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Project Title: **A Novel Biomarker for Beryllium Sensitization in Humans**

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RESEARCH PROJECT NO. DE-FG07-96ER62331

"A NOVEL BIOMARKER FOR BERYLLIUM SENSITIZATION IN HUMANS"

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This research project will develop a novel biomarker of early beryllium sensitization in humans through identification of beryllium reactive T-cells in peripheral blood. The new method is based on isolating the relevant lymphocytes from peripheral blood using the *HPRT* T-cell mutation assay, which selects mutant cells based on their resistance to 6-thioguanine. Such mutant populations of T-cells are expected to be enriched for cells that are proliferating *in vivo* as a result of their beryllium sensitization. The underlying hypothesis has been verified in a number of studies in autoimmune diseases and transplantation. The T-cell receptor (TCR) genes of the *in vivo* arising *HPRT* mutants are then determined. The specific aims of the overall project are:

1. To identify the *in vivo* proliferating T-cell clones in sensitized individuals by selecting for *HPRT* mutants,
2. To determine T-cell receptor (TCR) β (and now α) gene usages and commonalties among these clones,
3. To demonstrate the beryllium reactivity of these clones,
4. To generate beryllium sensitized T-cells *in vitro* from peripheral blood of the same individuals,
5. To determine TCR β (and α) gene usages and commonalties of the *in vitro* derived cells,
6. To compare TCR β (and α) gene patterns between the *in vivo* and *in vitro* derived clones, and
7. To develop a quantitative PCR (qPCR) method for amplifying the common and therefore relevant TCR genes directly from peripheral blood as the novel biomarker of early beryllium sensitization.

There have been unavoidable delays in obtaining blood samples from beryllium sensitized individuals. Many of these sent from Oak Ridge, Tennessee may not have been from sensitized patients as some were negative for the Beryllium Lymphocyte Proliferation Test (BLPT). For this reason, additional samples were sought from the National Jewish Hospital in Denver, CO. Physicians there care for Rocky Flats workers who have been unequivocally sensitized to beryllium, i.e. patients with berylliosis. Studies of T-lymphocytes obtained from the lungs of these patients have been characterized at National Jewish as to their TCR gene usage (supervised by Drs. Newman and Kotzin). We have now developed a collaboration to study approximately 10-15 patients whose lung lymphocytes (obtained by bronchioalveolar lavage [BAL]) have been characterized as to their TCR gene usage. These lung T-cells are unequivocally associated with beryllium sensitization. We in Vermont will use our peripheral blood mutation assay to identify clonal representatives of these same T-cells in peripheral blood. Such representatives will allow us to identify the TCR gene or genes for use as a biomarker to detect beryllium sensitization. We will develop a qPCR technique to amplify these TCR cDNAs directly from peripheral blood T-cells.

The cumulative work accomplished on this project to date (32 months) is summarized below with a specific breakdown of those studies performed in the last 12 months.

T-cell Cloning Assays for *HPRT* Mutants

A total of 23 cloning assays have now been completed on blood samples from 17 individuals from Oak Ridge, TN. Seven of these cloning assays were performed during the last year. All available samples from this source have now been tested. The mutant frequencies (MF) for the total set of 23 cloning assays remains in the range of $1-87 \times 10^{-6}$. Of the additional seven tested this year, one had an elevated mutant frequency value (66×10^{-6}), bringing the total of elevated values to four for this set of samples.

We have begun receiving blood samples from the National Jewish Hospital. Five have been received and have been tested by cloning assay. Two of these provided insufficient cells for a meaningful assay. Of the three successful assays, the MFs were 9.3 , 36.3 and 36.6×10^{-6} , respectively. (Two are elevated.)

TCR β Gene Analyses

An additional two wild type and 20 mutant isolates obtained from the Oak Ridge group of samples have been studied this past year. Several others are in various stages of study. Formal analysis of results will be done after all TCR gene patterns have been analyzed.

Approximately 19 wild type and 44 mutant isolates have now been obtained from the National Jewish Hospital samples. Their TCR gene analyses will begin soon. The TCR usages of these isolates will be compared with TCR gene usages determined for the BAL cells in Colorado for the same individual. Clonal identity will document that the T-cells involved in the lung reactions are also present in blood, as detected in the *HPRT* mutant fraction.

The TCR α gene assay has been developed in this laboratory as outlined in the last progress report. We have not begun formal studies of TCR α gene usage in the DNAs extracted from *HPRT* mutants and will withhold this until the TCR β gene patterns have been determined.

Studies of Beryllium Reactivity

No further studies of beryllium reactivity of *HPRT* mutant clones have been attempted this past year.

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

No further attempts have been made to develop T-cell lines directly from the blood samples from Oak Ridge subjects. These studies will be reinitiated once we have a sufficient source of samples from National Jewish Hospital.

Summary and Interpretation of Results to Date

Four of 23 cloning assays from samples of subjects obtained from Oak Ridge have had elevated mutant frequencies. Therefore, the conclusion that this set does not in general have an increased number of *HPRT* mutants is probably correct. Also, the additional mutants studied for TCR β gene usage have shown no large clonal amplification among the mutants.

One of the difficulties in interpreting the TCR β gene usage patterns has been uncertainty as to the beryllium sensitization status of the study subjects. For this reason, we now study samples from unequivocally sensitized patients from Denver, CO. As we know that there are TCR β gene usage patterns that dominate in the BAL cells of these patients, we have a "target" for TCR patterns to look for in peripheral blood.

Preliminary inspection of our peripheral blood TCR β gene usage patterns by the investigators at National Jewish Hospital has revealed some patterns that are identical to or similar to patterns used by lung infiltrating lymphocytes in their patients. Although from different individuals, this is a very encouraging observation and suggests that we may find identical clonal representatives of lung infiltrating lymphocytes in the peripheral blood from the same patient

Planned Activities

Studies of the next year will be as follows:

1. A request has been made for a no-cost extension of the current grant for one year. Our studies will therefore go through September 30, 2000.
2. We will study (by cloning assay) approximately 10-15 samples from National Jewish.
3. Peripheral blood *HPRT* mutant T-cell TCR β gene usage patterns will be determined and compared with those found in the BAL cells. Based on these usage patterns and commonality with those seen in lung-derived lymphocytes, isolates will be selected to determine TCR α gene usage.
4. Mass cultures of peripheral blood T-lymphocytes obtained from these new subjects (known to be sensitized to beryllium) will be stimulated with PHA or beryllium *in vitro*. TCR gene (β and α) usage will be determined using the method of qPCR to identify differential patterns between the specific (beryllium) vs. polyclonal activator (PHA) stimulated cultures. This differential will further identify the patterns that mark *in vivo* derived *HPRT* mutant isolates as likely candidates for antigen challenge studies. These patterns will be compared with the patterns described for both the BAL and *HPRT* mutant peripheral blood samples.
5. Selected *HPRT* mutant clones will be stimulated with beryllium to determine reactivity. We will also develop beryllium sensitive cell lines directly from peripheral blood using the new samples from individuals unequivocally sensitized to beryllium.

These studies proposed for the next year will allow us to determine, with confidence, if we can or cannot isolate beryllium sensitized T-cells from the peripheral blood of beryllium sensitized subjects. If we can, the qPCR methods as described will allow us to develop the novel biomarker of beryllium sensitization. If we cannot, we will have evidence that beryllium sensitized cells are limited to the lungs.

Information Access

There are no formal publications of this work to date. The results are available through the Progress Reports at the Department of Energy.