

# **Environmental Management Science Program**

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## **A Novel Biomarker for Beryllium Sensitization in Humans**

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### Research Objective

Beryllium reactive T-lymphocytes can be used as an indicator of sensitization. Traditionally, their presence is detected by an *in vitro* proliferation assay. However, this test is capricious (results varying from day to day in the same laboratory) and insensitive (rarely positive before clinical symptoms). The objective of this project is to obtain and characterize beryllium reactive T-cells from peripheral blood using the *hprt* T-cell mutation assay. T-cells are selected on the basis of their mutation of the *hprt* gene which renders them insensitive to 6-thioguanine in culture. Such mutant populations are expected to be enriched for cells which are proliferating *in vivo* as a result of the sensitizing process. This hypothesis has been verified in a number of studies.

The seven specific aims of this study will: (i) identify the *in vivo* proliferating T-cell clones in sensitized individuals by selecting for *hprt* mutants, (ii) determine T-cell receptor (TCR) gene usages and commonalities among these clones, (iii) demonstrate reactivity to beryllium of these clones, (iv) generate beryllium sensitized T-cells *in vitro* from peripheral blood of the same individual, (v) determine TCR gene usages and commonalities for these *in vitro* derived cells, (vi) compare TCR gene patterns between the *in vivo* and *in vitro* derived clones, and (vii) develop a quantitative PCR (qPCR) method for amplifying the common (and therefore relevant) TCR genes directly from peripheral blood. The last of these is the novel biomarker of early beryllium sensitization.

This report summarizes studies of the first 20 months of this project.

### Research Progress and Implications

#### T-cell Cloning Assays for *hprt* Mutants

Twenty-four (24) peripheral blood samples from 17 individuals reported to be sensitized to beryllium have been obtained. Sixteen cloning assays on samples from ten individuals have found *hprt* mutant frequencies (Mfs) that range from 0.1-87.0 x 10<sup>-6</sup>. All save three of these (two in one individual) are within the normal range. Therefore, unlike some autoimmune/hypersensitivity diseases, these results provide little support for the expectation that the sensitized individuals will have elevated MF values because of T-cell proliferation. (The remaining peripheral blood mononuclear samples have been cryopreserved for future study.)

#### TCR Gene Analyses

Sixty-nine wild type and 303 *hprt* mutant isolates, respectively, have been obtained from cloning assays. No evidence of common gene usage was found by sequence analysis of TCR gene variable (BV), junctional (BJ) or hypervariable (CDR3) regions, except for clonal amplifications. Although small clonal amplifications (based on identical TCR gene sequences in two or more isolates) were found among the mutants in many individuals, only one relatively large clone was found, and that in two separate blood samples from one individual.

#### Studies of Beryllium Reactivity

Because of limited cell numbers, it was necessary to establish autologous lymphoblastoid cell lines as antigen presenting cells. Seven such lines from seven individuals have been established. Others will be as peripheral blood mononuclear cell samples are thawed.

Three beryllium stimulation assays have been performed using random mutant isolates, none of which represented an *in vivo* clonal amplification. All were negative as manifest by lack of sustained

antigen driven cell growth *in vitro*. Further challenge assays are being deferred until the hundreds of isolates available for study are prioritized.

### **Development of a TCR Gene Assay**

Studies of TCR gene usage in immunologically reactive cells define only a portion of the genetically encoded antigen binding potential of these cells. Because no commonality of TCR gene usages have been apparent in studies to date, TCR gene usage of the isolates will be determined. Therefore, the TCR gene assay was adapted to our laboratory for this use. As this assay requires multiplex RT-PCR and sequencing of the multiple TCR gene segments (there are 33 TCR gene variable region (AB) families), this adaptation required the acquisition/development of many suitable primers for multiplex PCR analysis. This consumed considerable time.

### **Generation of Beryllium Reactive T-cell Clones *in vitro***

Attempts to develop T-cell lines directly from peripheral blood have not succeeded using samples from three putatively beryllium sensitized individuals. However, on review of the Beryllium Lymphocyte Proliferation Test (BLPT) results on the 24 blood samples received thus far from the Oak Ridge Laboratory, it was found that all were negative for the samples received. These individuals, however, did have positive test results in the past. We have recently established an alternative source of clinical material.

### **Summary and Implications of Results to Date**

We have determined that putatively beryllium sensitized individuals do not have elevated *hpvt* T-cell mutant frequencies. Also, there are no large clonal “mutant runs” among the mutant isolates from these individuals. This may indicate that, in the individuals from whom samples were received, there were no ongoing T-cell proliferations at the time the samples were obtained. (Note that the BLPT status of all the samples received to date suggest that some or all of these samples may not be from individuals sensitized to the degree expected.) Alternatively, this may indicate that sensitized T-cells amplifying in body compartments (e.g. lungs) do not leak to the peripheral blood in this disorder. Another possibility (which may be more likely) is that multiple TCR gene products interact with beryllium antigen complexes and that the reactive cells present among the *hpvt* mutants will not be identifiable by common TCR gene usage.

### **Planned Activities**

Studies in the coming months will be directed to sorting through these alternatives.

1. Analysis of the TCR gene usage for the mutant and wild type isolates currently available will be accomplished. Common usage pattern here will identify clones as likely candidates that represent beryllium sensitized clones.
2. TCR gene ( and ) usage patterns will be determined in mass cultures of peripheral blood T-cells obtained from beryllium sensitized individuals and stimulated with PHA or beryllium. This will identify differential TCR usage patterns between the specific (beryllium) vs. polyclonal activator (PHA) stimulated cultures. This differential will identify the patterns that identify *in vivo* derived *hpvt* mutants as likely candidates for antigen challenge studies.
3. Continuation of the antigen challenge studies as prioritized by steps 1 and 2.

Additional studies will employ beryllium rather than PHA in some *hpvt* T-cell cloning assays. Finally, another source of beryllium sensitized individuals has been developed. All studies will be repeated using samples from individuals who have high BLPT test results on the samples under study. Beryllium sensitized T-cell lines will be directly obtained from peripheral blood from these individuals.

## **Other Access To Information**

There are no formal publications of this work to date. These results are available through the progress reports at the Department of Energy.