ORIGINAL RESEARCH

# Modulation of Airway Epithelial Antiviral Immunity by Fungal Exposure

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## Abstract

Multiple pathogens, such as bacteria, fungi, and viruses, have been frequently found in asthmatic airways and are associated with the pathogenesis and exacerbation of asthma. Among these pathogens, *Alternaria alternata* (Alt), a universally present fungus, and human rhinovirus have been extensively studied. However, their interactions have not been investigated. In the present study, we tested the effect of Alt exposure on virus-induced airway epithelial immunity using live virus and a synthetic viral mimicker, double-stranded RNA (dsRNA). Alt treatment was found to significantly enhance the production of proinflammatory cytokines (e.g., IL-6 and IL-8) induced by virus infection or dsRNA treatment. In contrast to this synergistic effect, Alt significantly repressed type I and type III IFN production, and this impairment led to elevated viral replication. Mechanistic studies suggested the positive role of NF-κB and mitogen-activated protein kinase pathways in the synergism and the attenuation of the TBK1-IRF3 pathway in the inhibition of IFN production. These opposite effects are caused by separate fungal components. Protease-dependent and -independent mechanisms appear to be involved. Thus, Alt exposure alters the airway epithelial immunity to viral infection by shifting toward more inflammatory but less antiviral responses.

**Keywords:** Toll-like receptor 3; antiviral; epithelium; *Alternaria*; rhinovirus

### Clinical Relevance

This is the first report about the interaction between two important asthma-associated pathogens. Alteration of the airway epithelial antiviral immunity by fungal exposure may play an important role in the pathogenesis of asthma and its exacerbation. Elucidation of this mechanism will advance our understanding of these complex airway diseases.

The prevalence of asthma has significantly increased in the United States and in other industrialized countries (1). The development of asthma depends on the complicated interactions between genetics and environmental exposures (2). The pathological features of asthma include chronic airway inflammation (mainly type 2 T-helper cells [Th2] type), mucus cell metaplasia (the cause of mucus overproduction), airway remodeling (collage deposition, thickening of the basement membrane, etc.), and bronchial hyperresponsiveness. The prelude of asthma development is usually a repeated environmental allergen exposure and sensitization (3, 4). However, not all allergens can cause asthma (5, 6), and there is no structural similarity among these allergens (7).

*Alternaria alternata* (Alt) was first found to be the major asthmagenic allergen (or asthmagen) in children raised in arid and semiarid regions (8). A European community respiratory health survey has found that sensitization to fungi (Alt or Cladosporium) was a powerful risk factor for severe asthma in adults (2). A recent National Institute of Environmental Health Sciences study has indicated that Alt exposure in homes throughout the United States was associated with active asthma symptoms (9). Thus, Alt exposure appears to be associated with asthma in children and adults across different demographic regions. Because of the heterogeneous nature of Alt, human exposure to Alt is complicated and may involve many fungal factors, such as proteases, chitin, glucan, and toxins (10–12). Airway epithelium appears to be first to be affected by Alt exposure. Several studies, primarily using cell line models, have found various Alt-induced epithelial responses, such as cytokine transcription (13, 14), secretion (15), ATP release (16), and calcium flux (15–17).

Rhinovirus (RV) is another asthma-related pathogen. It is a small, positive-

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stranded RNA virus (18). In healthy individuals, RV infection is the major cause of the common cold, which has no effective treatment and imposes great economic burden (18). RV infection also plays a significant role in the development of asthma and in triggering asthma exacerbation. The wheezing illness associated with RV infection during pregnancy is a strong predictor for asthma development (19). In the Childhood Origins of Asthma birth cohort study (20), data have shown that episodes of wheezing associated with RV infections early in life was the most robust predictor of the risk of asthma. The odds ratio for the development of asthma was not further enhanced when wheezing episodes linked to RV and RSV were compared with those due to RV infection alone (20). These data suggest that early-life RV infection significantly increases the risk of asthma development independent of RSV. Asthma exacerbation represents a precipitation of the symptoms in otherwise stable patients with asthma and has been an important credential for the diagnosis of severe asthma. Eighty-five percent of acute asthma exacerbations in children and approximately 60% in adults are associated with the presence of viral infection (21, 22). Among all the viruses, RV is the dominant viral pathogen detected in approximately 60% of viral exacerbations in children and adults (21, 23, 24). Consistently, RV was also the major viral pathogen detected in children and adults hospitalized for asthma exacerbations (25, 26). Thus, besides its critical role in the development of asthma, respiratory RV infection is also the predominant risk factor associated with exacerbations of asthma.

