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Original Article

Effects of allergic stimulation and glucocorticoids on miR-155 in CD4$^+$ T-cells

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Abstract: Rationale: MicroRNAs (miRNAs) are emerging as important regulators of allergic inflammation and potential therapeutic targets. We sought to identify which miRNAs are expressed in CD4$^+$ T-cells and determine whether allergic stimuli or glucocorticoids alter their expression. Methods: After IRB approval, blood was collected from dust mite (DM) allergic rhinitis subjects (n=20), non-allergic controls (n=8), and asthmatics (n=16). Peripheral blood mononuclear cells were incubated with dust mite extract (DME), diluent control, or DME + dexamethasone (0.1 μM). CD4$^+$ T-cells were collected by magnetic bead column, and RNA was isolated by guanidinium/phenol-chloroform extraction. MicroRNA expression was measured using Nanostring microarray and quantitative real time PCR (qPCR). Results: We identified 196 miRNAs that were stably expressed in circulating CD4$^+$ T-cells. Allergen stimulation of CD4$^+$ T-cells with DME differentially induced miR-155 expression in cells of DM-allergic subjects as compared to non-allergic subjects. Induction of miR-155 expression was also observed with anti-CD3/anti-CD28 simulation and phorbol-12-Myristate-13-Acetate (PMA) treatment, and further augmented by calcium inophore and bromocyclic AMP in the latter treatment. The level of miR-155 expression was positively associated with expression of the T_H2 cytokines IL-5 and IL-13. Inhibition of miR-155 in Jurkat T-cells inhibited the production of these cytokines. Glucocorticoids attenuated the effects of dust mite allergen, raising the possibility that inhibition of this miRNA could be a mechanism through which glucocorticoids exhibit their anti-inflammatory effects. The CD4$^+$ T-cells had a higher level of miR-155 expression in asthma compared to in allergic rhinitis and non-asthmatics. The inhibitory effects of glucocorticoids on CD4$^+$ T-cell miR-155 expression were lost in severe asthmatics. Conclusion: Mir-155 is differentially expressed in allergic T-cells exposed to DM extract compared to in non-allergic cells and it is inhibited by glucocorticoids. MiR-155 may play a role in mediating allergic inflammation in T-cells and could be an anti-inflammatory target of steroids. This pathway may be de-regulated in severe asthma.

Keywords: microRNA, asthma, biomarker, allergic rhinitis, glucocorticoids, T-cell

Introduction

Allergic diseases, including asthma and allergic rhinitis, are common illnesses that cause significant healthcare burden. Asthma is a syndrome characterized by lower airway inflammation and it affects approximately 300 million people worldwide and 8.4% of the U.S. population [1]. Allergic rhinitis represents upper airway inflammation (nasal mucosa, sinuses) and affects 400 million people worldwide and over 15% of the U.S. population [2, 3]. These diseases are characterized by high morbidity, high cost, and poor quality of life. For example, asthma produces an average of 4-5 missed days of school and work per person annually and is estimated to cost over 56 billion dollars per year to treat [1]. One of the central gaps in the field is that the pathogeneses of these diseases are poorly understood, particularly regarding how regulation of inflammatory pathways is impaired. Allergic rhinitis and asthma frequently co-exist, which raises the possibility that similar mechanisms are altered in both diseases. Moreover, the first line of therapy for these diseases is glucocorticoids (GCs), which are anti-inflammatory drugs whose mechanism of action is not completely understood. The goal of this study was to elucidate common pathways in the mechanism of these diseases and determine whether glucocorticoids inhibit these pathways.

MicroRNAs (miRNAs) are small, non-coding, single-stranded ribonucleic acid (RNA) molecules

that regulate expression of target messenger RNAs (mRNAs) [4, 5]. MicroRNAs within the multi-protein RNA-induced silencing complex (RISC) repress translation of transcripts or induce their degradation by binding to the 3' untranslated region (3'UTR) [6]. In some cases, miRNAs may upregulate expression of genes [7]. Thus, the ability of miRNAs to modulate expression of genes encoding various cytokines and inflammatory mediators implicate them as important regulators of inflammatory and immune responses. Circulating miRNAs are emerging as central players in immunity and have been demonstrated to aid in diagnosis and treatment monitoring for diseases such as cancer [8-10].

We previously demonstrated that unique subsets of miRNAs are differentially expressed in the blood of asthmatics, subjects with allergic rhinitis, and healthy subjects with neither disease [4, 5, 11]. We also discovered that miRNA expression profiles were able to distinguish between eosinophilic and non-eosinophilic subtypes of asthma, suggesting that these could be diagnostic and phenotypic biomarkers [11]. Our results also raised the question about the potential role of miRNAs in disease pathogenesis. In particular, miRNAs have now been shown to be essential for induction of allergic airway inflammation and eosinophilic inflammation in mouse models of asthma [12, 13].

