion, AHR, and T_H2 production of IL-5 and IL-13 in the lung after HDM exposure. The anti-inflammatory effect of ant-miR-145 was similar to dexamethasone; however, its effect on T_H2 cytokine production was not as pronounced. Dexamethasone treatment completely suppressed the production of both IL-5 and IL-13 from antigen-reactive T_H2 cells, whereas ant-miR-145 was able to reduce expression only by approximately 50%. However, this partial reduction in T_H2 cytokine production by ant-miR-145 treatment was clearly therapeutically beneficial. Interestingly, blocking the function of miR-126 with antagomirs also only partially reduced T_H2 cytokine levels despite pronounced inhibition of inflammatory lesions and AHR.¹² This difference may also reflect the broader actions of steroids on other pathways not under the influence of these miRNAs.

Interestingly, although miR-21 and let-7b were highly expressed during allergic inflammation, blockade of their function was ineffective at modulating the expression of disease. Very recent studies have also shown that these miRNAs are expressed in the lung in various models of allergic airways disease.^{13,43}

However, the role for these 2 miRNAs in regulating allergic airways disease is unclear. miR-21 is activated by signal transducer and activator of transcription 3 signaling⁴⁴ and is frequently identified as one of the most upregulated miRNAs in lung carcinoma. In part, this is thought to be a result of the ability of miR-21 to inhibit directly the tumor suppressor phosphatase and tensin homolog.⁴⁵ miR-21 has been postulated to induce allergic inflammation by promoting the degradation of IL-12 transcripts and promoting a pro-T_H2-type immune environment.¹³ let-7b has previously been implicated as a regulator of the innate immune response through the modulation of IFN- β . It is able to exert this effect through a seed sequence binding site in the 3' untranslated region of the IFN- β mRNA sequence.³⁹ Systemic administration of locked nucleic acids targeting let-7 family function (antisense oligonucleotides) inhibited T_H2 cytokine production, eosinophil accumulation, normalized *MUC5AC* transcripts, and suppressed AHR in an ovalbumin model of allergic asthma.⁴³ However, in this study, *in vitro* analysis showed that T_H2-cell IL-13 mRNA was a direct target of let-7 family members, indicating that locked nucleic acid treatment (blockade of function) should increase T-cell production of this cytokine that would potentially exacerbate disease. The difference in observations between this investigation and our study may reflect the mode of administration of the antagonists (systemic vs intranasal) and/or the models used and the contribution of potential targets of the miRNA in the inflammatory response. Alternatively, redundant signaling between multiple members of the let-7 family may have masked the role of let-7b when its function was specifically inhibited by antagomir treatment in our study. The more general targeting of the let-7 common seed sequence may also be required to disclose a proinflammatory role for this miRNA cluster in response to HDM.

Abnormal expression of miRNA is beginning to be associated with inflammatory diseases of the lung, skin, and joints.^{11,17} However, there are very limited functional data on the contribution of miRNA to pathogenesis and whether targeting these molecules is a potential new approach to treatment. Here we demonstrate that miR-145 plays a central proinflammatory role in the development allergic airways inflammation to HDM and that blocking its function significantly reduces the severity of inflammatory lesions and AHR. Importantly, the efficacy of targeting 1 miRNA was equivalent to the therapeutic effect of steroid treatment. Thus, targeting miRNAs or their key downstream protein targets may provide a more specific way to deliver anti-inflammatory therapy. Our study highlights the importance of understanding the contribution of miRNAs to the pathogenesis of human allergic disease and chronic inflammatory conditions.

We thank Shannon Waldron, Fiona Eyers, and Stuart Reeves for their technical assistance.

Key messages

- miR-145 plays a critical role in regulation of allergic airways disease.
- Inhibition of miR-145 is comparable to dexamethasone treatment.

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METHODS

Treatment of established inflammation model

BALB/c mice were sensitized to HDM, and 12 days later, they were exposed to 4 consecutive low-dose HDM challenges. They were killed 24 hours after the final challenge for analysis. Twelve hours before the final HDM challenge—that is 36 hours before they were killed—mice received either a single dose of antagomir (50 μ g/50 μ L) specifically targeting miR-145 or scrambled control sequence intranasally or a single dexamethasone treatment or vehicle control intraperitoneally (3 mg/kg in 200 μ L SAL).

