STATEMENT

Synopsis of Research Report 146

Role of T Cells in Mouse Airways in Response to Inhaled Acrolein

BACKGROUND

Acrolein is a reactive aldehyde that injures the airways in humans and other species. It is an important air toxicant, one of a large and diverse group of air pollutants that, with sufficient exposure, are known or suspected to cause adverse human health effects. Even though ambient levels of air toxics are generally low, these compounds are a cause for public health concern because large numbers of people are exposed to them over prolonged periods of time. In the United States, these compounds are not regulated by the National Ambient Air Quality Standards but are subject to other rules set by the U.S. Environmental Protection Agency.

Dr. Michael Borchers, of the University of Cincinnati College of Medicine, submitted an application, "T Cell Sub-Populations Regulate Airway Inflammation and Injury Following Acrolein Exposures," under Request for Applications 03-2, the Walter A. Rosenblith Award, which was established to provide support for outstanding investigators beginning an independent research career. Dr. Borchers proposed to study the role of γδ T cells, a minor subpopulation of T cells that use the γ and δ chains as their antigen-specific T-cell receptor (TCR) and are found predominantly at mucosal epithelial sites such as the airways and the gastrointestinal tract. The HEI Research Committee thought it would be valuable for Dr. Borchers to evaluate the role of both γδ T cells and αβ T cells, the major subpopulation of T cells, which uses a different two-chain molecule as its antigen-specific TCR, and Dr. Borchers agreed.

APPROACH

Dr. Borchers used 8–12-week-old wild-type (C57BL/6J) mice and mice genetically deficient in either αβ T cells or γδ T cells. Mice were exposed to 0.5 or 2 ppm acrolein vapor, or were sham-exposed (to filtered air), for 6 hours per day, 5 days per week, for 1, 2, or 4 weeks. Immediately after exposure, bronchoalveolar lavage (BAL) was performed and total and differential cell numbers in BAL fluid were determined. The numbers of epithelial cells detected in BAL fluid were used as a marker of epithelial-cell injury. BAL supernatant was assayed for levels of mucin (Muc5ac). Lung tissue was assessed for mucous-cell metaplasia, by light microscopy, and lung cells were evaluated for expression of activated caspase 3, a marker of apoptosis (programmed cell death). The investigators also measured levels in lung cells of the cytokines interferon-γ (IFN-γ) and granulocyte–macrophage colony-stimulating factor (GM-CSF), which are produced by T cells and which activate macrophages. For biologic end points, the investigators analyzed data by using one-way analysis of variance with differences between means considered significant when $P < 0.05$.

To identify genes expressed differentially in αβ T cells and γδ T cells in the lung, total cellular RNA was extracted from highly purified populations of αβ T cells and γδ T cells from wild-type C57BL/6 mice exposed to 2.0 ppm acrolein or filtered air for 1 week. RNA samples were used to assess changes in gene expression by microarray analysis using slides containing more than 30,000 DNA sequences (70-base-pair probes). Borchers and colleagues used appropriate statistical tests to identify genes whose expression changed significantly. To confirm findings for some of the changes in gene expression identified by microarray experiments, the investigators performed quantitative real-time

polymerase-chain-reaction (qRT-PCR) assays on RNA from 15 selected genes. They used two programs, MAPPFinder and PANTHER database, to organize the measured changes in gene expression into several discrete biologic pathways; in this way they could determine the types of biologic processes and functions that had been affected in the αβ or γδ T cells by the exposure to acrolein.

RESULTS AND INTERPRETATIONS

Acrolein exposures of wild-type mice and mice deficient in γδ T cells resulted in increased epithelial-cell sloughing (resulting in increased epithelial-cell numbers in BAL fluid), apoptosis (measured as cells stained with active caspase 3—most of which were found in distal airways and airspaces) and increases in macrophage numbers; these effects appeared to be somewhat more pronounced in mice deficient in γδ T cells than in wild-type mice. By contrast, mice deficient in αβ T cells that were exposed to acrolein did not show effects on epithelial-cell injury, apoptosis, or macrophage numbers. In the airways of all strains of mice, acrolein exposure also resulted in similar, but small, increases in the mucus-cell index (reflecting the total amount of mucus in airways and the number of airways affected) and similar increases in levels of the cytokines IFN-γ and GM-CSF.