$CD4^+$ T-cells play central roles in allergic inflammation and may be highly regulated by miRNAs. In particular, T_H2-differentiated T-cells are a major driver of allergic inflammation [14]. These T-cells produce the type 2 cytokines IL-4, IL-5 and IL-13, which are crucial for recruitment of eosinophils into the airway, airway inflammation hyper-reactivity, and remodeling [14]. Type 2 cytokines also promote IgE class-switching and thus are central to both development of allergy and chronic inflammation [14]. The role of miRNAs in T-cell biology, as it pertains to pathogenesis of allergy, is not well studied. However, it is becoming evident that some miRNAs are crucial for T-cell differentiation and identifying those that regulate allergic inflammation may be important to understanding their roles in allergy pathogenesis and finding potential anti-inflammatory therapeutic targets [15-19].

In this study, we sought to determine whether miRNAs are induced by allergens in $CD4^+$ T-cells and how glucocorticoids affect this process. We identified miR-155 as a potential mediator of inflammation in $CD4^+$ T-cells and target of glucocorticoid effects. The implications of these findings and a model of miR-155 in allergic rhinitis and asthma are discussed herein.

Methods

Research subjects

After Institutional Review Board (IRB) approval, informed consent was obtained from all subjects. Venous blood samples were collected from a total of 44 subjects: 20 dust mite-allergic individuals with allergic rhinitis (positive skin test (≥4 mm wheal on skin prick test or >0.35 IU/ml serum ImmunoCAP test to *Dermatophagoides farinae*), 8 non-allergic (no positive skin prick tests or serum ImmunoCAP to a panel of 20 relevant indoor and outdoor aeroallergens in the northeast U.S.A.), and 6 dust mite-allergic severe asthmatics. Diagnosis of asthma was based on history consistent with asthma and evidence of airway hyper-reactivity (FEV1 change with albuterol ≥12% and 200 ml or increase in FEV1 of ≥12% and 200 ml with maximal anti-inflammatory treatment). Severity was based on NHLBI guideline classification [20].

Isolation of $CD4^+$ T-cells and culture

Using the standard Ficoll-Paque method, peripheral blood mononuclear cells (PBMCs) were isolated from the blood samples [21]. A concentration of 10^6 PBMCs/ml were seeded in 6-well cell culture plates. Cells were treated with 50 μg/mL of *Dermatophagoides farinae* dust mite extract (DME, obtained from GREER labs), DME + 0.1 μM dexamethasone (dex), or PBS control and incubated at 37°C for 48 hours. We selected *Dermatophagoides farinae* extract as this specific lot contained the lowest amount of endotoxin (486 EU/ml) of all the dust mite extracts available from the manufacturer at that time (GREER). $CD4^+$ T-cells were isolated from the cell cultures by immunomagnetic selection (Dynabeads™ CD4 Positive Isolation Kit, Life Technologies) per manufacturer protocol. Cells were harvested for miRNA analysis and media was collected for measuring levels of IL-4, IL-5, and IL-13, which were measured by ELISA per manufacturer (R&D Biosystems).

Analysis of miRNA expression

RNA was isolated from cell pellets using the TRIzol method, followed by column isolation

Table 1. Subject characteristics

Characteristic	Healthy (n=8) n (%) or mean ± SEM	Allergic (n=20) n (%) or mean ± SEM	Asthmatic (n=16) n (%) or mean ± SEM
Age (Years)	33.6 ± 3.93	38.9 ± 3.6	39.9 ± 3.66
Sex (Male/Female)	4/4 (50/50)	10/10 (50/50)	3/13 (19/81)
Race (White/Black/Asian)	6/0/2 (75/0/25)	16/0/4 (80/0/20)	13/3/0 (81/19/0)
Ethnicity (Hispanic/Non-Hispanic)	1/7 (12.5/87.5)	1/19 (0.05/99.95)	0/16 (0/100)
BMI (kg/m^2)	25.5 ± 2.48	27.3 ± 1.62	35.1 ± 2.65
Allergic Rhinitis	0 (0)	20 (100)	14 (88)
Spirometry			
FVC (L)	4.13 ± 0.69	4.41 ± 0.73	3.15 ± 0.23
FVC%	94.2 ± 5.32	100.2 ± 8.69	85.3 ± 4.58
FEV1 (L)	3.40 ± 0.51	3.43 ± 0.70	2.22 ± 0.16
FEV1%	95 ± 3.11	81.3 ± 8.79	72.9 ± 4.59
FEV1/FVC	0.84 ± 0.02	0.77 ± 0.01	0.70 ± 0.03