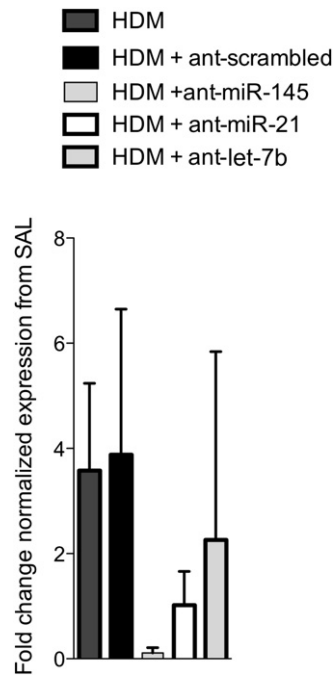


FIG E1. miRNA-specific qPCR for miR-145 was conducted on RNA isolated from the airways and normalized to the expression of ribosomal RNA 18s. Data are expressed as mean fold change from expression levels of SAL controls $\pm$ SEM (n = 4-8). * $P \leq .05$.

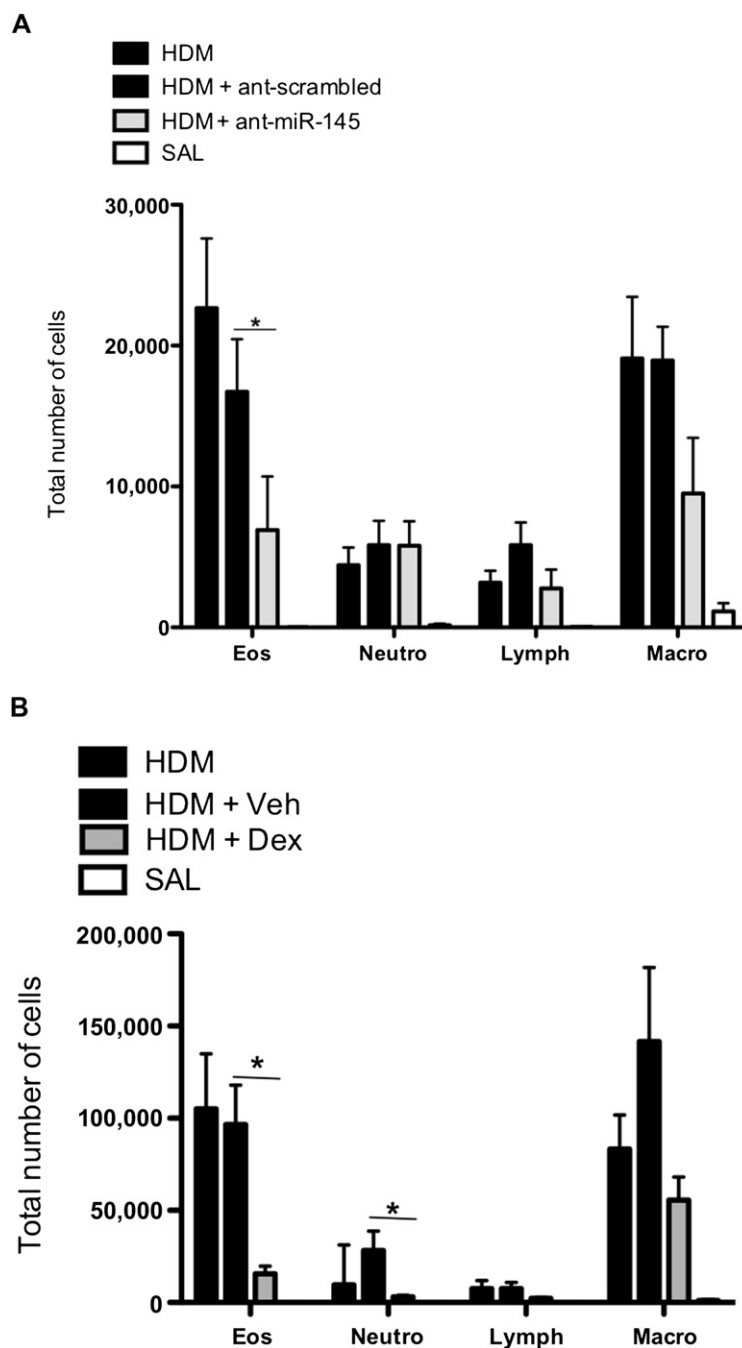


FIG E2. Total number of cells in bronchoalveolar lavage fluid of allergic house dust mite sensitized and challenged mice treated with specific antagomir 145 (*ant-miR-145*), scrambled control sequence (*ant-scrambled*), no treatment (*HDM*) and nonallergic saline controls (*SAL*; **A**) or dexamethasone (*Dex*) or vehicle control (*Veh*; **B**). * $P \leq .05$.

