Interactions of Allergens and Irritants in Susceptible Populations in Producing Lung Dysfunction: Implications for Future Research

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Environmental agents, when applied in combination or sequentially, can induce a wide variety of adverse health effects in humans. To determine the effects of sequential allergen challenge and acid exposure on human bronchial epithelial cell function, we subjected normal, nonallergic control and ragweed-allergic individuals to bronchoscopic segmental ragweed challenge *in vivo*. We harvested bronchial epithelial cells by brush biopsy both before challenge and 24 hr after challenge and exposed cells to an acid stress *in vitro* (pH 5 for 3 hr), followed by a 1-hr recovery period at normal pH. In normal, nonallergic subjects, segmental allergen challenge produced no effects on ciliary activity; pH 5 exposure produced reduced ciliary activity (a decrease in the percent of the initially active area), with significant recovery after cells were returned to normal pH. Ciliary activity from allergic subjects was also inhibited by pH 5 exposure; however, activity was not recovered when cells were placed in medium of normal pH. Ciliary activity in allergics who developed a stress response postantigen challenge, as determined by an induction of the 27 kDa stress (heat shock) protein, displayed no ciliary dysfunction when exposed to a pH 5 stress. In this case, a stress sufficient to provoke a heat shock (stress) protein (HSP) response (but not one that produced more severe lung injury and did not provoke an HSP response) protected cells from a subsequent acid stress. Because of our observations and recent findings reported in the literature, we suggest that in order to define the wide variety of health effects of environmental agents, control as well as at-risk populations should be studied and the ability to define potentially beneficial as well as detrimental effects should be built into the experimental design. Inclusion of different and novel end points also should be considered. *Key words*: acid aerosols, allergens, ciliary activity, heat shock (stress) proteins, irritants, pollutants, segmental allergen challenge. — *Environ Health Perspect* 109(suppl 4):605–607 (2001).
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Environmental pollutants have been associated with a wide variety of adverse health effects in humans, including pulmonary dysfunction (*1*). For example, exposure to ozone has been reported to produce both physiologic and biochemical/inflammatory changes in humans (*2–7*). Similarly, acid aerosols have been reported to produce pulmonary dysfunction (*8,9*). The effects of such exposures might be expected to be magnified when the exposed individual either has a degree of pre-existing lung dysfunction such as asthma, and/or when multiple different exposures are performed (*10–13*).

The bronchial epithelium is a key cellular element in asthmatics, both as a disrupted barrier in asthmatics and as a rich source of cytokines and chemokines (*1,14*). Previous work in our laboratory focused on alterations in the bronchial epithelium associated with disease states such as allergic asthma, and as a target for environmental insults (*14,15*). For example, we demonstrated that human bronchial epithelial cells are a rich source of cytokines when studied *ex vivo* after *in vivo* segmental antigen challenge of allergic volunteers (*14*). We also observed that an insult as mild as inhaling four puffs (90 μg/puff) of the β-agonist albuterol results in a stress response in bronchial epithelial cells studied *ex vivo*, as shown by an increase in the 72- to 73-kDa family of heat shock (stress) proteins (HSPs) (*15*).

On the basis of our own observations and the emerging literature concerning increased effects of multiple different environmental pollutants/irritants on various physiologic and biochemical end points, we sought to determine the effects of sequential treatment of human bronchial epithelial cells on a different end point—ciliary function of human epithelial cells. Alterations in ciliary activity might be expected to occur before gross histologic changes in the bronchial epithelium that have been described as characteristic of asthmatics. Our strategy was to perform segmental allergen challenge of volunteers *in vivo* as a first stress of the epithelial cells and harvest bronchial epithelial cells 24 hr after antigen challenge by bronchial brush biopsy. Then we would provide a second stress by exposing epithelial cells to an acid environment (pH 5 for 3 hr, *in vitro*) to simulate an acid aerosol exposure (*16*). We further sought to determine the extent to which these exposures produced stress in the epithelium by quantitating the 27-kDa HSP in epithelial cells, a protein abundant in unstressed lung that has been implicated in actin polymerization. Finally, we studied both

normal and potentially at-risk populations by studying individuals in three different groups: nonallergic nonasthmatic healthy controls, ragweed-allergic subjects with allergic asthma and allergic rhinitis, and ragweed-allergic subjects with allergic rhinitis but without asthma.