using the Direct-Zol RNA MicroPrep kit (Zymo Research, Irvine, Ca). RNA quality was assessed by Bioanalyzer (Model 2100, Agilent) and samples were submitted (on dry ice) to the Johns Hopkins School of Medicine Deep Sequencing and Microarray Core for Nanostring miRNA array analysis (nCounter Human V2 miRNA Expression Assay panel, Nanostring, Seattle, WA). The nCounter assay panel consisted of six positive controls, eight negative controls, five control mRNAs (ACTB, B2M, GAPDH, RPL19 and RPLP0), and 800 miRNAs. Data analysis was performed on the NanoString nSolver, version 2.5 software. Samples were normalized according to the geometric mean of the top 100 most highly expressed miRNAs. The minimum threshold to consider a miRNA for further analysis was a cutoff value corresponding to twice the standard deviation of negative control probes plus the means in at least half of the samples.

For analysis by qPCR, the qScript microRNA cDNA Synthesis Kit (Quanta BioSciences, Gaithersburg, MD) was used to reverse transcribe 250 ng of total RNA to cDNA. MiRNAs were quantified using quantitative PCR (qPCR) performed with the CFX384 Real-Time System. Mixes included primers specific to the miRNAs of interest (250 nmol/L), 1 μL of diluted cDNA (diluted 1:10), and the iTaq Universal SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA) in a total volume of 10 μL. Primers to each miRNA were synthesized by Integrated DNA Technologies (Coralville, IA) and placed in a unique position on 96-well plates at a stock concentration of 1 μmol/L. A multichannel EDP-3 Plus electronic pipette (Bio-Rad Laboratories) was used to transfer the primer to a 384-well plate. The specific primers were then mixed with cDNA, SYBR Green mix, and universal primer. Expression of miRNAs was measured by using the CFX384 Real-Time System (Bio-Rad Laboratories). Reactions were run in triplicate using the following 2-step program: 40 cycles at 95°C for 10 seconds, followed by 60°C for 30 seconds. Sample Ct values were normalized to SNORD44 using the ddCT method.

Statistical analysis

Expression of miRNAs with/without DME in the allergic and non-allergic group was determined by ANCOVA with Bonferroni post-test for multiple comparisons. Association between miRNA and cytokine expression was analyzed by linear regression. For all other analysis, two tailed T-test or ANOVA with Bonferroni adjustment for multiple comparisons were used. The significance level of all analyses was set at 0.05.

Results

Subject demographics

Our study included a total of 44 participants (**Table 1**). Across the healthy, allergic, and asthmatic categories, the average age was in the fourth decade of life. In the healthy and allergic groups, the male to female ratio was 1:1. The asthmatic group had a greater percentage of females, which is consistent with the demographics in our asthma clinic. Of the 16 asthmatic patients, 14 also had allergic rhinitis. The average FEV1 (L) in the healthy and allergic gro-

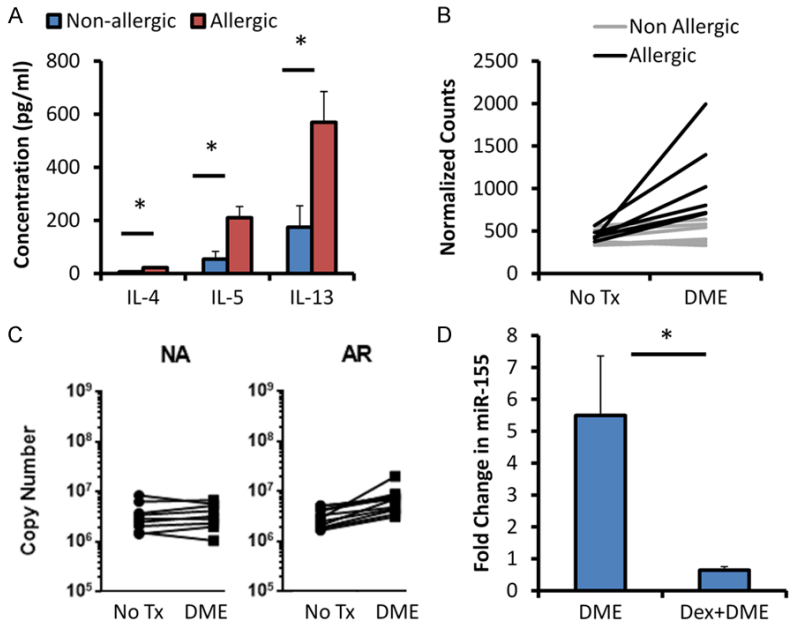

Figure 1. Effects of allergic stimuli on miR-155 expression. A. Dust mite extract induces T_H2 cytokine expression in $CD4^+$ T-cells of dust mite-allergic subjects. B. Nanostring miRNA array analysis demonstrates that miR-155 is induced in $CD4^+$ T-cells by DME. C. Validation of miR-155 induction using qPCR in non-allergic (NA) and allergic rhinitis (AR) subjects. D. Dexamethasone (dex) inhibits the effects of DME on miR-155 expression. (*) indicates P<0.05.