Airway epithelium is the primary infection site of RV (27). Although direct viral particle binding to the cell surface can induce some effects (28), the majority of studies have focused on the replicating virus and its intermediate double-stranded RNA (dsRNA) (29–32). We (29, 32) and others (30, 31) have demonstrated a diverse pathway network (i.e., TLR3, PKR, MDA5/RIG1) that leads to proinflammatory cytokine productions and the IFN-mediated antiviral defense in airway epithelium. Among these pathways, TLR3 is the initial sensor of RV infection, and its activation regulates the activity of RIG1/MDA5 pathways (33). When engaging their cognate ligands, TLR3 dimerize, trigger the recruitment of intracellular proteins, and initiate the signaling via an adaptor protein-TICAM1 (or TRIF) (34). The downstream signaling pathways mediated by TICAM1 include the activation of nuclear factor-κB (NF-κB), extracellular signal–regulated kinase (ERK), p38, and c-Jun N-terminal kinase (JNK), which lead to proinflammatory cytokine production (34). In addition, TLR3-TICAM1 activates the IFN pathway (34). IFN-β and IFN-λ have been linked to the epithelial anti-RV defense reported by us (32) and others (35). The repressed IFN response in asthma, which led to uncontrollable RV replication, has been associated with RV-induced asthma exacerbation (35, 36).

Despite the facts that multiple pathogens are found in asthmatic airways and that host–pathogen interactions (37–39) are important for the development and exacerbation of asthma, almost all recent studies are centered on the single pathogen, which may not reflect the nature of this complex disease. Thus, in the present study, we have made the first attempt to explore the potential interaction between Alt and RV, the two most important pathogens in asthma.

## Materials and Methods

### Reagents and Expression Constructs

Lyophilized cake of *Alternaria* filtrates was purchased from GREER (Lenoir, NC) and dissolved in cell culture media to make a 100× stock solution. RV16 stock was amplified and purified, and the titer was determined based on the previous published protocol (40). Synthetic dsRNA was from InvivoGen (San Diego, CA). Protease inhibitor mix was from Sigma (St. Louis, MO). Monoclonal antibody targeting RV16 coat protein was generated as described elsewhere (32). Antibodies targeting phosphorylated (p)-ERK, p-IκBα, and p-IRF3 were purchased from Cell Signaling Technology (Danvers, MA). p-P38 and p-JNK antibodies were from BD Transduction Laboratories (San Jose, CA). Anti-actin antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). IL-6 and IL-8 ELISA kits were purchased from R&D systems (Minneapolis, MN). The IFN-β ELISA kit was from Invitrogen (Carlsbad, CA). The medium concentration of IL-6 or IL-8 was reported in pg/ml, and IFN was reported in international units/ml (32). TBK1, kinase inactive mutant TBK1 (K38A), and IFN reporter were generous gifts from K. Fitzgerald (University of Massachusetts).

### Cell Culture, Alt Exposure, and RV Infection

Human tissues were obtained from the National Disease Research Interchange or from University Medical Center. The University of Arizona Institutional Review Board approved all procedures involved in tissue procurement. Primary airway tracheobronchial epithelial cell cultures were established as described elsewhere (41, 42). Because of the high resistance to viral infection of the well-differentiated airway epithelial cells, monolayered primary cells (mainly basal cells) were used in the study to achieve high viral infection. To account for the donor variation, every experiment involving primary cells was repeated on at least three independent

**Table 1:** Real-Time Primers

| Gene | Primers |
|---|---|
| *IFN-β* | Forward: ATTGCCTCAAGGACAGGATG; reverse: GCTGCAGCTGCTTAATCTCC |
| *IFN-λ1* | Forward: GGACGCCTTGGAAGAGTCACT; reverse: AGAAGCCTCAGGTCCCAATTC |
| *RV16* | Forward: GCTGTGCAGTTGGATGTGAT; reverse: AAAGCCATGATGCAATCTCC |
| *IL-6* | Forward: GTAGTGAGGAACAAGCCAGAGC; reverse: TCAGGGGTGGTTATTGCATCTA |
| *IL-8* | Forward: GCTCTGTGTGAAGGTGCAGTT; reverse: TGTGGTCCACTCTCAATCACTC |
| *GAPDH* | Forward: CAATGACCCCTTCATTGACC; reverse: GACAAGCTTCCCGTTCTCAG |

*Definition of abbreviation*: RV, rhinovirus.

donors. A549 cells were cultivated in RPMI media plus 10% FBS. Alt was first titrated in A549 and primary epithelial cells for the induction of IL-8. The dose of 21.5 μg/ml was found to be the optimal dose. Higher doses of Alt induced cytotoxicity, presumably due to its intrinsic protease activity. Heat-inactivated Alt was prepared by heating Alt stock solution to 70°C for 30 minutes. For protease inactivation, Alt stock was incubated with protease inhibitor mix for 30 minutes. For RV infection, a multiplicity of infection of 10 was used as described elsewhere (32). For the combined treatment, RV/dsRNA and Alt were coincubated with cells for the indicated time points. Immediately before the sample collection (RNA or protein), the cells were thoroughly washed to remove residual RV or Alt.