We expected to see that the initial insult (allergen challenge) produced some degree of epithelial cell dysfunction, which would be magnified by the second insult (acid exposure). We further anticipated that at least one of the at-risk groups would be more susceptible to the insults than normal, nonallergic controls. In many of these hypotheses, our expectations were not met. These results have been discussed, both in terms of their specific implications for the models, stressors, and human subjects used in this work, as well as their possible implications for future work where at-risk populations are subjected to multiple environmental insults.

Materials and Methods

Subjects

All these procedures were reviewed and approved by the Thomas Jefferson University Institutional Review Board, and all subjects gave written, informed consent. We have previously described in detail the criteria for subject selection, their physiologic characterization, and techniques used for bronchoscopy and segmental allergen challenge (*17*). Briefly, all subjects were nonsmokers in good health, had no chronic illnesses, and were taking no chronic medication. Nonallergic, nonasthmatic controls had no symptoms of either asthma or hay fever, had normal pulmonary function, were not responsive to intradermal injection of short ragweed allergen, and were not responsive to inhaled methacholine.

Ragweed allergic subjects had appropriate symptoms for their subject category (rhinitis alone for subjects with rhinitis but not asthma; rhinitis and intermittent chest tightness, dyspnea, and/or wheezing for asthmatics), a positive skin test to intradermally injected ragweed, and had normal or near normal pulmonary function. In general, asthmatics were hyperresponsive to inhaled methacholine [$PC_{20} < 8$ mg/mL (18)], whereas rhinitics were not. Asthmatics were taking only an inhaled β-agonist on an as-needed basis.

Bronchoscopy, Segmental Allergen Challenge, and Bronchial Brush Biopsy

Bronchoscopy, segmental allergen challenge, and bronchial brush biopsy are described in detail elsewhere (16,17). Briefly, baseline samples (bronchoalveolar lavage, followed by bronchial brush biopsy to obtain epithelial cells) in one lung were collected from subjects, followed by segmental ragweed challenge in the contralateral lung during a first bronchoscopy. Twenty-four hours later, the antigen-challenged segment was similarly sampled (bronchoalveolar lavage and bronchial brush biopsy).

Sample Handling, Cell and Albumin Quantitation, and Quantitation of 27-kDa HSP and Ciliary Activity

These procedures are described in detail elsewhere (16). Briefly, bronchoalveolar lavage cells were counted, and cell differential counts were performed on Wright-stained cytospins (Accustain; Sigma Diagnostics, St. Louis, MO, USA). Albumin concentration was quantitated by enzyme-linked immunoabsorbent assay in cell-free bronchoalveolar lavage fluid. Strips and clusters of bronchial epithelial cells in four areas were video recorded and the areas of active ciliary activity were quantitated using BIOQUANT system IV image analysis software (R & M Biometrics, Nashville, TN, USA). The protocol employed included a determination of active ciliary area at baseline, after 3 hr of acid treatment (pH 5) and a 1-hr recovery period at pH 7.4. Data are presented as the percent of the initially active ciliary area. (In these experiments, little decrease in ciliary beat frequency was observed; rather, areas of initially active epithelium became inactive.) The 27-kDa HSP was then quantitated (Western analysis) in lysed epithelial cell samples subjected to sodium dodecyl sulfate gel electrophoresis, followed by blotting onto nitrocellulose, and probed using a monoclonal anti-HSP27 antibody (StressGen Biotechnologies Corp., Victoria, BC, Canada). This antibody also recognized a 76-kDa protein (which was apparently not a HSP) which was present in a constant amount in the various samples and used as an internal standard.