ups (3.40 ± 0.51 and 3.43 ± 0.70, respectively) were greater than in the asthmatics (2.22 ± 0.16). BMI was greater in the asthmatic group (35.1 ± 2.65) compared to non-asthmatic groups (25.5 ± 2.48 in healthy, 27.3 ± 1.62 in allergic), which is also consistent with our asthma clinic demographics.

Expression of miRNAs in $CD4^+$ T-cells

As expression of miRNAs in T-cells have not been well studied, we first sought to determine which miRNAs were expressed in $CD4^+$ T-cells. After isolation of $CD4^+$ T-cells from the blood of n=12 subjects (n=6 allergic and n=6 non-allergic), RNA was isolated and subjected to analysis using the Nanostring nCounter V2 Human miRNA platform. We identified 196 miRNAs that were reproducibly expressed in $CD4^+$ T-cells (Supplementary Data) and the top 20 expressed miRNAs are shown in Figure S1. We did not observe any differences between the miRNA expression pattern in the allergic rhinitis and non-allergic group in the absence of treatment.

We next asked whether DME-stimulation induced differential changes in subjects with allergic rhinitis (AR) vs. non-allergic subjects (NA). PBMCs from each group were incubated with DME for 48 hours with the intent of identifying which miRNAs were potentially induced by allergic stimulation of $CD4^+$ T-cells. The pro-T_H2 effect of allergen exposure on T-cells was confirmed by measuring up-regulation of IL-4, IL-5, and IL-13 in the media of the cells. All three cytokines were induced in the cells of AR compared to in NA subjects (**Figure 1A**). Expression of miRNA ± DME in AR and NA subjects was screened by Nanostring nCounter miRNA assay. The only miRNA that's expression was altered between the two groups of subjects was miR-155, 2.5 ± 0.5-fold change in AR group vs. 1.1 ± 0.2 in the NA group, P=0.04 (**Figure 1B**).

We next sought to validate these results using a different platform (qPCR) as well as with additional subjects. The qPCR analysis produced similar results and the induction of miR-155 by DME-stimulation was confirmed in subjects with allergic rhinitis (n=12) vs. non-allergic subjects (n=8), 2.4 ± 0.5-fold change vs. 1.1 ± 0.1 (mean ± SEM), respectively, P=0.015 (**Figure 1C**).

As glucocorticoids are potent anti-inflammatory molecules that inhibit allergic inflammation, we asked whether they could inhibit miR-155 as part of their mechanism of action. We recruited an additional n=8 dust mite allergic subjects and stimulated PBMCs with DME ± dexamethasone (dex, 100 nM). As expected, DME induced miR-155 expression (5.5 ± 1.9, fold change DME vs. No Tx) and this induction was effectively blocked by the addition of GCs (0.6 ± 0.1, fold change DME + dex vs. No Tx), **Figure 1D**.

miR-155 is induced by T-cell receptor-dependent and independent stimuli

We next sought to determine whether treatment of $CD4^+$ T-cells with a variety of T-cell receptor dependent and independent stimuli also induced miR-155 expression. Stimulation of the T-cell receptor using anti-CD3/anti-CD28