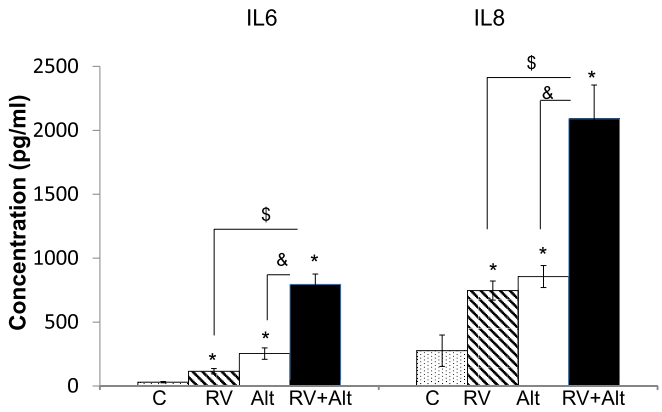


**Figure 1.** *Alternaria alternata* (Alt) enhanced rhinovirus (RV)-induced cytokine production in epithelial cells. Epithelial cells were infected with RV 16 (multiplicity of infection, 10) and treated with Alt (21.5 μg/ml) individually or in combination. Culture medium was collected 24 hours later and was analyzed by ELISA for IL-6 or IL-8. $^{*}P < 0.05$ ($n$ = 5) when the treated samples (Alt, RV, or ALT+RV) were compared with the nontreated control (C). $^{\$,\&}P < 0.05$ ($n$ = 5) when RV-infected or Alt-treated samples were compared with the samples with cotreatment (RV+Alt).

### Real-Time PCR

Real-time PCR was performed as described previously (43). The relative mRNA amount in each sample was calculated based on the $\Delta\Delta C_t$ method using housekeeping gene *GAPDH*. Results were usually calculated as fold induction over control as described previously (29), except for the RV positive-stranded RNA, which was presented in relative viral RNA abundance (32). All primer sequences are listed in Table 1.

### Transient Transfection and Dual-Luciferase Reporter Assay

For the dual-luciferase reporter gene assay, cells were transfected with IFN luciferase reporter plus TBK1 or plus TBK1 (K38A) together with the Renilla luciferase expression plasmid pGL4.74 (Rluc/TK) (Promega, Madison, WI). At 24 hours after transfection, the cells were treated with or without Alt for 3 hours and lysed with passive lysis buffer (Promega). Firefly and Renilla luciferase activities were measured with the dual-luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity. The experiment was performed in triplicate and expressed as mean ± SD.

### Western Blot

Total cellular protein was collected based on the methods described previously (29). Equal protein load for total cellular proteins was confirmed using the staining of antiactin antibody.

### Statistical Analysis

Experimental groups were compared using a two-sided Student's $t$ test, with significance level set as $P < 0.05$. When data were not distributed normally, significance was assessed with the Wilcoxon