Results

Table 1 presents subject demographics and bronchoalveolar lavage cell and albumin concentrations. Two considerations became apparent as these data were obtained and analyzed. First, in the end points chosen for this study—induction of a 27-kDa HSP response and alterations in ciliary activity—allergic asthmatics and subjects with allergic rhinitis, but not asthma, responded similarly (data not shown). Therefore, these two groups have been combined and a primary comparison was made between ragweed-allergic and nonallergic subjects. Second, segmental antigen challenge can produce an extreme, nonphysiologic injury in the antigen-challenged segment. Such a large inflammatory signal, although helpful in dissecting the elements of the inflammatory response, can overwhelm other cellular events. In fact, as shown below, a severe *in vivo* inflammatory response overwhelmed the stress response in bronchial epithelial cells in that no induction of the 27-kDa HSP was observed. For that reason, we divided subjects into two groups, those who had a mild inflammatory response and those who had a severe response. The response was classified as mild if bronchoalveolar lavage albumin levels increased < 50 μg/mL above the preantigen challenge baseline, and severe if the increase was > 50 μg/mL. Increases in bronchoalveolar lavage albumin concentrations < 50 μg/mL are typically seen in subjects who undergo whole-lung antigen inhalation challenge (19).
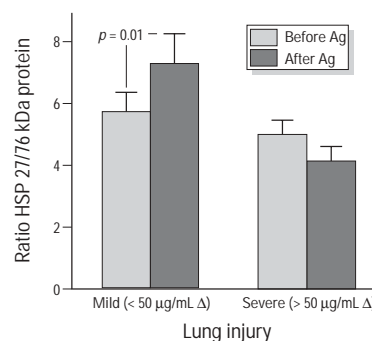
Our three subject groups included normal, nonallergic subjects who developed a mild inflammatory response (mild injury), allergic subjects who developed a mild inflammatory response (mild injury), and allergic subjects who developed a severe inflammatory response (severe injury). There were 10–15 subjects in each group, and subject demographics and pulmonary function were similar in all three groups (Table 1). Nonallergic subjects had minimal response to segmental allergen challenge, with a slight increase in bronchoalveolar cells and a non-significant increase in albumin concentration. The allergic–mild injury group had a slight but significant increase in albumin concentration and a 2-fold increase in bronchoalveolar cell concentration. The allergic–severe injury group had a 9-fold increase in cell concentration and a 12-fold increase in albumin.

Figure 1 shows that subjects with a mild injury had an induction of a stress response, as shown by an increase in the 27-kDa HSP, whereas those with a severe injury did not. Figure 2 shows ciliary activity in these two subject groups before segmental antigen challenge (Figure 2A) and after segmental antigen challenge (Figure 2B). Nonallergic, nonasthmatic controls displayed significant inhibition of ciliary activity after pH 5 exposure, with a significant recovery of ciliary activity after returning to a normal pH; this pattern of response was not altered by segmental allergen challenge *in vivo*. The allergic individuals, both those who would subsequently be found to have a mild injury produced by antigen challenge and those who would be found to
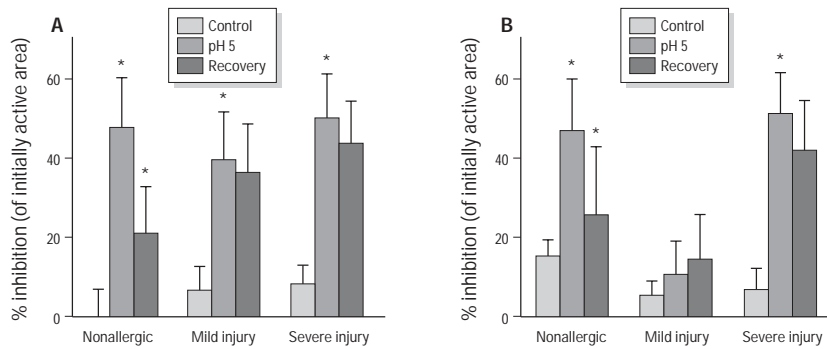

Figure 1. Induction of stress response (27-kDa HSP) in subjects with a mild degree of lung injury but not in those with severe injury. Data are presented in comparison with an internal standard, a 76-kDa protein observed in all gels. Adapted from Hastie et al. (16) with permission of the American Physiological Society.

Table 1. Subject demographics and BAL albumin and cell concentrations.

