House Dust Mite Extract Increases Rhinovirus Infection and Attenuates Antiviral Immunity in Respiratory Epithelial Cells

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Abstract

Human rhinovirus (HRV) is associated with both the development and exacerbation of asthma in at-risk children. HRV targets respiratory epithelial cells and activates the pattern recognition receptors MDA5 and TLR3 to express type I and III interferons (IFNs) and antiviral IFN-stimulated genes (ISG). Allergic sensitization increases the risk of asthma, and house dust mites (HDM) are major indoor allergens that are ubiquitous in bedding and other household environments. While the role of HDM in allergic inflammation is well understood, less is known about their effect on innate antiviral immunity. We compared the effect of pretreatment with HDM (*Dermatophagoides pteronyssinus*) extract on ISG expression and STAT1/2 activation in response to infection with HRV-16, stimulation with the synthetic dsRNA analog poly(I:C), or IFNβ in two human respiratory epithelial cell lines: A549 and BEAS-2B. ISG expression was measured by qRT-PCR, and STAT1/2 and IRF3 phosphorylation and HRV-16 infection were measured by western blot. We found that pre-treatment with HDM extract in both cell lines increased HRV infection and decreased ISG expression. To explore the mechanism behind this, we either added or transfected poly(I:C), which activates TLR3 alone or TLR3 and RIG-I/MDA5, respectively. We found that HDM extract blocked IRF3 and STAT1/2 activation, and inhibited ISG expression. Since HDM extract did not affect ISG expression in response to IFNβ, the effects of HDM extract are downstream of RIG-I/TLR3 activation and upstream of subsequent type I IFN stimulation. To explore the proteins responsible for these effects, we compared HDM extracts from different vendors and asked whether other indoor allergens similarly repress antiviral responses in these two cell lines. Interestingly, there were differences in ISG expression when comparing extracts from different vendors. Furthermore, we found that German cockroach and *Alternaria alternata* (mold) extracts also suppressed ISG expression after poly(I:C) stimulation, but only in the BEAS-2B cell line. Thus, different indoor allergens may attenuate antiviral host defense through different mechanisms. Defining mechanisms by which indoor allergens impair antiviral host defenses may suggest strategies to intervene in the development of allergic asthma in children.

Materials and Methods

Cell Culture: The BEAS-2B human bronchial epithelial cell line (ATCC) was cultured in BEGM Bronchial Epithelial Cell Growth Medium (Lonza, Walkersville, MD) supplemented with BEGM Bullet Kit. A549 cells (ATCC) were cultured in F12K medium supplemented with 10% fetal bovine serum. All cell lines were maintained at 37°C, 5% CO_2.

Virus Culture: Recombinant VSV-GFP was propagated in Vero cells when the cells were >80% confluent.

Treatment: House dust mite extract (LoTox™ *D. pteryonyssinus* Antigen, Indoor Biotechnologies unless otherwise noted) was added to cell cultures. After 24 hours, cells were either infected with HRV-16, VSV or stimulated with the synthetic dsRNA analog poly(I:C) (Millipore) or IFNβ (PBL). Poly(I:C) was either transfected into the cells using FuGene6 (Promega, Madison, WI) or added to the culture alone.

Western Blot: Protein expression in whole cell lysates was determined using STAT1, STAT2, P-STAT1, P-STAT2 antibodies (Cell Signaling Technologies, Danvers, MA), TLR3, P-TLR3 antibodies (Thermo Scientific), and GFP and Actin antibodies (Santa Cruz Biotechnology, Santa Cruz, CA)

RT-qPCR: ISGs were measured as described in Panda *et al* (2019).

Statistics: Statistical differences between each treatment within each cell line were calculated using a one-way ANOVA.