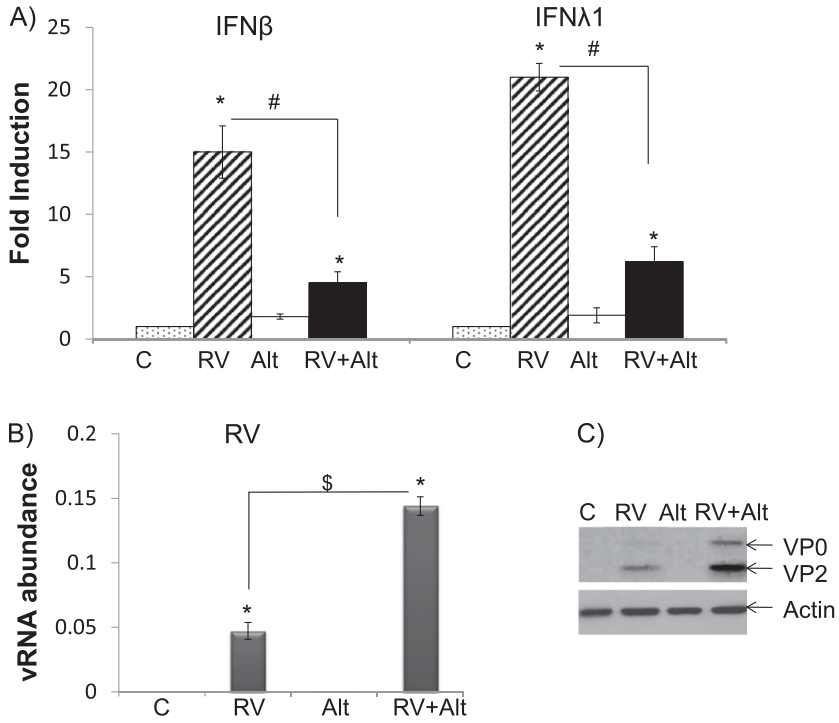


**Figure 2.** Alt repressed RV-induced IFN expression and enhanced RV replication in epithelial cells. Cells were infected with RV 16 and treated with Alt individually or in combination. RNA was collected 24 hours later and was analyzed by real-time PCR. Fold induction of IFN-β and IFN-λ1 (*A*) or relative abundance of viral RNA (vRNA) (*B*) were determined. $^{*}P < 0.05$ ($n$ = 5) when the treated samples (Alt, RV, or Alt+RV) were compared with the control. $^{\#}P < 0.05$ ($n$ = 5) when the samples with cotreatment (RV+Alt) were compared with RV-infected samples. $^{\$}P < 0.05$ ($n$ = 5) when RV-infected samples were compared with the cotreatment (RV+Alt) samples. (*C*) Cellular protein was collected and analyzed by Western blot. VP0 and VP2 = RV coat protein. Actin was used as a loading control. The image is representative of at least three independent experiments.

matched-pairs signed-ranks test, and $P < 0.05$ was considered to be significant.

## Results

### Alt Enhanced RV-Induced Proinflammatory Cytokines but Repressed RV-Induced IFN Production

Because airway epithelium is the primary site of Alt exposure and viral infection, we tested if Alt had any effect on RV-induced epithelial signaling. Alt significantly enhanced productions of IL-6 and IL-8 induced by RV infection (Figure 1). IL-6 concentration was 115, 253, 795 pg/ml in the samples of RV infection alone (RV), Alt exposure alone (Alt), and the combined treatment (RV+Alt), respectively, and IL-8 was 746, 856, and 2,089 pg/ml, respectively. Thus, this enhancement was not a merely additive effect; rather, it was a synergistic effect. Alt also strongly repressed gene expression of type I IFN (IFN-β) and type III IFN (IFN-λ1) (Figure 2A). Consistently, this repression led to significant increases of viral RNA (Figure 2B) and coat protein (Figure 2C), suggesting the increase of RV replication and production. Thus, Alt-induced impairment of IFN production appeared to increase the susceptibility of these cells to RV infection.

### Alt Enhanced dsRNA-Induced Proinflammatory Cytokines but Repressed IFN Production

We (29, 32) and others (30, 31) have demonstrated a diverse pathway network (i.e., involving TLR3 and MDA5/RIG1) that mediates epithelial proinflammatory cytokine production and IFN-mediated antiviral defense. Because there is no established ligand for MDA5/RIG1, we tested if Alt affected TLR3 signaling using its specific ligand, dsRNA. First, we tried to define the kinetics of Alt- or dsRNA-induced gene expression. Alt significantly increased the expression of proinflammatory cytokines (e.g., IL-6 and IL-8) (Figures 3A and 3B) but not of any IFN genes (data not shown). The gene expression was significantly elevated at 3 hours, peaked at 6 hours, and then decreased at 24 hours. dsRNA treatment also induced the expression of IL-6 (Figure 3C) and IL-8 (Figure 3D). In contrast to Alt, dsRNA robustly induced the expression of type I (Figure 3E) and III IFN (Figure 3F). The kinetics of IFN elevation were more transient as compared with cytokine induction. Both IFN's gene expressions peaked at 3 hours, rapidly decreased at 6 hours, and almost completely fell back to the control level at 24 hours. Therfore, we used the early time points (i.e., 3, 6 h) to examine the interaction between Alt and dsRNA at the level of gene expression and used the 6-hour time point for the combined secretory protein level in the media. We found that Alt significantly enhanced dsRNA-induced expression of IL-6 and IL-8 (Figure 4A) but robustly repressed expression of IFN-β and IFN-λ1 (Figure 4C) at 3 hours. The samples from the 6-hour treatment had identical results (data not shown). Consistently, at the protein level, Alt significantly increased dsRNA-induced IL-6 secretion (Figure 4B) while repressing dsRNA-induced IFN-β secretion (Figure 4D). Thus, the opposite effects of Alt on RV-induced epithelial gene expression (Figures 1 and 2) may be mediated by the interactions between the Alt and TLR3 signaling pathways.