Property	Nonallergic–mild injury	Allergic–mild injury	Allergic–severe injury
n	10	13	15
Gender	8 male/2 female	11 male/2 female	7 male/8 female
Age (years)	26 ± 1	27 ± 2	29 ± 2
Asthma	0/10	7/13	10/15
FEV_1 (L)	4.45 ± 0.2	3.89 ± 0.3	3.26 ± 0.2
FEV_1/FVC (%)	87 ± 1	81 ± 2	85 ± 1
BAL albumin (μg/mL)			
Baseline	27.2 ± 11.3	52.4 ± 12.2	56.7 ± 10.1
24 hr after antigen	30.6 ± 7.3	69.7 ± 14.2*	664 ± 220**
Cells/mL × 10^4			
Baseline	13.9 ± 1.0	16.2 ± 2.7	14.8 ± 2.0
24 hr after antigen	18.9 ± 2.4*	35.6 ± 9.7*	135 ± 37.3**

Abbreviations: BAL, bronchoalveolar lavage; FEV₁, forced expiratory volume in 1 sec: FVC, forced vital capacity.
Values given are mean ± SEM. Adapted from Hastie et al. (16) with permission of the American Physiological Society. *p < 0.05 for baseline vs 24 hr after antigen challenge. **p < 0.05 for allergic–mild injury vs allergic–severe injury.

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Figure 2. Ciliary activity (percent inhibition of initially active area) before (*A*) and after (*B*) segmental antigen challenge. *$p < 0.05$ vs control. **$p < 0.05$ vs pH 5 condition. Adapted from Hastie et al. (*16*) with permission of the American Physiological Society.

have a severe injury, demonstrated ciliary dysfunction after pH 5 exposure that did not recover after cells were returned to a normal pH (an inability to recover). Segmental antigen challenge that produced a severe injury had no effect on this pattern of response. Subjects who had a mild injury induced by antigen challenge (those who also had a stress response as shown by an induction of the 27-kDa HSP), had preserved ciliary activity when exposed to pH 5. This did not change after cells were returned to a normal pH.

Discussion

In summary, at baseline before *in vivo* antigen challenge, normal, nonallergic individuals displayed ciliary dysfunction when exposed to pH 5 and recovered when pH returned to normal. Atopic individuals showed similar dysfunction when exposed to pH 5, but did not recover when pH was normalized. Production of a mild allergic injury did not synergistically or additively result in an increase in ciliary dysfunction but rather protected ciliary activity from an acid stress. In this case, multiple insults (allergen challenge followed by acid exposure) had fewer effects in a susceptible population (allergic subjects) than in control subjects. Therefore, low levels of injurious agents cannot always be assumed to produce detrimental effects in all populations, including asthmatics.

These observations suggest that some at-risk subject groups (atopic individuals) respond differently to some stressors than normal controls; this finding was not surprising. However, we also observed that a mild stress or injury could protect cells from a second stress. Such a protective effect was unexpected and appears to be related to the cells' ability to generate a stress response that includes the induction of HSPs.

We suggest that these findings have implications for experiments designed to explore the effects of multiple, different environmental agents on pathophysiologic processes in humans. First, as suggested by investigators for a number of years, it is useful to study both control and at-risk populations. To that well-established practice, we would add the caveat that it could be informative to observe both beneficial as well as detrimental effects. Second, in both our studies (*15,16*) as well as in those of others (*4,6,7,13*), large intersubject variability has been noted. Efforts to examine reasons for such variability using molecular and genetic approaches, including the use of genetically altered animals, could prove to be most informative. Finally, the use of nontraditional end points could be informative. Such end points could include the rate and severity of infection (*20*) and the response to medication (*21*). Recent studies have reported that cigarette smokers are both more likely to develop severe pneumococcal disease (*20*) and, if asthmatic, respond less well to inhaled corticosteroids (*21*) than nonsmokers. A variety of epithelial cell end points could also prove informative, as these cells are among the first of our cells to sample the environment and its toxins. Such end points include

- mediator and cytokine synthesis and release;
- induction of stress response;
- mucociliary activity and clearance;
- resistance to infection;
- apoptosis; and
- factors involved in injury and repair.

In summary, environmental agents, particularly when applied to susceptible populations in increased amounts or in the form of multiple different insults, have the potential to produce a wide variety of effects, many of which are likely to be adverse. However, to define the wide variety of effects of environmental agents, control as well as at-risk populations should be studied, the ability to define potentially beneficial as well as detrimental effects should be built into the experimental design, and the inclusion of different and novel end points should be considered.