Immunologic Responses to Isocyanates in Sensitized Asthmatic Subjects*

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Isocyanates, highly reactive compounds used extensively in industry, are the most commonly reported cause of occupational asthma in industrial populations. Sensitized individuals may develop immediate and/or late asthmatic responses following isocyanate exposure. The mechanisms by which isocyanates cause asthma are not well defined. The limited information available suggests that isocyanate asthma is mediated via predominantly non-IgE mechanisms, and that isocyanates may act like a foreign low molecular weight hapten, inducing antigen-specific T-cell responses and airway inflammation. However, the nature of these T-cell responses and the precise characteristics of the airway inflammation remain unclear. Studies have been initiated to characterize the lung inflammatory and T-cell responses to isocyanates in subjects with isocyanate asthma.

Table 1—Airway Histologic Type in Patients With Isocyanate Asthma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=5)</th>
<th>Baseline</th>
<th>Postchallenge</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Mean, Range)</td>
<td>306</td>
<td>306</td>
</tr>
<tr>
<td>Basement membrane thickness, μm</td>
<td>3.44 (2.41-4.86)</td>
<td>9.8</td>
<td>11.78</td>
</tr>
<tr>
<td>Eosinophils*</td>
<td>5 (0-13)</td>
<td>40</td>
<td>28</td>
</tr>
<tr>
<td>CD4+ T cells</td>
<td>307 (182-437)</td>
<td>376</td>
<td>616</td>
</tr>
<tr>
<td>CD8+ T cells</td>
<td>169 (18-390)</td>
<td>274</td>
<td>577</td>
</tr>
</tbody>
</table>

*Five to eight biopsy specimens per subject were analyzed using hematoxylin-eosin. 303, 306, and 309 refer to the three patients with isocyanate asthma. Total area of a section of basement membrane was measured and divided by its length. Cells within 125 μm of the surface epithelium were counted. Results are expressed as average in microns or as average number of cells per square millimeter.

†Two biopsy-specimens were stained for CD45 and CD3. Cells within 125 μm of the surface epithelium were counted. Results are expressed as the average number of cells per square millimeter.
peripheral blood mononuclear cells using a panel of monoclonal antibodies directed toward T-cell surface antigens to characterize the lymphocyte subsets present. Control samples were obtained from nonasthmatics with no history of isocyanate exposure. All studies were approved by the Yale Human Investigation Committee.

**RESULTS**

Each subject reacted to specific isocyanate challenge with symptomatic and spirometric changes consistent with late and/or dual asthmatic responses. Light microscopy showed increased numbers of total inflammatory cells and eosinophils in all three subjects compared with nonasthmatic controls (Table 1). Increased total basement membrane thickness was also noted in two of three subjects (Table 1). Immunohistochemistry using several different monoclonal antibodies demonstrated increased T cells in the airways of these isocyanate asthmatics, including CD4+, CD8+, and CD25+ T cells. Baseline bronchoscopy on patient 306, 3 months away from exposure, showed fewer T cells on airway biopsyspecimen, with no significant change in basement membrane thickening or the number of eosinophils (Table 1).

The total cell counts and differential counts (1 to 10% lymphocytes, ≤1% eosinophils, 1 to 3% neutrophils, and 85 to 95% macrophages) on the BAL cells were comparable to those from normal volunteers. Two of the subjects had BAL CD4/CD8 ratios in the low normal range (1.1 to 1.3), and the third had a markedly reduced ratio of 0.27, with normal peripheral blood CD4/CD8 T-cell ratios (2.04 to 2.47), suggesting a possible selective recruitment of CD8+ T cells to the lung. Three-color flow cytometry showed that the BAL lymphocytes were clearly distinct from those present in peripheral blood, with more BAL T cells expressing the activation antigen HLA-DR+ and memory immunophenotype (CD45+ RA-RO+) compared with blood (Fig 1, left [A]). Baseline bronchoscopy performed 3 months away from exposure on one subject showed fewer memory T cells, a normal CD4/CD8 ratio, and persistent activated T cells, compared with after isocyanate challenge (Fig 1, left [A]).

Similar flow cytometry studies were performed on peripheral blood mononuclear cells before and up to 48 h after isocyanate challenge. Two of the isocyanate asthmatics showed an increase in total peripheral lymphocytes, total T cells, and T cells expressing HLA-DR+ and IL-2R+ after challenge (Fig 1, right [B]). These patients also showed a shift in their CD4+ cells from a "naive" immunophenotype (CD45+ RA-RO+) to memory phenotype (CD45+ RA-RO+) in their peripheral blood and BAL (Fig 1, left [A]).