### NF-κB and Mitogen-Activated Protein Kinase Were Involved in the Synergistic Effect of Alt on TLR3 Signaling

TLR3-mediated proinflammatory cytokine expression has been reported to be regulated by the mitogen-activated protein kinase (MAPK) and NF-κB pathways (44). Either dsRNA or Alt individually activated NF-κB (increased P–I-kappa B) and MAPKs (increased p-ERK, P38, and JNK) (Figure 5). As expected, the combined treatment of dsRNA and Alt further enhanced the activation of these pathways. Thus, the synergism of Alt and dsRNA on the proinflammatory cytokine expression may be mediated by the greater activation of the NF-κB and MAPK pathways by the combined treatment as compared with the individual treatment.

### Alt Repressed TBK1-IRF3

IRF3 has been reported to be the key transcriptional factor that drives IFN expression downstream of TLR3 (44). Therefore, we tested TBK1, an upstream kinase of IRF3. In this assay, TBK1 overexpression in airway epithelial cells

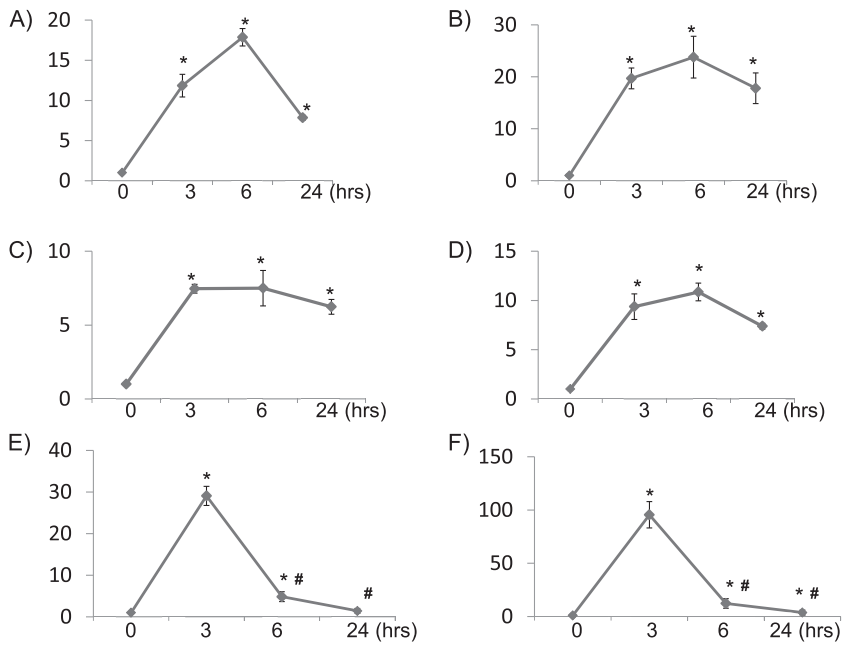


**Figure 3.** Time course of Alt- or double-stranded RNA (dsRNA)-induced gene expression in epithelial cells. Epithelial cells were treated with Alt (*A*, *B*) or dsRNA (*C–F*) individually. RNA was collected at the indicated time point and was analyzed by real-time PCR. IL-6 (*A*, *C*), IL-8 (*B*, *D*), IFN-β (*E*), and IFN-λ1(*F*) expression was determined. $^{*}P < 0.05$ ($n = 5$) when the treated samples (Alt or dsRNA) were compared with the control. $^{\#}P < 0.05$ ($n = 5$) when dsRNA-treated samples at later time points (i.e., 6 or 24 h) were compared with the samples treated with dsRNA for 3 hours.

