House dust mite proteins reduce the spread of Respiratory Syncytial Virus in the BEAS-2B bronchial epithelial cell line.

Maria Rife[1], Andrew Freiman[1], Philippa Hillyer[1], Ronald L. Rabin[1]
[1]Laboratory of Immunobiology, CBER, USFDA, Silver Spring, MD

Abstract

House dust mites (HDM) are a predominant causative agent of airway hypersensitivity and asthma. The HDM group 1 allergens include Der p 1, a cysteine protease that contributes to sensitization and symptom exacerbation. Respiratory syncytial virus (RSV) infection of infants and small children can lead to severe pneumonitis. RSV activation of TLRs and RIG-I-like receptors induces proinflammatory cytokines and chemokines, which exacerbate disease, and type I/III interferons (IFNs) which induce expression of antiviral IFN stimulated genes (ISGs). Since RSV in children is coincident with HDM exposure, we used BEAS-2B human bronchial epithelial cells to explore the effects of HDM on RSV infection.

BEAS2B cells were exposed to HDM extract or Der p 1 alone before, during, or after RSV infection. Surprisingly, HDM extract or Der p 1 decreased RSV infection in a dose and time-dependent manner. In a viral entry assay, HDM extract reduced the area of foci, rather than their number, suggesting that one or more HDM proteins attenuate cell to cell spread. Preliminary experiments point to a role for the cysteine protease activity of Der p 1. We also measured expression of a panel of representative proinflammatory mediators and ISGs. Compared to RSV alone, Der p 1 increased expression of some proinflammatory mediators, while HDM extract decreased expression of a panel of ISGs. In the absence of RSV, HDM extract increased proinflammatory mediators while Der p 1 did not change gene expression. These results were unchanged following heat treatment at 65°C for 1 h. Elucidating mechanisms by which HDM proteins enhance protection against RSV may reveal novel antiviral mediators that locally control RSV infection.

Introduction

House dust mites (HDM) are ubiquitous small arthropods that thrive in a humid indoor environment and are predominant indoor allergens. HDM contains allergenic proteins belonging to proteolytic group 1 and non-proteolytic group 2. The enzymatic activity of group 1 includes Der p 1, a cysteine protease that may contribute to sensitization and symptom exacerbation due to the proteolytic cleavage of tight junctions. The group 2 allergen Der p 2 is structurally homologous with MD-2, a co-receptor of TLR4, and facilitates and enhances TLR4 signaling to induce the production of inflammatory cytokines and chemokines.

Since RSV in children is coincident with HDM exposure, we asked how HDM allergens affect RSV infection and host defense. Specifically, what are the critical host proteins that mediate local control of RSV and what are the effects of HDM proteins on RSV infection in vitro?

Materials and Methods

Virus culture: Recombinant RSV strain D46 6120 A2 expressing enhanced green fluorescent protein from an added gene between the P and M genes (rgRSV) was propagated in Vero cells at an input multiplicity of infection (MOI) of 0.01 PFU/cell and grown for 6-8 days in Optipro serum free medium (Life Technologies, Grand Island, NY) supplemented with 4 mM L-glutamine (Life Technologies). Cells were harvested and the virus was purified over a sucrose gradient. Virus was resuspended in RPMI 1640 supplemented with 2mM L-glutamine, snap-frozen, and stored at -80°C until use.

Materials and Methods

Cell culture: Human type II pulmonary epithelial cells, A549 cells (ATCC, Manassas, VA), were grown in Ham's F12K medium (Life Technologies) supplemented with 10% FBS (Quality Biological, Gaithersburg, MD). Human bronchial epithelial cells, BEAS-2B cells (ATCC), were grown in BEBM media (Lonza, Walkersville, MD) supplemented with BEGM SingleQuotsTM Supplements and Growth Factors (Lonza). Cells were maintained at 37°C, 5% CO2 without antibiotics. For experiments, cells were seeded in 24-well plates and grown to 90% confluence. For infection, virus was diluted in appropriate medium containing 20ug/mL gentamicin (Lonza). Cells were inoculated with 0.25 to 0.5 mL per well for 2 hours with shaking at 37°C, 5% CO2. For A549 cells, FBS in medium was reduced to 5% during infection. At 2 hpi, the virus-containing medium was removed and replaced with fresh medium. Cells were exposed to house dust mite extract (Indoor Biotechnologies, Charlottesville, VA) or purified Der p 1 (Indoor Biotechnologies) or Der p 2 (Indoor Biotechnologies) 24 hours prior to, during or 2h after infection or stimulation. Cells were imaged at intervals to 48 hours or harvested or lysed for PCR.

