that is followed up for development of asthma, not a retrospective collection of cases (or claimants), with its inevitable selection bias.

The observations on humans following short-term irritant inhalation injury leave fundamental questions unanswered, the most important being what determines whether the airways undergo complete recovery, reverting to normal structure and function, or if chronic, either variable (asthma) or progressive (bronchiolitis obliterans), airways obstruction results from the injury. Figure 6 illustrates some possible pathways of the postinjury biological processes.

The development of new knowledge in occupational lung diseases depends on the contribution of multiple disciplines—biologists, epidemiologists, statisticians, and industrial hygienists, to name only a few. Either those in fundamental research or in the investigation of exposure-response relationships in working populations can generate hypotheses that can be tested by the other. My emphasis has been on describing observed relationships in human populations exposed to workplace inhalants, and suggesting approaches by the basic scientist that may confirm and explain some of these relationships. The ultimate public health objective is to prevent or successfully intervene in occupationally related lung disease.

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Beryllium Stimulates Release of T Helper 1 Cytokines Interleukin-2 and Interferon Gamma From BAL Cells in Chronic Beryllium Disease*

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Chronic beryllium disease (CBD) is an occupationally acquired lung disease that begins as a sensitizing cell-mediated immune response to beryllium antigen that, over

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time, results in the development of noncaseating granulomas. Previous studies demonstrated that this antigen-driven process involves the proliferation of CD4+ T lymphocytes. We hypothesize that disease results from changes in T helper 1 (Th1) cytokine release, leading to unopposed amplification of the inflammatory response by effector macrophages and Th1 lymphocytes. To test this hypothesis, BAL cells from beryllium-diseased patients (n=5) and a nonexposed normal population (n=4) were cultured in the presence or absence of 10 μM beryllium sulfate and in the presence or absence of interleukin (IL)-2 neutralizing antibody for specified intervals up to 7 days. Protein concentrations of the Th1 cytokines IL-2 and gamma interferon (IFN-γ) and for the Th2 cytokine IL-4 were measured by enzyme-linked immunosorbent assay (ELISA), and corresponding mRNA levels were determined by semiquantitative polymerase chain reaction (PCR). T lymphocyte proliferation was measured by tritiated thymidine incorporation.

BAL cells from CBD patients showed: (1) beryllium-stimulated release of IL-2 and IFN-γ, but not IL-4; (2) high levels of IFN-γ (7 to 10 ng/mL) sustained to 168 h in culture; (3) parallel timecourses for cytokine mRNA synthesis and protein release; and (4) decreased, but not arrested, beryllium-stimulated T lymphocyte proliferation following neutralization of IL-2. These data suggest that Th1 lymphocytes participate in the beryllium disease process, beryllium-stimulated IFN-γ mRNA and protein demonstrate an extended timecourse, and IL-2 participates directly in beryllium-stimulated T lymphocyte proliferation, but other T lymphocyte mitogenic cytokines may be involved. Future studies of the role of Th1 cytokines in CBD will be extended to beryllium-specific, herpes simplex virus Saimiri-transformed BAL T lymphocyte cell lines currently under development in our laboratory.

### Immunologic Responses to Isocyanates in Sensitized Asthmatic Subjects*

Carrie A. Redlich, MD, MPH; Robert J. Homer, MD, PhD; Brian R. Smith, MD; Joel A. Wirth, MD; and Mark R. Cullen, MD

Isocyanates, highly reactive compounds used extensively in industry, are the most commonly reported cause of occupational asthma in industrial populations.1 Sensitized individuals may develop immediate and/or late asthmatic responses following isocyanate exposure.2 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.2,3 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.

### Materials and Methods

Three patients with isocyanate asthma and current exposure were studied. Following baseline pulmonary function testing and methacholine challenge, a workplace exposure to the patient’s own work materials was conducted, followed by hourly spirometry and observation. Twenty-four hours after the isocyanate challenge, patients underwent bronchoscopy, including BAL and multiple endobronchial airway biopsies. Blood was drawn prechallenge, 24 h, and/or 48 h after exposure for a number of different immunologic and other assays. One subject (case 306) was also studied at “baseline,” 3 months after removal from isocyanate exposure. Airway histologic type was characterized, and the inflammatory cells were quantitated using a digital morphometry system. Immunohistochemistry was performed with a panel of several different monoclonal antibodies to further characterize the airway inflammatory infiltrate. Three-color flow cytometry was performed on BAL and

### Table 1—Airway Histologic Type in Patients With Isocyanate Asthma

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Controls (n=5)</th>
<th>Baseline 306</th>
<th>Postchallenge 306</th>
<th>Postchallenge 309</th>
<th>Postchallenge 303</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basement membrane thickness, μm*</td>
<td>3.44 (2.41-4.86)</td>
<td>9.8</td>
<td>11.78</td>
<td>13.14</td>
<td>3.45</td>
</tr>
<tr>
<td>Eosinophils*</td>
<td>5 (0-13)</td>
<td>40</td>
<td>28</td>
<td>85</td>
<td>33</td>
</tr>
<tr>
<td>CD45f</td>
<td>307 (182-437)</td>
<td>376</td>
<td>616</td>
<td>698</td>
<td>510</td>
</tr>
<tr>
<td>CD3f</td>
<td>169 (18-390)</td>
<td>274</td>
<td>577</td>
<td>752</td>
<td>493</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.

fTwo 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.