Transfection with Cadherin-related Family Member 3 Variant Increases Eosinophil Adhesion to Transfected Cells and Superoxide Anion Generation

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Abstract

Rationale: A coding single nucleotide polymorphism (rs6967330; $C_{529}Y$) in cadherin-related family member 3 (CDHR3) is related to severe exacerbations of childhood asthma. Furthermore, CDHR3 is a receptor for rhinovirus (RV) C, which is closely linked to wheezing illnesses. A genetic variant increases CDHR3 (receptor) expression, RV-C binding and progeny yields, and the severity of illnesses, suggesting that CDHR3 may contribute to the pathogenesis of asthma exacerbation. To test the hypothesis that CDHR3 upregulates the effector functions of eosinophils, we examined whether transfection of CDHR3 variants could differentially modify eosinophil adhesion to transfected cells or superoxide anion (O_2^-) generation.
Methods: HeLa cells were transfected with plasmids encoding wild-type CDHR3 (C_{529}) or CDHR3 Y_{529} variant, and incubated with eosinophils obtained from healthy volunteers. Eosinophil adhesion to transfected HeLa cells was measured using eosinophil peroxidase assays or using fluorescence-labeled eosinophils. Eosinophil O_2^- generation was measured as superoxide dismutase-inhibitable reduction of cytochrome C.
Results: Transfection with plasmids encoding CDHR3 Y_{529} variant increased eosinophil adhesion to transfected cells as compared with that of wild-type CDHR3 (C_{529} 6.4 ± 1.1%; Y_{529} 7.9 ± 1.3%; $P < 0.01$ vs C_{529}). Furthermore, transfection of CDHR3 Y_{529} variant induced greater eosinophil O_2^- generation compared to that of wild-type CDHR3.
Conclusions: These findings suggest that transfection with the asthma-risk variant of CDHR3 up-regulated eosinophil functions such as adhesion and O_2^- generation. These effects may contribute to the development of eosinophilic airway inflammation during exacerbations of childhood asthma, especially with the CDHR3 variant.

Introduction

1. A coding single nucleotide polymorphism (SNP) (rs6967330; $C_{529}Y$) in CDHR3 is associated with severe exacerbations of childhood asthma[1]. This SNP directed greater CDHR3 protein expression on the cell surface.
2. CDHR3 is a receptor for rhinovirus C (RV-C)[2]. CDHR3 rs6967330; $C_{529}Y$ increases the expression of CDHR3 (receptor) protein on the cell surface, resulting in increased RV-C binding and progeny yields. Moreover, this SNP is associated with increased RV-C illnesses in vivo
3. Eosinophils play important roles in the development of asthma exacerbation.

Hypothesis

We hypothesized that transfection with CDHR3 variant increases eosinophil adhesion to transfected cells and superoxide anion generation

Aim

The objective of this study was to examine whether transfection of CDHR3 variants could differentially modify eosinophil adhesion to transfected cells or O_2^- generation.

Materials and methods

1. Eosinophils were obtained from healthy volunteers, and their adhesion to CDHR3 was measured using eosinophil peroxidase assays.
2. Eosinophil O_2^- generation was measured as superoxide dismutase-inhibitable reduction of cytochrome C. Eosinophil-derived neurotoxin (EDN) concentrations in cell media were measured as a marker of degranulation.
3. HeLa cells were transfected with plasmids encoding wild-type CDHR3 (C_{529}) or CDHR3 variant (Y_{529}) or lipofectamine only (Lipo) as described previously[2], and eosinophil adhesion to transfected cells or O_2^- generation was measured

Results

1. CDHR3 induces eosinophil adhesion

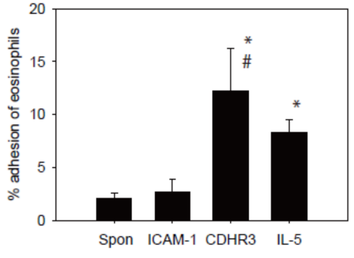

Figure 1. Effect of CDHR3 on eosinophil adhesion. Eosinophils (1×10^5 cells/ml) collected from non-allergic donors were incubated with or without IL-5 (100 pM), and their adhesion to rh-CDHR3 or rh-ICAM-1 was measured. Data are shown as means ± SEM of 6 experiments using cells from different donors. * P < 0.05 vs. spontaneous adhesion (Spon). # P < 0.05 vs. ICAM-1.

2. IL-5 enhances CDHR3-induced eosinophil adhesion

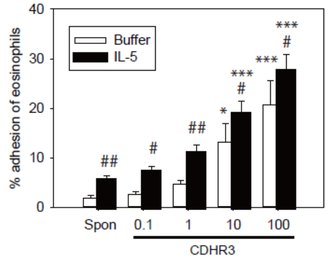

Figure 2. Effect of IL-5 on CDHR3-induced eosinophil adhesion. Eosinophils (1×10^5 cells/ml) collected from non-allergic donors were incubated with or without IL-5 (100 pM), and their adhesion to various concentrations of rh-CDHR3 (0.1-100 μg/mL) was measured (n = 6). * P < 0.05 and *** P < 0.001 vs. spontaneous adhesion (Spon). # P < 0.05 and ## P < 0.01 vs. buffer (without IL-5).