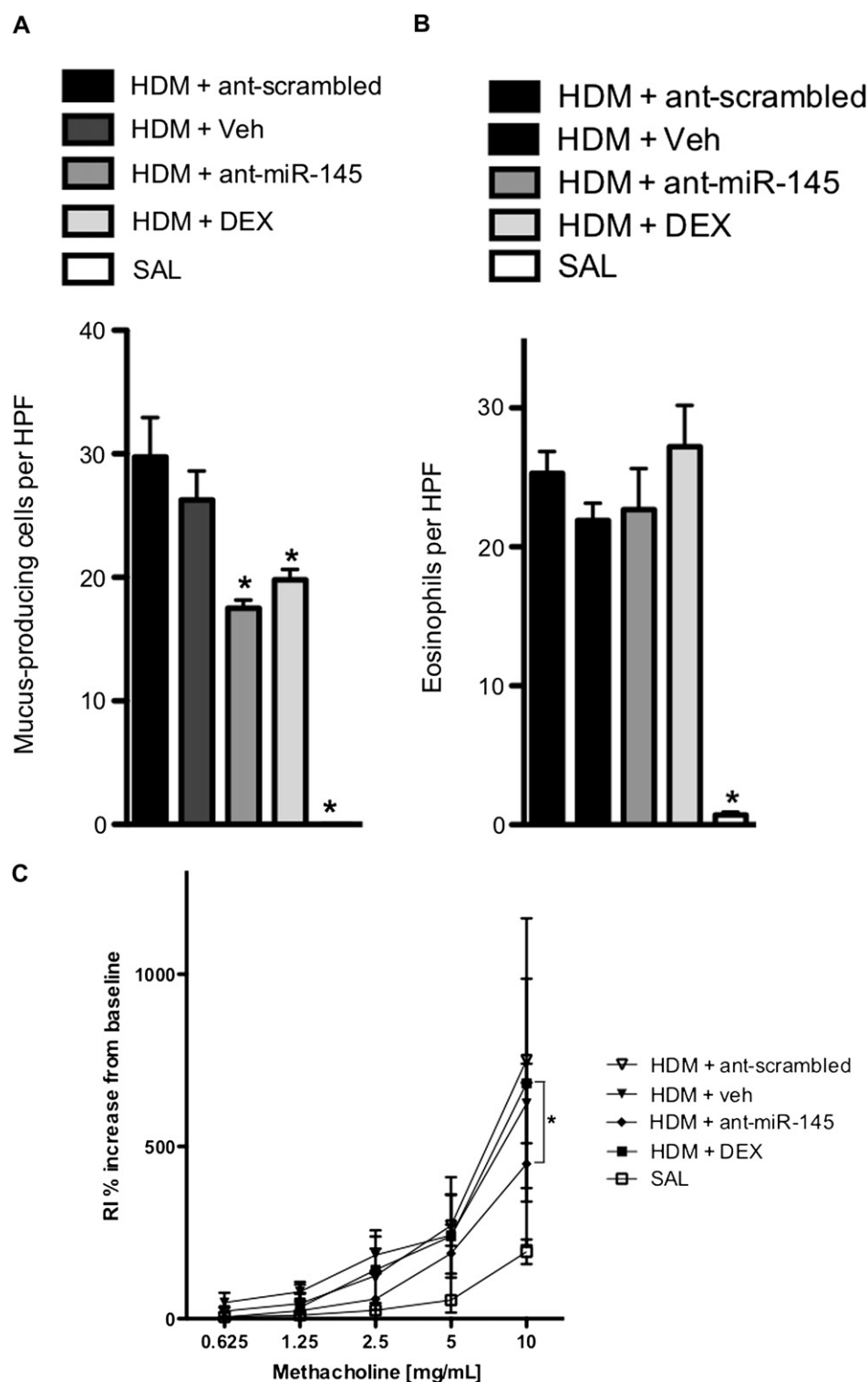


FIG E3. Both antagomir targeting miR-145 and dexamethasone display limited ability to reverse established allergic inflammation within the lung. Lung sections from mice treated with a single dose of antagomir targeting miR-145 (*HDM + ant-miR-145*), scrambled control sequence (*HDM-ant-scrambled*), dexamethasone (*HDM + DEX*), or vehicle control (*Veh*) were stained with periodic acid-Schiff (PAS) or Congo Red (CR) for the detection of mucus-producing cells (**A**) or peribronchial eosinophils (**B**), respectively. Cells were identified morphologically and enumerated from 10 high-power fields (HPFs) at $\times 1000$ magnification. **C**, Lung resistance (RI) is presented as a percentage change over baseline measurement (SAL) in response to inhaled methacholine. Results are mean $\pm$ SEM ($n = 6$). * $P \leq .05$.