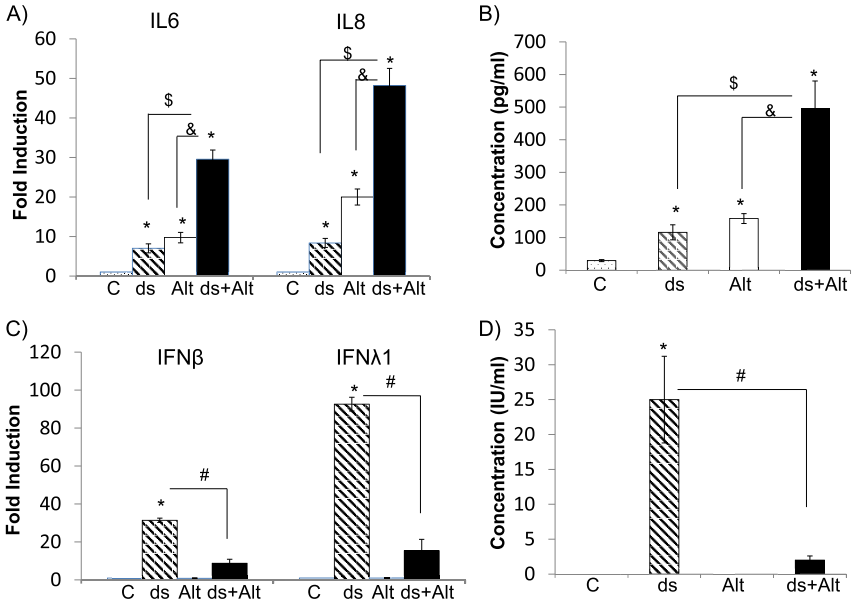


**Figure 4.** Alt enhanced dsRNA (ds)-induced cytokine production but repressed IFN production in epithelial cells. The cells were treated with ds (25 μg/ml) and/or Alt (21.5 μg/ml) individually or in combination. Real-time PCR was used to determine the expression of proinflammatory cytokines (i.e., IL-6, IL-8) (*A*) and IFN-β and IFN-λ1 (*C*). Culture medium was collected and analyzed by ELISA for the secretions of IL-6 (*B*) or IFN-β (*D*). $^{*}P < 0.05$ ($n = 5$) when the treated samples (Alt, ds, or ds+Alt) were compared with the control. $^{\$,\&}P < 0.05$ ($n = 5$) when ds- or Alt-treated samples were compared with cotreatment (ds+Alt) samples. $^{\#}P < 0.05$ ($n = 5$) when the samples with cotreatment (ds+Alt) were compared with ds-treated samples.

significantly increased IFN reporter activity, and this activation was repressed when the cells were treated with Alt (Figure 6A). As a negative control, a TBK1 mutant (K38A) was used. This construct contains a point mutation in the catalytic site of TBK1 (Lysine → Alanine) that renders the kinase inactive. As expected, TBK1-K38A failed to activate IFN reporter and did not respond to Alt. Consistently, Alt significantly repressed IRF3 activation, causing a decreased level of p-IRF3 (Figure 6B). Thus, the antagonistic effect of Alt on dsRNA-induced IFN expression appeared to be mediated by its repression of the TBK1-IRF3 pathway.

### Separate Fungal Components Are Responsible for the Opposite Effects of Alt on the Two Branches of Virus-induced Epithelial Immunity

It has been reported that heat-sensitive fungal proteases are responsible for Alt-induced cytokine expression (13–17). Therefore, we tested if the combined effects of Alt and dsRNA were also heat sensitive and protease dependent. Consistent with the previous reports, heat-inactivated Alt failed to induce IL-6 expression, and its synergism with dsRNA treatment was lost (Figure 7A). In addition, preincubation of protease inhibitor mix with Alt partially abolished Alt-induced IL-6 expression and its synergism with dsRNA (Figure 7C). We had the same observation on IL-8 expression (data not shown). Thus, heat-sensitive, protease-dependent fungal components appeared to be responsible for the Alt-induced proinflammatory cytokine production and its synergism with dsRNA. However, IFN expression had a very different profile (for clarity, only IFN-β data are shown; IFN-λ1 had the same profile). Although heat-inactivated Alt lost the repressing effect on IFN expression (Figure 7B), protease inhibitor treatment had no effect on dsRNA-induced IFN expression (Figure 7D), suggesting that this effect was not caused by the established fungal proteases. Altogether, our data indicate that separate fungal components were responsible for the opposite effects of Alt on the two branches of virus-induced airway epithelial immunity.

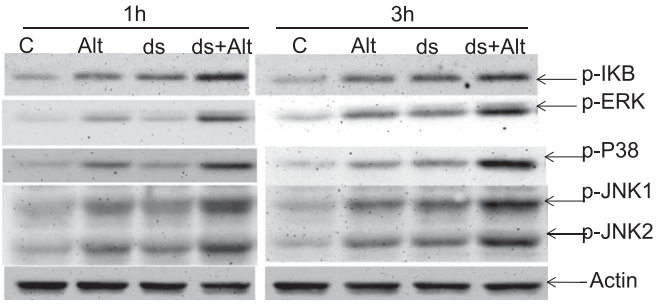


**Figure 5.** Alt enhanced ds-activated cellular signaling in epithelial cells. The cells were treated with ds (25 μg/ml) and/or Alt (21.5 μg/ml) individually or in combination (ds+Alt) for the indicated time points, and cellular protein was collected for Western blot analysis. p, phosphorylated. Actin was used as a loading control. The image is representative of at least three independent experiments.