Results

3. Anti αM or β2 integrin Ab suppresses CDHR3-induced eosinophil adhesion

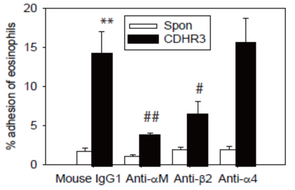

Figure 3. Effect of anti-integrin-mAbs on eosinophil adhesion to CDHR3. Eosinophils from non-allergic donors were pre-incubated with mouse IgG1, anti-αM integrin mAb, anti-β2 integrin mAb or anti-α4 integrin mAb (3 μg/ml, respectively) for 15 min. The adhesion of the eosinophils to rh-CDHR3 was then assessed (n = 6). ** P < 0.01 vs. spontaneous adhesion (Spon). # P < 0.05 and ## P < 0.01 vs. mouse IgG1.

4. CDHR3 induces eosinophil O_2^- generation and degranulation

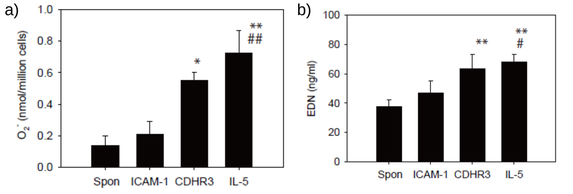

Figure 4. (a) Effect of CDHR3 on eosinophil O_2^- generation. Eosinophils (1×10^6 cells/ml) from non-allergic donors were incubated with or without IL-5 (100 pM) in CDHR3 or ICAM-1-coated plates (10 μg/ml). The generation of eosinophil O_2^- was examined (n = 6). The maximum value over the next 240 min of eosinophil O_2^- generation were shown. * P < 0.05 and ** P < 0.01 vs. spontaneous O_2^- generation (Spon). # P < 0.05 and ## P < 0.01 vs. ICAM-1. (b) Effect of CDHR3 on EDN release. Eosinophils (1×10^6 cells/ml) from non-allergic donors were incubated with or without IL-5 (100 pM) in CDHR3 or ICAM-1-coated plates (10 μg/ml) for the 240 min. Levels of EDN in cell-free supernatants were quantified using ELISA (n = 6). ** P < 0.01 vs. spontaneous EDN release (Spon). # P < 0.05 vs. ICAM-1.

5. Transfection with CDHR3 Y_{529} increases eosinophil adhesion and O_2^- generation

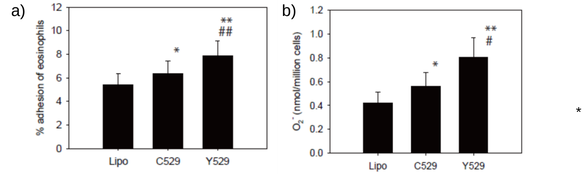

Figure 5. Effect of CDHR3 $C_{529}Y$ gene mutation on eosinophil functions. (a) Effect of CDHR3 $C_{529}Y$ gene mutation on eosinophil adhesion to HeLa cells transfected with plasmid DNA. HeLa cells were transfected with plasmids encoding wild-type CDHR3 (C_{529}) or CDHR3 variant (Y_{529}) or lipofectamine only (Lipo), and eosinophil adhesion to transfected cells was measured (n = 6). * P < 0.05 and ** P < 0.01 vs. Lipo. ## P < 0.01 vs. C_{529}. (b) Effect of CDHR3 $C_{529}Y$ gene mutation on eosinophil O_2^- generation. HeLa cells were transfected with plasmids encoding wild-type CDHR3 (C_{529}) or CDHR3 variant (Y_{529}) or lipofectamine only (Lipo), and eosinophils were incubated with transfected HeLa cells for 240 min. The generation of eosinophil O_2^- was examined (n = 6). The maximum value of eosinophil O_2^- generation was shown. * P < 0.05 and ** P < 0.01 vs. Lipo. # P < 0.05 vs. C_{529}

Results

6. Transfection with CDHR3 Y_{529} increases eosinophil adhesion

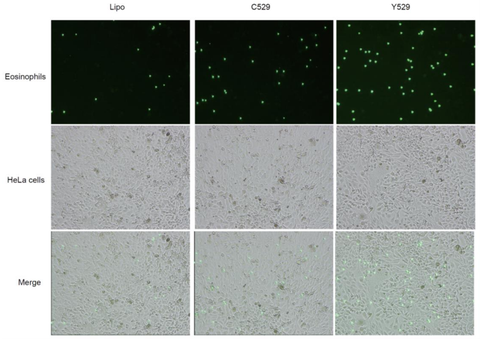

Figure 6. Representative figures of fluorescence-labeled eosinophil adhesion to transfected HeLa cells. Eosinophils were labeled with calcein-AM and then incubated with the transfected HeLa cells.

Discussion

1. We found that transfection with CDHR3 variants up-regulated eosinophil functions Genetic variation in CDHR3 could enhance airway inflammation and perhaps modify the risk for developing asthma through effects on eosinophil activation, as well as by increasing the risk of RV-C induced illnesses.
2. Whether CDHR3 expression is increased in the airway of patients with asthma is incompletely understood. Everman et al. reported that CDHR3 is exclusively expressed on ciliated cells, and that expression is greatest in cells undergoing ciliation as compared to mature ciliated cells[3], suggesting that eosinophil adhesion to ciliated cells could be greatest in airways with active cell differentiation or repair.

Conclusions

1. CDHR3 upregulates eosinophil adhesion, O_2^- generation and degranulation through αMβ2 integrin
2. Transfection with CDHR3 variants up-regulated eosinophil functions
3. These effects may contribute to the development of eosinophilic airway inflammation during exacerbations of childhood asthma, especially with the CDHR3 variant. (Nakagome K, et al. Allergy. 2020 (in press).

Reference

(1) Bønnelykke K, et al. Nat Genet. 2014.
(2) Bochkov YA, et al. Proc Natl Acad Sci USA. 2015.
(3) Everman JL, et al. JACI 2019.