Results and Discussion

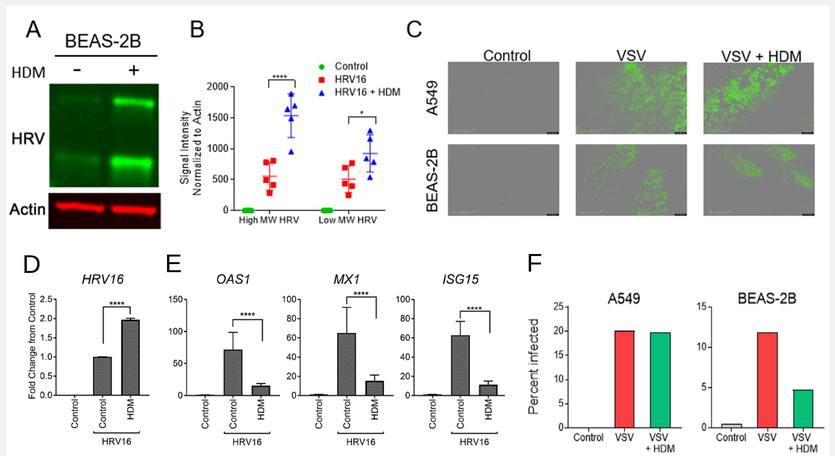


Figure 1. In BEAS-2B cells, house dust mite extract increases HRV infection and decreases ISG expression in response to HRV, but decreases VSV infection. *A549 or BEAS-2B cells were treated with 10 μg/mL HDM for 24 hours, then infected with HRV16 for 48 h or VSV at an MOI of 0.0004 (A549), or 0.002 (BEAS-2B) for 18 h. Immunoblots of cell lysates were probed with anti-HRV-A16 (A). Densitometry was performed on the HRV proteins in three experiments performed in duplicate and normalized to actin (B). Fluorescence images were captured at 18 hpi (C) and the percent VSV infected area (GFP+ area) was calculated (E). HRV16, OAS1, MX1, and ISG15 expression was measured by qPCR at 48 hpi (D-E). Data are presented as mean ± SD of three experiments performed in duplicate. *p ≤ .05, **p ≤ .01, ***p ≤ .001, ****p ≤ 0.0001.*

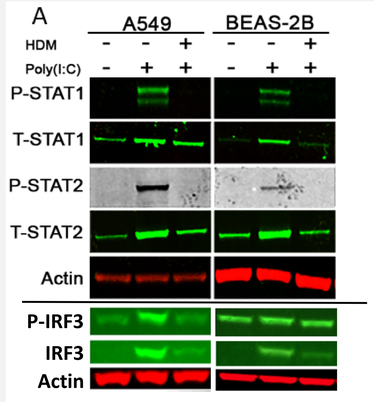


Figure 2. House dust mite extract inhibits poly(I:C) induced, but not IFNβ induced STAT activation and ISG expression. *A549 or BEAS-2B cells were treated with 10 μg/mL HDM for 24 hours, then transfected with 1 μg/mL Poly(I:C) for 24 hours (A-B) or stimulated with 0.25 ng/mL IFN-β for 6 hours (C). Immunoblots of cell lysates probed with anti-P-STAT1, anti-T-STAT1, anti-P-STAT2, anti-T-STAT2, anti-IRF3, and anti-pIRF3 are presented (A,C). The expression of OAS1, MX1, and ISG15 was determine by qPCR (B). Data presented as mean ± SD n = 3-6. *P < .05, **P < .01, ***P < .005.*

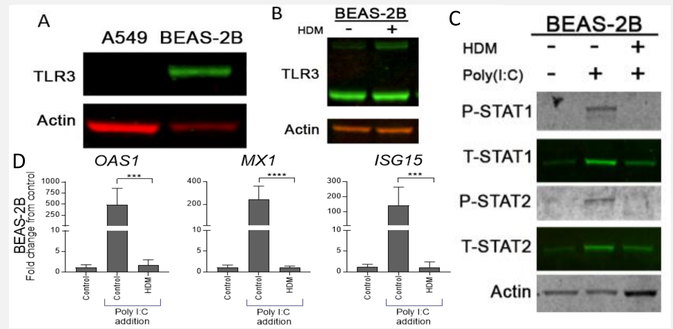
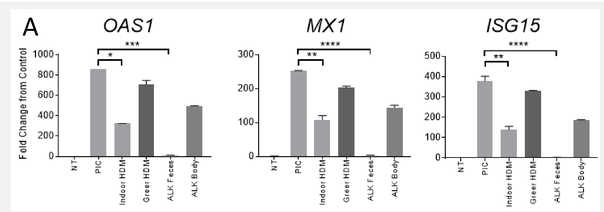