Time lapse imaging: Scans of the infected cultures were taken at 1 – 4 h intervals using an Incucyte S3 (Essen Biosciences, Ann Arbor, MI). RSV infection was quantified by measuring percent green objects in confluent cultures.

RT-qPCR: IFN gene expression was measured as described in Hillyer et al (1). All other genes were measured using gene expression assays from Life Technologies.

Results

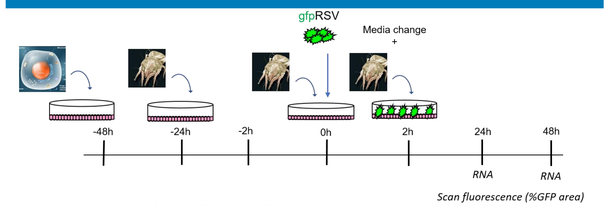
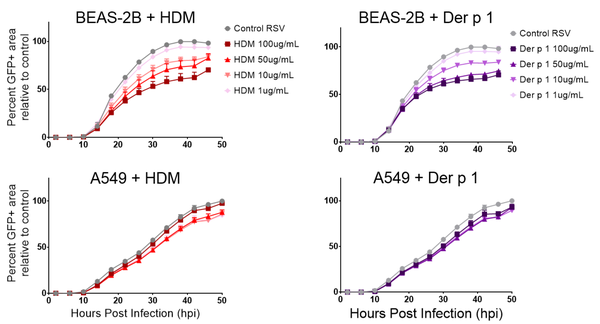
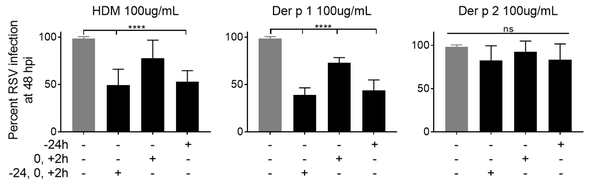


Figure 1. Workflow for studies on the effects of HDM on innate immune responses of lung epithelial cells. A549 or BEAS-2B epithelial cells are grown to confluence for 48 hours prior to viral infection. For RSV experiments, the virus is removed after 2 hours and replaced with a volume of fresh medium. Cells were exposed to house dust mite extract or purified Der p 1 or Der p 2 24 hours prior to, during or 2h after infection or stimulation. Cells were imaged at intervals to 48 hours or harvested or lysed for PCR.



Results



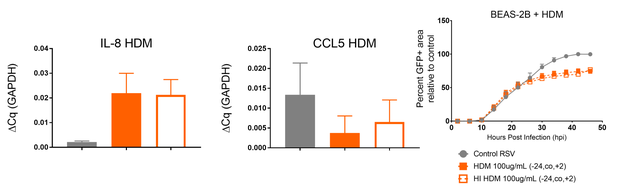


Figure 2. House dust mite extract and Der p 1 inhibit RSV spread in a dose and time dependent manner in BEAS-2B cells. BEAS-2B cells (A) treated with the indicated doses of HDM extract, Der p 1 or Der p 2 at -24, 0 and 2h relative to RSV infection or (B) treated with 100µg/ml of HDM extract, Der p 1 or Der p 2 at the indicated time points relative to RSV infection. RSV MOI = 0.3. Data are presented as normalized percent mean infection relative to RSV alone at 48h with mean ± SD for three experiments performed in duplicate. *p ≤ .05, **p ≤ .01, ***p ≤ .001, ****p ≤ 0.0001. Statistical differences between each treatment within each cell line were calculated using a one-way ANOVA with Tukey's test for multiple comparisons.