## Discussion

The precise mechanism underlying the interaction between Alt and virus/dsRNA is unclear. Ligand engagement of TLR3 activates two general branches of signaling pathways: one leads to the production of proinflammatory cytokines, and the other activates IFN transcription (34). A single adaptor protein, TICAM1 (or TRIF), is responsible for these two responses via separate protein domains (44). TRAF6 and RIP1 have been reported to activate TAK1, which further activates the NF-κB pathway. TAK1 also activates MAPK, which in turn leads to the activation of the AP-1 family of transcription factors. NF-κB and AP-1 are key factors driving the transcription of proinflammatory cytokines. An additional adaptor, NAP1, is required to activate TBK1-IKKε, which activates IRF3 (44). In our study, these two branches were affected in the opposite manners. However, the precise targets are unclear.

A large portion of the studies on Alt-induced cellular signaling have centered on protease-dependent pathways (13, 14, 16, 17). Our study confirms this notion by demonstrating that fungal proteases were indeed involved in the induction of

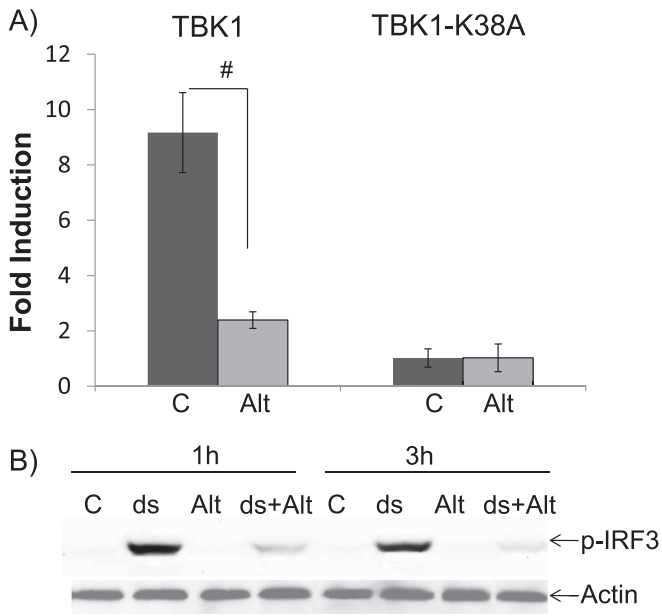


**Figure 6.** Alt repressed TBK1-IRF3. (*A*) Alt repressed TBK1 activity in airway epithelial cells. Cells were cotransfected with IFN reporter and with TBK1 or its inactive mutant (K38A) as well as Rluc/TK for the normalization of transfection efficiency. At 24 hours after transfection, cells were treated with Alt for 3 hours and lysed for luciferase assay. For luciferase, $^{\#}P < 0.05$ ($n = 6$). (*B*) Alt repressed ds-activated IRF3 in epithelial cells. p-IRF3, phosphorylated (activated) IRF3. Actin was used as a loading control.

proinflammatory cytokines. Furthermore, we have shown that these proteases were responsible for the enhancement of virus-induced cytokine productions by Alt. Because most of the common asthma-causing allergens (e.g., cockroach and house dust mite) have intrinsic protease activities (45, 46) that are associated with the sensitization and asthma development, the synergistic effects between these proteases and RV infection may exacerbate the symptom by markedly enhancing the inflammatory responses in the asthmatic airways. Proteases can induce multiple cellular responses, including the activation of proteinase-activating receptors (47) and receptor tyrosine kinases (48), the generation of reactive oxygen species (49), the disruption of epithelial barrier function (16, 50), and cell death (15, 49). Future study is required to delineate these pathways and to determine how they act individually or in combination with RV infection to promote airway inflammation.

We have also found that Alt represses virus-induced IFN production by a protease-independent mechanism. IFN-dominant Th1 responses are indispensable for the defense against fungal infection (51). Thus, Alt-induced repression of IFN production may represent an adapted response by fungus to survive the attacks from the host defense system. A recent report on the repression of IFN by Alt in dendritic cells (52) supports this notion. Viruses have been well established to evade host defense by compromising the IFN system through various mechanisms (53). However, this area has not been elaborated in the fungal infection/exposure. There are multiple reports on defective virus-induced IFN production in airway epithelial cells (35, 36, 54–56) and in other cell types from patients with asthma (57–61). This IFN defect may lead to uncontrollable viral production and plays a critical role in RV-induced asthma exacerbation (35, 36). Indeed, the defective IFN induction in patients with asthma was associated with greater airway hyperresponsiveness (55). Whether or not Alt is involved in some of these conditions and whether or not other allergens (e.g., cockroach or house dust mite) have IFN-repressing activity is unclear. However, considering the universal presence of Alt across the demographic regions, its IFN-suppressing property may have an important adjuvant effect in the development and exacerbation of asthma when present together with these other allergens.