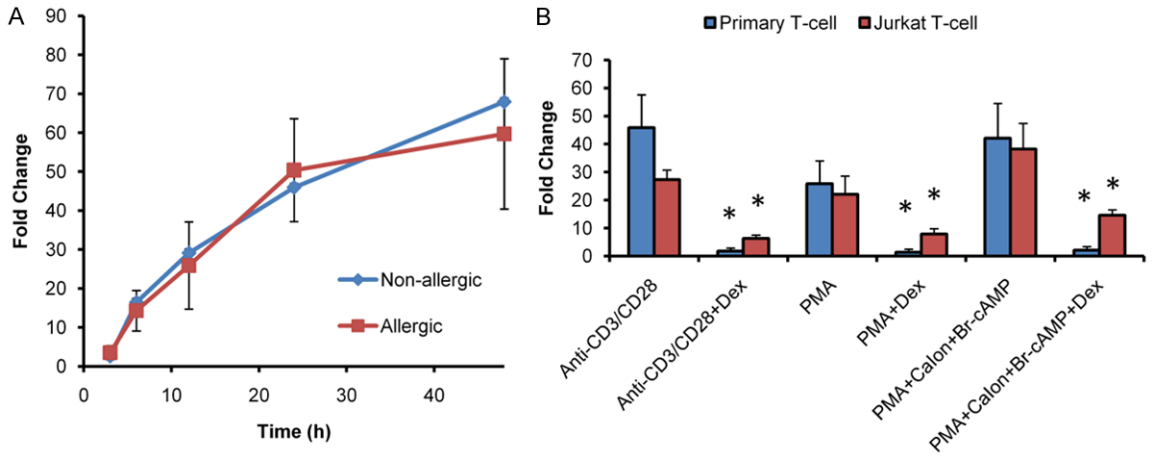

Figure 2. Effects of anti-CD3/anti-CD28 and PMA on miR-155 expression. A. Time course of anti-CD3/anti-CD28 stimulation of miR-155 in allergic and non-allergic subjects. B. Effects of Anti-CD3/anti-CD28, PMA, calcium ionophore (Calon), and bromocyclic AMP (Br-CAMP) ± dex in primary T-cells and Jurkat T-cells.

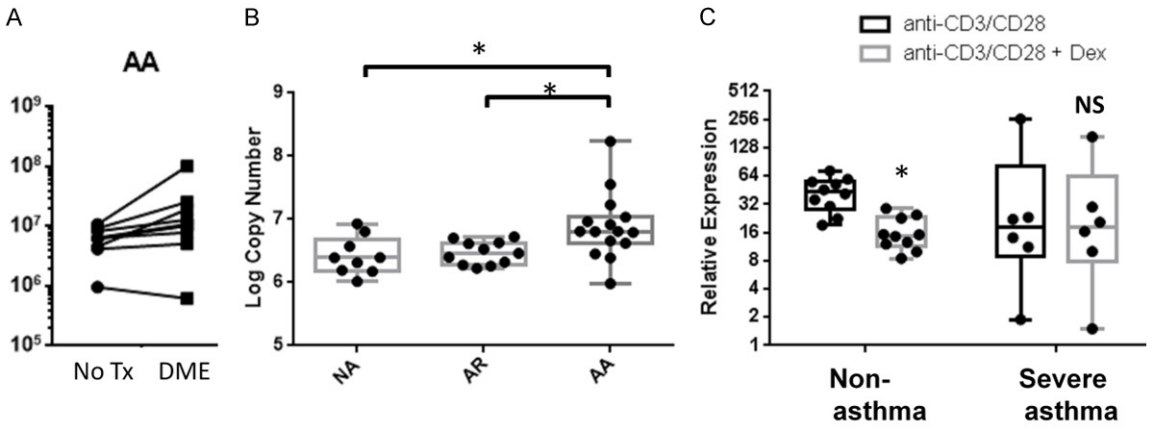

Figure 3. miR-155 expression in asthmatics. A. Effect of DME on miR-155 expression in dust mite-allergic asthmatics. B. Expression of miR-155 in $CD4^+$ T-cells of allergic asthmatics (AA) compared to in allergic rhinitis (AR) and non-allergic, non-asthmatic (NA) subjects. C. Effects of dex on anti-CD3/anti-CD28 stimulated miR-155 expression in severe asthmatics and non-asthmatics.

led to robust changes in miR-155 expression in cells from both allergic (n=5) and non-allergic subjects (n=5, **Figure 2A**). Induction of miR-155 was observed in as little as 1 hour and continued to rise 48 hours after stimulation. We also measured effects in Jurkat T-cells, a leukemic cell line. Similar effects were observed in Jurkat cells (n=5 replicates, **Figure 2B**), indicating that this cell line could be useful to interrogate the mechanisms of miR-155 as, in contrast to primary human cells, this cell line is easily transfected.

Next, the effect of the phorbol ester PMA, a non-T-cell receptor stimulus, was evaluated. Treatment with PMA also induced miR-155 expression, which was enhanced by the addition of calcium inophore and bromocyclic AMP (**Figure 2B**). These effects were observed in primary T-cells from human donors as well as in Jurkat T-cells. As was the case with DME-stimulation, the effects of anti-CD3/anti-CD28 and PMA were inhibited by GCs (dex 100 nM) in both primary and Jurkat cells. The inhibitory effect of dexamethasone was not as great in Jurkat T-cells, which is consistent with prior observations that these cells contain a mutation in one allele of the glucocorticoid receptor, which attenuates the effects of GCs [22].

