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A Novel Biomarker for Beryllium Sensitization in Humans

Final Report – 09/23/1996 – 09/22/2000

R. J. Albertini

April 2001

Work Performed Under Contract No. DE-FG07-96ER62331

For
U.S. Department of Energy
Assistant Secretary for
Environmental Management
Washington, DC

By
The University of Vermont
Burlington, VT

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P.I. Richard J. Albertini

SUMMARY OF RESEARCH AND MAJOR FINDINGS

Beryllium-reactive T-lymphocytes can be used as indicators of sensitization. Traditionally, their presence was detected by an *in vitro* proliferative assay (Beryllium Lymphocyte Proliferation Test = BLPT). However, this test is capricious and insensitive. The objective of this current project was to obtain and characterize beryllium-reactive T-cells from peripheral blood using the *HPRT* T-cell mutation assay. T-cells were selected on the basis of their *in vivo* mutation of the *HPRT* gene which renders them insensitive to the cytotoxicity of 6-thioguanine in culture. Such mutant populations are expected to be enriched for cells that are proliferating *in vivo* as a result of the beryllium sensitization process. This hypothesis of mutant populations being enriched for *in vivo* proliferating cells has been verified for several autoimmune diseases in a number of studies in various laboratories.

The major specific aims of the project were:

- To identify the *in vivo* proliferating T-cell clones in sensitized individuals by selecting for *HPRT* mutants,
- To determine T-cell receptor (TCR) β gene usages and commonalties among these clones, and
- To develop a quantitative PCR (qPCR) method for amplifying the common (and, therefore, relevant) TCR genes directly from peripheral blood. This will become the novel biomarker of beryllium sensitization.

Blood samples from 16 "beryllium sensitized" subjects were received from the Oak Ridge National Laboratories, including replicates from several individuals. *HPRT* MF values determined by a cloning assay revealed them to be in the expected ranges. Approximately 400 *HPRT* mutant and 100 wild type (non-mutant) isolates were recovered from these assays for determinations of TCR β gene usage. Although clonal amplifications were documented in several subjects, there were no obvious commonalties of BV or BJ segment usages, or CDR3 motifs, among the individuals. Difficulty in obtaining sufficient cell numbers precluded functional studies. The study subjects available at the Oak Ridge National Laboratories were not ill at the time of study, and several showed negative BLPTs. This, coupled with the heterogeneous patterns of T-cell proliferation, led to a study of patients with active berylliosis being cared for and studied at the National Jewish Hospital in Denver, Colorado.

Mutant frequency values were determined for three Colorado patients (two samples from one patient). TCR β gene usage patterns for 37 mutants and 21 wild type isolates revealed a striking preponderance of BV 2:BJ 1s2 usage in all three patients. One patient recently "patch-tested" for beryllium sensitization showed a large amplification of one BV 2:1s2 clone and the presence of another. This demonstration concluded the studies performed under this grant.

FUTURE STUDIES (Pending Additional Support)

TCR β gene usage patterns for the peripheral blood mutant T-cells from these patients will be compared with usage patterns of their bronchoalveolar lavage (BAL)-derived T-cells. If sufficient cells can be obtained, functional studies will be done.

Our current hypothesis is that clonal amplifications of TCR BV 2:BJ 1s2 T-cells among the *HPRT* mutant peripheral blood mononuclear cells may be a peripheral biomarker of beryllium sensitization. Verification of this requires study of additional patients with known beryllium sensitization. Quantitative PCR methods can then be devised to simplify the testing.