Figure 3. House dust mite extract inhibits ISG expression and STAT signaling through TLR3 in BEAS-2B cells. *A549 or BEAS-2B cells were treated with 10 μg/mL HDM for 24 hours, followed by the addition of 1 μg/mL Poly(I:C) to the culture for 24 hours (B-D). Immunoblots of cell lysates probed with anti-TLR3 (A-B), anti-P-STAT1, anti-T-STAT1, anti-P-STAT2, and anti-T-STAT2 (C) are presented. The expression of OAS1, MX1, and ISG15 was measured by qPCR (D). Data are presented as mean ± SD n = 3-6. *P < .05, **P < .01, ***P < .005.*



Vendor	Source Material
Indoor Biotech	HDM Bodies & Feces
Greer	HDM Bodies
ALK	HDM Feces
ALK	HDM Bodies

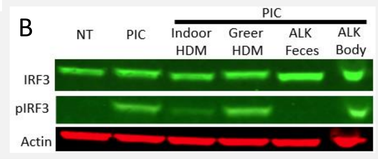


Figure 4. The impact of HDM from different vendors on ISG expression and IRF3 activation varies in BEAS-2B cells. *BEAS-2B cells were treated with 10 μg/mL HDM Extract for 24 hours, then transfected with 1 μg/mL Poly(I:C) for 24 hours. Immunoblots of cell lysates probed with anti-IRF3 and anti-pIRF3 are presented (A). OAS1, MX1, and ISG15 expression was measured by qPCR (B). The advertised source material of each vendor extract is noted. Data are presented as mean ± SD of three experiments performed in duplicate. *p ≤ .05, **p ≤ .01, ***p ≤ .001, ****p ≤ 0.0001.*

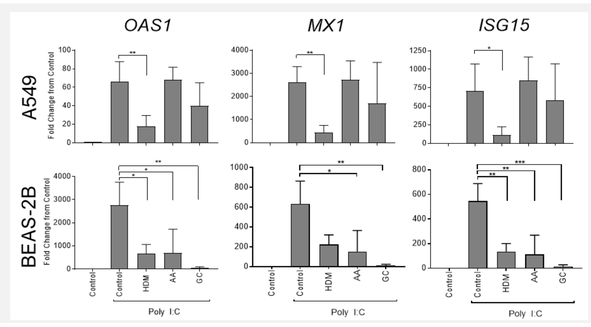


Figure 5. Inhibition of Poly I:C induced ISG expression is allergen and cell type dependent. *A549 or BEAS-2B cells were treated with 10 μg/mL HDM, 5 μg/mL Alternaria alternata (AA), 10 μg/mL German Cockroach (GC) for 24 hours, then transfected with 1 μg/mL Poly(I:C) for 24 hours. The expression of OAS1, MX1, and ISG15 was measured by qPCR. Data are presented as mean ± SD n = 6. *P < .05, **P < .01, ***P < .005.*

Conclusions

- House dust mite extract increases HRV, but not VSV infection levels, and decreases ISG expression in BEAS-2B cells
- House dust mite extract inhibits Poly I:C induced, but not IFNβ induced STAT activation and ISG expression
- Different indoor allergens may attenuate antiviral host defense through different mechanisms
- **A component of HDM feces interferes with poly(I:C) induced ISG expression and IRF3 phosphorylation in BEAS-2B cells, presenting a likely mechanism by which HDM increases HRV infection of respiratory epithelial cells**