These data together point to a conserved induction of miR-155 by a variety of stimuli, sug-

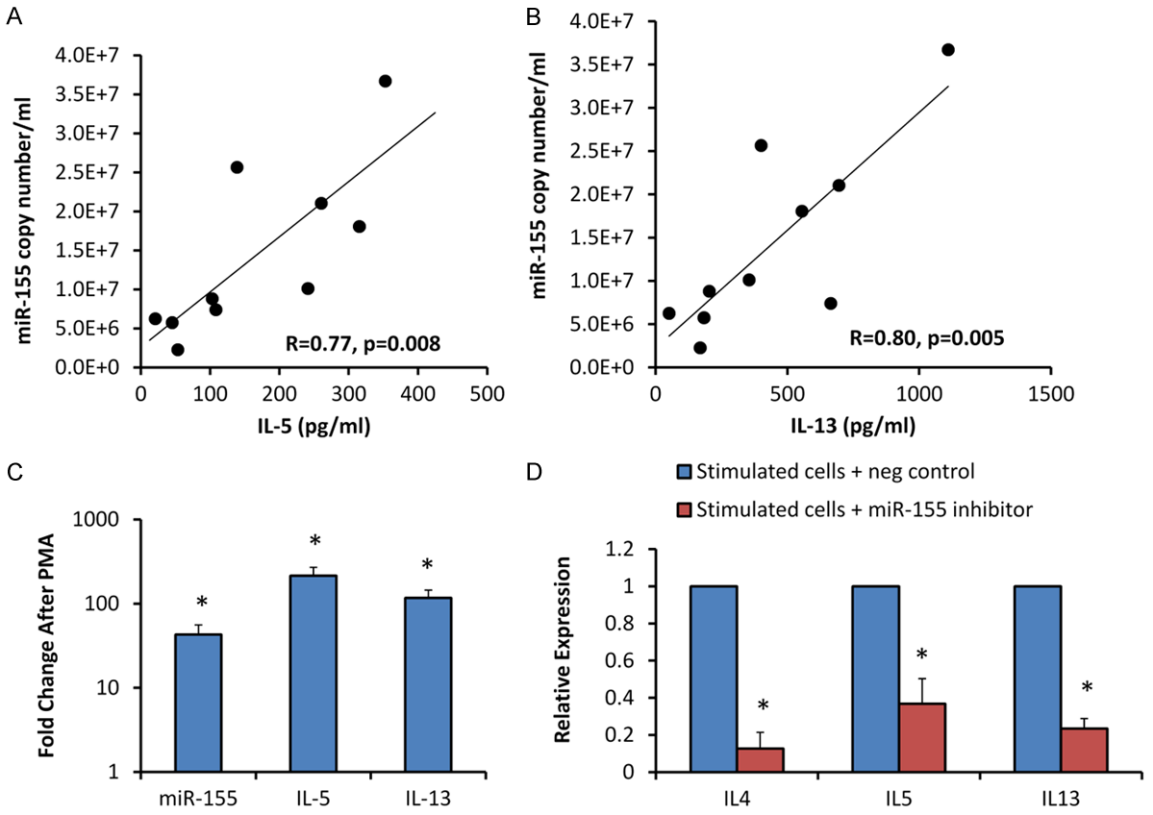

Figure 4. Association between miR-155 and T_H2 cytokines. miR-155 levels in $CD4^+$ T-cells are proportional to expression of IL-5 (A) and IL-13 (B) in DME-stimulated T-cells from allergic donors. (C) miR-155 as well as IL-5 and IL-13 are induced by PMA. (D) Transfection of a miR-155 inhibitor in Jurkat cells attenuates the production of T_H2 cytokines.

gesting a broad role in T-cell immunity. Moreover, it suggests that the Jurkat cell line could be a useful tool to understand the mechanisms of miR-155 biogenesis and function.

miR-155 expression and regulation by GCs in asthma

We next selected 6 subjects with dust mite-allergic asthma and asked whether expression of miR-155 or its induction with DME was altered in asthma. DME treatment induced expression of miR-155 in allergic asthmatic (AA) T-cells, with a 2.7 ± 0.9 fold change, P=0.036, similar to what was observed in AR (**Figure 3A**). Interestingly, the level miR-155 was higher in unstimulated T-cells in AA (**Figure 3B**), raising the possibility that this miRNA may have a role in $CD4^+$ T-cells in asthma.

Inhibitory effects of glucocorticoids on miR-155 is attenuated in severe asthma

A major barrier to effective treatment of severe asthma is that many subjects have a sub-optimal response to glucocorticoids. We sought to assess the effects of T-cell stimulation on miR-155 expression in severe, difficult-to-treat asthma, and determine whether the inhibitory effect of glucocorticoids was maintained. We recruited six severe asthmatics who met a clinical definition of glucocorticoid-insensitivity (no change in asthma symptoms or lung function after 1 week of treatment with 40 mg of prednisone daily). PBMCs were isolated from their blood, stimulated with anti-CD3/anti-CD28, and the level of miR-155 were measured ± dex (100 nM). Addition of GCs did not inhibit miR-155 expression, in contrast to the effects observed in non-asthmatics (**Figure 3C**).