The present data have to be interpreted with caution for several reasons. First, we used *Alternaria* filtrates, instead of live

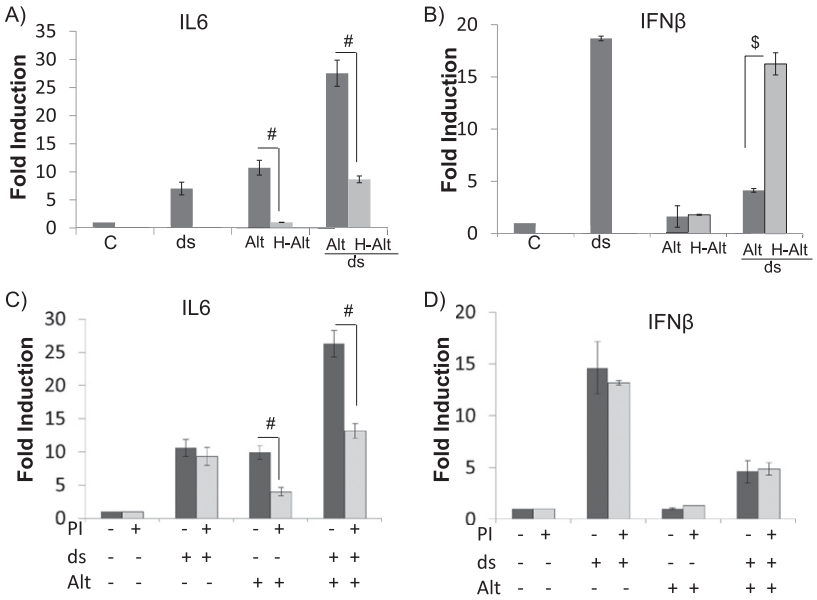


**Figure 7.** Separate fungal components are involved in the effects of Alt on virus-induced epithelial immunity. The cells were treated with ds (25 μg/ml) or Alt (21.5 μg/ml) individually or in combination for 3 hours, and RNA samples were analyzed by real-time PCR analysis. (*A*, *B*) Alt effects were heat sensitive. H-Alt = heat-inactivated Alt. $^{\#,\$}P < 0.05$ ($n = 5$) when Alt-treated samples were compared with H-Alt–treated samples. (*C*) Alt's effect on IL-6 expression was protease dependent. Alt was preincubated with protease inhibitor mix (PI) for 30 minutes before the treatments. The same percentage of DMSO (solvent for PI) was added to non-PI samples as the negative control. $^{\#}P < 0.05$ ($n = 5$). (*D*) Alt's effect on IFN-β was protease independent.

fungi, in all the experiments. Despite their wide usage in various previous studies (13–17), the filtrates contain a complex mixture of secretory products from an established fungal culture, and some of these products may reflect specific responses to the artificial *in vitro* growth environment. Thus, how representative these filtrates are as compared with the real-life Alt exposure is unclear. Nonetheless, the filtrates have been successfully used in a mouse model to generate many *bona fide* asthma-like pathologies (62), which strongly supports their applicability in asthma research. Second, although Alt is one of major indoor environmental allergens and although RV infection (symptomatic and asymptomatic) is widespread, the degree to which they coexist in the human airway is unclear. It is also likely that persistent exposure to Alt may permanently alter the epithelial antiviral immunity. Further studies in the areas of epidemiology and/or molecular biology are warranted to test these hypotheses. Third, the fungal life cycle has different phases. Filtrates represent the secretory products from a mature fungal growth that may be different from those from the spore germination. Fungal spores have been recently found to activate the TLR3 pathway by gene expression profiling in an epithelial cell line (63). Along this line, Daines and colleagues (64) have reported that fungal spores induced a very different immune response in mice as compared with the filtrates. In these experiments, the mice exposed to the spores had a strong neutrophilic inflammation but a weak IgE production, whereas the mice exposed to the filtrates had classical asthma-like Th2 responses. Thus, further studies are needed to completely understand the agonistic or antagonistic effect of Alt exposure on the epithelial antiviral immunity at different stages of the fungal life cycle.

This is the first attempt to understand the interaction between the two pathogens that are critical to the asthma pathogenesis. Alt exposure affected virus-induced immunity in an imbalanced manner, in which proinflammatory cytokine production was significantly enhanced and IFN production was severely repressed. Complete elucidation of this interaction at molecular level will advance our understanding of the complex nature of asthma and its exacerbation. ■

**Author disclosures** are available with the text of this article at www.atsjournals.org.

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