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 a 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.

On the basis of our own observations and the emerging literature concerning the 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 stressed 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.
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                |
| FEV1 (L) | 4.45 ± 0.2             | 3.89 ± 0.3          | 3.26 ± 0.2           |
| Age (years) | 26 ± 1               | 27 ± 2              | 29 ± 2               |
| Gender   | 8 male/2 female         | 11 male/2 female    | 7 male/8 female      |
| 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          | 67.8 ± 14.2*        | 664 ± 220**          |
| Cells/mL × 10⁶ |                        |                     |                      |
| Baseline | 139 ± 10               | 16.2 ± 2            | 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; PVC, 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.
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 more 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 and points should be considered.

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.

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REFERENCES AND NOTES

1. Koenig J OR air pollution and asthma. J Allergy Clin Immunol 104:717–722 (1999).
2. Young WA, Shaw DB, Bates DV. Effect of low concentrations of ozone on pulmonary function in man. J Appl Physiol 29:165–170 (1966).
3. Golden JA, Nadel JA, Bouchoy HA. Bronchial hyperreactivity in healthy subjects after exposure to ozone. Am Rev Respir Dis 128:271–274 (1983).
4. Devlin RB, McDonnell WF, Mann R, Becker S, House DE, Schneiemachers D, Koren HS. Exposure of humans to ambient levels of ozone for 6.6 hours causes cellular and biochemical changes in the lung. Am J Respir Cell Mol Biol 4:72–81 (1991).
5. Atis RM, Christian D, Hearne PD, Kent KF, Finkbeiner WE, Balmes JR. Ozone-induced airway inflammation in human subjects as determined by airway lavage and biopsy. Am Rev Respir Dis 142:323–327 (1990).
6. Weinmann GG, Liu MC, Proud D, Weidenbach-Gerbase M, Hubbard W, Frank R. Ozone exposure in humans: inflammatory small and peripheral airway responses. Am J Respir Crit Care Med 152:1175–1182 (1995).
7. Krishna MT, Spiral D, Meng O-H, Willson N, Miecell D, Biscione G, Frey A, Polak J. Holgate S. Effects of ozone on epithelium and sensory nerves in the bronchial mucosa of healthy humans. Am J Respir Crit Care Med 156:943–950 (1997).
8. Reesche MT, Dolevich M, Obninsk G, Wolff RK. Effects of TVL levels of SO2 and H2SO4 on bronchial clearance in exercising man. Arch Environ Health 33:24–32 (1978).
9. Balmes JR, Fine J, Gordon T, Shepard D. Potencial bronchostimulator stimuli in acid fog. Environ Health Perspect 79:163–166 (1989).
10. Flampton MW, Morgan PE, Cox C, Levy PC, Condon J, Spears D, Goff BF, Uelt MJ. Sulphuric acid aerosol followed by ozone exposure in healthy and asthmatic subjects. Environ Res 69:1–14 (1995).
11. Linn WS, Anderson KR, Shamos DA, Edwards SA, Web TL, Hackney JD, Gong H Jr. Controlled exposures of young asthmatics to mixed oxidant gases and acid aerosol. Am J Respir Crit Care Med 152:885–891 (1995).
12. Jorres R, Rowak M, Nagyagius H. The effect of ozone exposure on antigen responsiveness in subjects with asthma or rhinitis. Am J Respir Crit Care Med 153:56–64 (1996).
13. Kehr HLR, Peden DB, Ball B, Pollenbeke L, Horstman D. Increased specific airway reactivity of persons with mild allergic asthma after 7.6 hours of exposure to 0.16 ppm ozone. J Allergy Clin Immunol 104:1198–1204 (1999).
14. Hastie AT, Evetts KB, Cho S-K, Zangrilli J, Shaver J, Police MB, Fish JE, Peters SP. IL-1β release from cultured bronchial epithelial cells and bronchoalveolar lavage cells from allergic and normal humans following segmental challenge with ragweed. Cytokine 8:730–738 (1996).
15. Hastie AT, Evetts KB, Shaver J, Police MB, Fish JE, Peters SP, β2-Agonist–elevated stress response in human bronchial epithelial cells in vivo and in vitro. Lung 175:387–398 (1997).
16. Hastie AT, Evetts KB, Zangrilli J, Shaver J, Police MB, Fish JE, Peters SP. HSP27-elevated stress response in mild allergic inflammation protects airway epithelium from H2SO4 effects. Am J Physiol 273:L727–L734 (1997).
17. Shaver JR, O’Connor J, Police M, Cho SK, Kane GC, Fish JE, Peters SP. Pulmonary inflammation after segmental ragweed challenge in allergic asthmatics and nonasthmatics. Am J Respir Crit Care Med 152:1189–1197 (1995).
18. PC20 is the provocative concentration causing a 20% fall in FEV1.
19. Collins DS, Dupuis R, Gleich G, Barretes KR, Koh YY, Police M, Albertyne KH, Fish JE, Peters SP. Immunoglobulin E–mediated increase in vascular permeability correlates with eosinophilic inflammation. Am Rev Respir Dis 147:677–683 (1993).
20. Nuorti JP, Butler JE, Peters SP. HSP27 elevated in mild allergic inflammation protects airway epithelium from H2SO4 effects. Am J Physiol 273:L727–L734 (1997).
21. Pedersen B, Dahl R, Karlstrom R, Peterson CGB, Venge P. Eosinophil and neutrophil activity in asthma in a one-year trial with inhaled budesonide. The impact of smoking. Am J Respir Crit Care Med 153:1519–1529 (1996).

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