**DISCUSSION**

Studies performed on three subjects with isocyanate asthma have demonstrated bronchial hyperreactivity to specific isocyanate challenge, airway inflammation, and increased numbers of T cells in the airways. These findings are consistent with the limited data from the literature showing airway inflammation and fibrosis, and increased numbers of eosinophils, T cells, and other inflammatory cells in patients with isocyanate asthma.2-4,6 These findings suggest that T-cell recruitment and activation may occur in subjects with

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**Figure 1.** Three-color flow cytometry on BAL and peripheral blood mononuclear cells from patient 306 at baseline and postchallenge. Left, A: blood and BAL "naive" and "memory" T-cell immunophenotypes. Right, B: peripheral blood T-cell activation markers.
isocyanate asthma, and may participate in the asthmatic responses seen following isocyanate exposure. The relative increase in CD8 T cells noted on BAL is also consistent with the limited reports in the literature. Increased numbers of CD8+ T cells have been identified in the peripheral blood following isocyanate challenge,7 and recently, CD8+ T-cell clones have been cultured from the bronchial biopsy specimens of two subjects with isocyanate asthma.8

Further characterization of the T-cell responses to isocyanates has been limited. The flow cytometry studies in these three patients have demonstrated that BAL lymphocytes are clearly phenotypically distinct from those present in peripheral blood, perhaps representing greater numbers of isocyanate-specific T cells. The shift to a more “memory” and activated T-cell immunophenotype following specific isocyanate challenge also suggests that isocyanates can induce specific T-cell responses, and that peripheral markers of isocyanate sensitization may be identifiable.

These findings are limited primarily by the small number of subjects and controls, and certain inherent limitations of clinical research. Some variability in the physiologic and immunologic responses was noted in the three patients, such as different patterns of airway responses (early vs late or dual), differences in the CD4/CD8 ratios, and different T-cell responses following isocyanate exposure. These findings suggest that isocyanates may result in a spectrum of immunologic responses.

Further studies to better characterize the T cells present in patients with isocyanate asthma are in progress. Whether isocyanates can stimulate the proliferation and/or activation of lymphocytes in vitro is also being investigated. In addition to a better understanding of the role of T cells in the pathogenesis of isocyanate asthma, these studies will also hopefully identify much needed markers of sensitization, early disease, and/or host susceptibility.

REFERENCES

Production of Reactive Oxygen Intermediates Following Exposure to Ozone*

Relative Contribution of Alveolar Macrophages

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Exposure to ambient levels of ozone air pollution causes decrements in lung function, epithelial injury, and airway inflammation. Release of reactive oxygen intermediates (ROI) by inflammatory cells may contribute to epithelial injury following ozone exposure. We sought to determine the relative role of alveolar macrophages (AM) in the overall burden of ROI generated by airway cells following ozone exposure, and to determine whether generation of ROI correlates with lung function responsiveness to ozone.

Twenty-four healthy nonsmokers were classified as responders (>15% fall in FEV1, n=12) or nonresponders (<5% fall in FEV1, n=12) following a 4-h screening exposure to 0.2 ppm ozone with exercise. Each subject underwent 2 subsequent identical exposures to ozone and 1 to air, separated by at least 3 weeks. BAL was performed on 3 occasions: immediately (early) and 18 h (late) after ozone, and either early or late after air exposure. Generation of superoxide anion (O2-) by the total lavage cell population was determined with and without thorobold myristate acetate (PMA) stimulation using superoxide dismutase-inhibitable cytochrome c reduction. Generation of H2O2 by AM was evaluated using flow cytometric determination of intracellular oxidation of 2,7-dichlorofluorescein, gating to exclude polymorphonuclear leukocytes (PMN) based on light scatter.

The concentration of PMN in BAL increased nearly fivefold 18 h after ozone exposure in both responders and nonresponders. In responders, stimulated O2- increased progressively (mean±SE, nmoles O2-/min: 11.73±1.40 after air, 13.90±1.87 early after ozone, 19.06±3.29 late after ozone, p=0.023). Unstimulated H2O2 production in AM from responders decreased progressively (fluorescence units: 5.37±1.10×105 after air, 4.20±1.14×105 early after ozone, 2.67±0.69×105 late after ozone, p=0.022). Stimulation with PMA caused only small increases in AM generation of H2O2. There were no significant changes in ROI production by cells from nonresponders. Influx of PMN may increase the burden of ROI to the airway epithelium; we speculate that AM production of ROI may be downregulated following exposure in ozone responders, thus limiting the potential for injury to the alveolar epithelium.

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