The expression of more than 1000 genes was altered in either αβ or γδ T cells isolated from wild-type C57BL/6J mice after a 1-week exposure to 2 ppm acrolein; about 75% of these changes were unique to either the αβ or the γδ T-cell subpopulation. Of the 15 genes selected for analysis by qRT-PCR to validate changes in gene expression observed in the microarray experiments, the investigators reported that most, but not all, qRT-PCR findings were consistent with those from the microarray experiments.

From the changes in gene expression detected in microarray experiments, the investigators singled out several genes in each subpopulation that were considered candidates for further study regarding their responses to acrolein exposure. Borchers and colleagues interpreted the data to suggest that the key changes found in αβ T cells only were in the expression of genes for cytokines and chemokines and their receptors, in particular, those associated with the activation of T cells and the consequent activation and accumulation of macrophages. They also reported that the key changes in gene expression found in γδ

T cells only were associated with genes involved in host-cell recognition, affecting the clearance of damaged host tissue, cellular interactions, and certain cytokines and chemokines and their receptors.

Using the programs to link microarray data to a gene database to identify biologic processes affected by acrolein exposure, the investigators found several intracellular pathways that were affected in αβ and γδ T cells. Some overlap between the subpopulations was noted, but some differences were also found in the pathways affected. On the basis of the most statistically significant findings, organic anion transport and mitochondrial apoptosis were concluded to be affected in αβ T cells, whereas peptide antigen binding and pathways including defense responses were concluded to be affected in γδ T cells.

CONCLUSIONS

In its independent review of the study, the HEI Review Committee thought that Dr. Borchers and colleagues had successfully designed and conducted a preliminary descriptive study in mice genetically depleted of one or other key subpopulation of T cells —those using either αβ or γδ as their antigen-specific receptor—to study the airway response to acrolein exposure via inhalation. The study provides useful information into the host response and pathology associated with acrolein exposure; it also provides useful preliminary descriptions of the roles of two groups of T cells during the course of the host response. The findings suggest that T cells play a role in the lungs' responses to acrolein exposure, and the data generally support the view that αβ and γδ T cell subpopulations have different roles in the response to acrolein: αβ T cells primarily promote the accumulation of macrophages and γδ T cells protect the integrity of airway epithelial cells.

The Review Committee generally agreed with the investigators' interpretations of the biologic responses to acrolein exposure and thought that the results showing dissociation between inflammation (attributable to macrophages and their products) and epithelial damage were interesting and compatible with recent data from other studies showing injury responses in the absence of inflammation. However, the Committee noted caution in interpreting the results of any study based on the use of mice that are entirely genetically deficient in αβ or γδ T cells; of particular concern are uncertainties about the possibilities of

unexamined developmental changes in these animals and compensatory increases in the remaining sub-populations of T cells. In addition, αβ and γδ T cells are heterogeneous, each containing numerous functionally specialized subsets; thus, mice deficient in all T cells of a given type might display a net effect of deficiencies of numerous subsets, some of which may cancel each other out.

The Committee also thought that the studies by Borchers and colleagues of changes in gene expression pointed to potentially useful directions for future research. The Committee agreed that several genes in each T-cell subpopulation were up-regulated, and several were down-regulated, by exposure to acrolein; moreover, some genes were expressed differently in the two T-cell subpopulations. However, the Committee found the investigators' interpretations of the differences in expression of key genes and involvement of different biologic pathways in αβ and γδ T cells after exposure to acrolein were interesting but speculative, requiring further experiments for confirmation or refutation. Nonetheless, the Committee concluded that the approaches and results of Borchers and colleagues will encourage more-mechanistic studies to explore the role of T cells in the pathology associated with exposure to acrolein.

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The Role of T Cells in the Regulation of Acrolein-Induced Pulmonary Inflammation and Epithelial-Cell Pathology

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