Association between miR-155 and Th2 cytokine expression in $CD4^+$ T-cells

MiR-155 has emerged as an important T_H2 target in mouse models of allergic disease. However, there is little known about its effects in human disease and the mechanism of action in both species has not been elucidated. As miR-155 is induced by allergic stimuli, we hypothe-

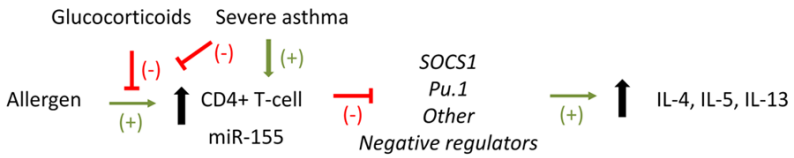

Figure 5. Proposed model of miR-155 in CD4$^+$ T-cells. miR-155 is induced by allergen presentation, which in turn contributes to expression of T_H2 cytokines, possibly by inhibiting negative regulators of T_H2 immunity. Glucocorticoids inhibit miR-155 production, though this pathway may be de-regulated in asthma.

sized that miR-155 promotes production of type 2 cytokines. This hypothesis was supported by the positive association between miR-155 expression and DME-induced T_H2 cytokine expression in T-cells. The number of copies of miR-155 post-DME treatment in CD4$^+$ T-cells of dust mite-allergic asthmatics was positively associated with levels of IL-5 (r=0.77, P=0.008) and IL-13 (r=0.80, P=0.005), as shown in **Figure 4A** and **4B**.

The effects of miR-155 on T_H2 cytokine expression were further probed in Jurkat T-cells, as these cells are readily transfectable and can be induced to produce IL-5 and IL-13. Consistent with the effects seen in primary CD4$^+$ T-cells, stimulation of Jurkat T-cells with PMA, calcium ionophore, and bromocyclic AMP produced a robust increase in miR-155 as well as in IL-5 and IL-13 (**Figure 4C**). In order to determine whether miR-155 was necessary for induction of these cytokines, Jurkat T-cells were transfected with a miR-155 antisense inhibitor. The miR-155 inhibitor attenuated production of T_H2 cytokines (**Figure 4D**), indicating that the miRNA augments expression of IL-4, IL-5, and IL-13.

Discussion

MiRNAs are emerging as important regulators of inflammation though their roles in allergic diseases and asthma remain to be well elucidated. In this study, we interrogated the expression and function of miRNAs in CD4$^+$ T-cells in allergic rhinitis and asthma. We identified 196 miRNAs that were detectable in CD4$^+$ T cells, which provides a useful baseline for future experiments that probe miRNA expression and function in these cells. Interestingly, the top 20 expressed miRNAs accounted for 92.8% of the total miRNA content (Figure S1), raising the possibility that a relatively small subset of miRNAs may be the most important to CD4$^+$ T-cell function. This subset included miR-155, the main candidate in our study.

Our finding that miR-155 was the sole miRNA differentially induced by DME in CD4$^+$ T-cells of allergic subjects as compared to in non-allergic subjects suggested a potentially important functional role. Our inability to find any other miRNAs that were differentially induced by DME could be multifactorial. This might be partially related to limitations in our study design, as miRNA expression was profiled in the total CD4$^+$ T-cell population instead of antigen-specific T-cells. We chose this study design to simplify our initial approach; however, as dust mite-specific T-cells represent only a small portion of the total T-cells in culture, it is possible that miRNAs with smaller magnitudes of changes could not be detected. These small magnitudes of change would essentially be dampened by the background of the non-antigen specific cells, where expression is unchanged.

Alternatively, it is possible that miR-155 is the central miRNA in allergic T-cell responses. The findings that both T-cell receptor-dependent (anti-CD3/anti-CD28) and independent (PMA) stimuli induce miR-155 in primary human T-cells as well as Jurkat leukemic cell line, suggest that the miRNA may have a conserved role in various T-cell processes. Along these lines, miR-155-deficient mice have identified the miRNA as a central player in a variety of disease models [23-25]. In particular, numerous studies have demonstrated that the miRNA is essential for airway inflammation [15, 26-30]. Consistent with these studies, our work presents human evidence that miR-155 is involved in CD4$^+$ T-cell allergic pathways. The CD4$^+$ cellular levels of miR-155 after DME treatment were proportional to the amount of T_H2 cytokines (i.e. IL-5 and IL-13) produced by these cells, indicating a link between these pathways. Inhibition of miR-155 by transfection of an antagomir in Jurkat T-cells repressed the production of these cytokines, demonstrating a role of the miRNA in augmenting their levels. However, the mechanisms tying miR-155 to T_H2 cytokine production remains to be completely elucidated. It has been shown that miR-155 inhibits inhibitors of cytokine production (i.e. SOCS1), thereby providing a mechanism to enhance their expression [31, 32]. In addition, miR-155 has been shown to target the tran-

scription factor PU.1, which could result in skewing towards T_H2 immunity [27, 33-35].