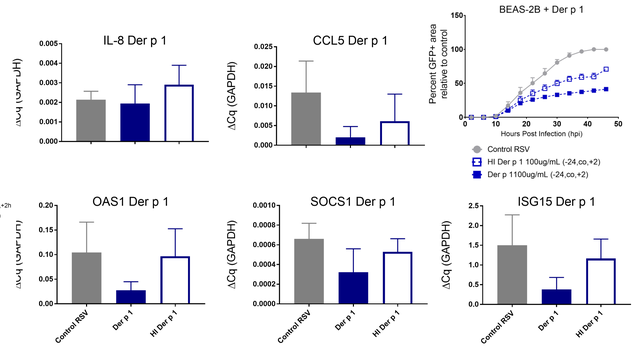


Figure 5. Heat inactivation (HI) effect of Der p 1 partially mitigated infection and some ISG and proinflammatory cytokine gene expression, with no significant change after HI HDM . BEAS-2B cells were treated with 100µg/ml of (HI) HDM extract or (HI) Der p 1 at the indicated time points relative to RSV infection. RSV MOI = 0.3. Gene expression was quantified using RT-qPCR. Data are presented as mean ± SD for three experiments performed in duplicate.

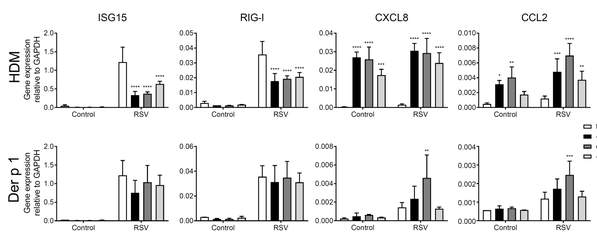


Figure 3. Differential effects of Der p 1 and HDM extract, which decreased ISGs and increased proinflammatory cytokine gene expression. BEAS-2B cells were treated with 100µg/ml of HDM extract or Der p 1 at the indicated time points relative to RSV infection. RSV MOI = 0.3. Gene expression was quantified using RT-qPCR. Data are presented as mean ± SD for three experiments performed in duplicate. Statistical differences between treated and controls were tested using a two- way ANOVA with Dunnett's post hoc test for multiple comparisons. *P < .05, **P < .005, ***P < .0005 ****P < .0001.

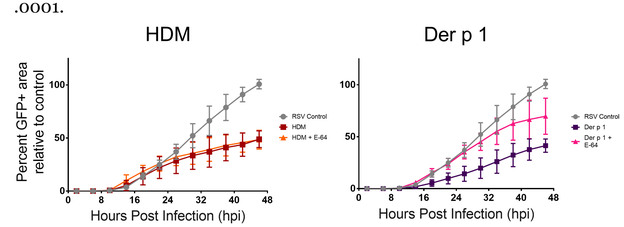


Figure 4. Cysteine protease E-64 treatment of Der p 1 partially effected Der p 1 inhibition of RSV spread, while E-64 treatment of HDM had no effect. BEAS-2B cells were treated with 100ug/mL HDM or Der p 1 incubated with 10ng/mL E-64. Data are presented as mean ± SD for four experiments performed in duplicate.

Conclusion

- Pre- or post-treatment with HDM extract *decreases* RSV infection in vitro
  - No effect on cell viability
  - Unaffected by heat inactivation
  - Decreases representative ISG expression and increases proinflammatory cytokines
- Der p 1 decreases RSV infection, but does not affect most ISGs tested, with some increases in proinflammatory cytokines
  - Partially mitigated by heat inactivation and E-64 treatment
- Therefore, Der p 1 reproduces the antiviral effect of HDM extract, but not most of the immunologic parameters that we measured (and that are often measured)
- Defining the mechanism by which Der p 1 decreases RSV infection will identify critical factors that selectively suppress RSV