We also evaluated the potential roles of miR-155 in asthma, as we previously identified this miRNA as a possible biomarker in plasma of asthmatics [11]. Herein, we discovered that miR-155 expression was higher in $CD4^+$ T-cells of asthmatic subjects, raising the questions of whether there may be intrinsic de-regulation of miR-155 in $CD4^+$ T-cells of asthmatics and if the miRNA could be targeted therapeutically. Regarding the latter question, we sought to determine whether glucocorticoids could modulate miR-155 expression, potentially as part of its anti-inflammatory mechanism of action. Indeed, we found that dexamethasone blunts the increase in miR-155 expression in $CD4^+$ T-cells stimulated with DME, anti-CD3/anti-CD28, or PMA. However, this effect was lost in severe asthmatics, a group of patients whose disease is difficult to treat and often insensitive to the anti-inflammatory effects of glucocorticoids. We acknowledge that our sample size is small and may not be generalizable to all severe asthmatics, but the findings raise the questions about whether this pathway is de-regulated in this group of patients and whether inhibiting the miRNA could be a novel approach to anti-inflammatory therapy.

In summary, we present a model whereby presentation of allergen to $CD4^+$ T-cells leads to production of miR-155, which upregulates T_H2 cytokine expressions, perhaps by targeting SOCS1, Pu.1, or other negative regulators of T_H2 immunity (**Figure 5**). This pathway may be de-regulated in asthma, where baseline expression of miR-155 is higher in $CD4^+$ T-cells. Glucocorticoids repress miR-155 expression, possibly as a mechanism of its anti-inflammatory effects. These effects appear to be blunted in severe asthmatics that are insensitive to glucocorticoids. Future work will be needed to validate and further dissect these mechanisms, but carries potential that this pathway could be tapped at various points for targeted therapeutics.

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Disclosure of conflict of interest

None.

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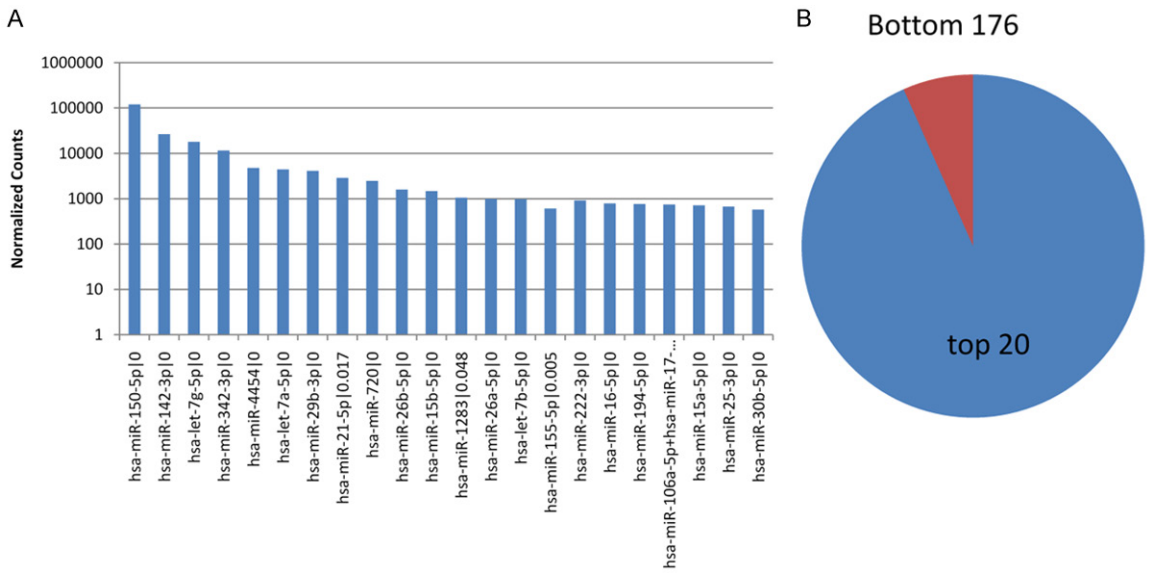

Figure S1. Top miRNAs expressed in CD4$^+$ T-cells. A. Expression of the top 20 miRNAs in CD4$^+$ T-cells. B. The top 20 miRNAs in CD4$^+$ T-cells account for over 90% of the total miRNA content in the cell.