

# A Novel Biomarker For Beryllium Sensitization In Humans

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# A NOVEL BIOMARKER FOR BERYLLIUM SENSITIZATION IN HUMANS

*Progress Report: October, 1996 to June, 1998*

- T-cell Cloning Assays for *HPRT* Mutants
- TCR $\beta$  Gene Analyses
- Studies of Beryllium Reactivity  
(Establish  $\beta$ -cell Lymphoblastoid Lines as APCs)
- Generation of Beryllium Reactive Clones *in vitro*  
(BLPT Results Largely Negative Thus Far)
- Development of TCR $\alpha$  Gene Assay

## A NOVEL BIOMARKER FOR BERYLLIUM SENSITIZATION IN HUMANS

### Studies for the Upcoming Year:

- TCR $\alpha$  Gene Analyses
- Mass T-cell Cultures for Sensitized Individuals
  - PHA
  - Beryllium
  - Define TCR $\alpha$  and  $\beta$  Usage Using QPCR  
(Identify usages being sought in mutants)
- Continue Antigen Challenge Studies
- Obtain Samples from Patients with Unequivocally Positive BLPTs

## ABSTRACT

This research project will determine the T-cell receptor (TCR) gene usages of beryllium reactive T-lymphocytes isolated directly from the peripheral blood of individuals exposed at a U.S. Department of Energy site. The objective is to develop a sensitive and novel biomarker for identifying early human sensitization to environmental beryllium. This is a collaborative project involving the Genetics Laboratory of the University of Vermont and both the Center for Epidemiological Research and the scientific staff of the Cytogenetics Program at the Oak Ridge Institute for Science and Education (ORISE). The >2000 beryllium exposed workers who have been contacted for participation in the ORISE study "Follow-up of Beryllium Workers at the Y-12 Plant/Efficacy of the Peripheral Blood Lymphocyte Proliferation (LPT) and other Non-Invasive Procedures for Diagnosis of Chronic Beryllium Disease" will provide the pool of potential participants for the proposed study. Beryllium reactive T-lymphocytes will be directly isolated from peripheral blood using a novel antigen-independent method of surrogate selection for *in vivo* arising *hprt* mutants as representatives of clones that are undergoing chronic proliferation. The T-cells undergoing chronic proliferation in beryllium sensitized individuals will be enriched for beryllium reactive cells. The TCR  $\beta$  gene usage of these T-cell isolates will be determined and their junctional (CDR3) regions sequenced. Beryllium reactive T-cell clones will also be recovered following *in vitro* beryllium stimulation of peripheral blood lymphocytes from these same individuals and the TCR gene CDR3 region sequences similarly determined. The TCR  $\beta$  genes used by the beryllium reactive isolates and their CRD3 region sequences will be compared within (*in vivo* vs. *in vitro* derived) and among individuals with attention to kinds and durations of beryllium exposure and HPA DPB Glu 69 status. A method for quantitating total body loads of these antigen reactive T-cells in individuals will be developed using quantitative polymerase chain reaction (QPCR) amplification of specific TCR gene sequences. Successful achievement of this overall objective will permit future studies aimed at the elucidation of the immunological mechanisms underlying sensitization, the comparison of cells involved in pulmonary and systemic sensitization and the definition of potential targets for immunotherapy.

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### Objectives

1. Identify T-cell subpopulations undergoing clonal amplification *in vivo* in beryllium sensitized individuals by surrogate selection for somatic mutants.
2. Identify common TCR BV, BJ and/or junctional region usage in the *in vivo* amplified clones.
3. Determine reactivity of representative isolates of *in vivo* amplified clones to beryllium and control antigens.
4. Generate and isolate beryllium reactive T-cell clones *in vitro* from the same beryllium sensitized individuals.
5. Identify common TCR BV, BJ and/or junctional region usage in the *in vitro* derived beryllium reactive T-cell clones.
6. Compare TCR BV, BJ and junctional region usage.
  - a. *In vivo* amplified T-cell clones
  - b. *In vitro* derived beryllium reactive T-cell clones
  - c. Within and among beryllium sensitized individuals
7. Q-PCR of specific VB-junctional and/or junctional-BJ regions that characterize beryllium reactive T-cell clones from PBLs as biomarkers of sensitization/disease activity.

TABLE 4

**TCR  $\beta$  GENE USAGE**  
**Beryllium Subject: AB**

Isolate #	BV # <sup>(3)</sup>	BV Sequence <sup>(4)</sup>	CDR 3 <sup>(5)</sup>	BJ Sequence <sup>(6)</sup>	BJ # <sup>(7)</sup>
		Experiment LS 913 - 2/28/97			
WT2 <sup>(1)</sup>	8	-	-	-	2s3
WT3	5	CASS	LAAPR	GEKLFFG	1s4
WT4	3	CAS	TEHANT	GELFFG	2s2
WT5	2s1	CSA	RSGDL	YNEQFFG	2s1
WT6	22/23 6	CASS CASS	EAPIMOA PTVASGGP	DTQYFG SQKHSVLRR	2s3 2s4 (FS)
WT8	14	CASS	FGTGVV	GELFFG	2s2
WT9	17	CASS	IPRGW	QFFG	2s1
WT10	7s2	CASS	QDGSPG	DTGELFFG	2s2
WT11	6	CASS	LDWDI	QETQYFG	2s5
WT12	2s1	CSA	PLELLRV <sup>(8)</sup>	SYNEQFFG	2s1
WT17	2s1	CSA	PLELLRV	SYNEQFFG	2s1
M1 <sup>(2)</sup>	13s2	CASS	YGAE	SCNTIYFG	1s3
M4	13s2 2	CASS CS	YGAE GRSGDIY	SGNTIYFG NEQFFG	1s3 2s1
M6	13s2	CASS	YGAE	SGNTIYFG	1s3
M3	14	CASS	YGA	NEQFFG	2s1
M2	13s2	CASS	RGHVGRD	SPLHFG	1s6
M9	12s2	CAIS	STSGNT	YNEQFFG	2s1
M10	12s2	CAIS	STSGNT	YNEQFFG	2s1
M13	12s2	CAIS	STSGNT	YNEQ LLRA	2s1
M5 <sup>(9)</sup>	2	CSA	PRRWPAS	QETQYFG	2s5
M18	21	CASS	LVSARD	TGELFFG	2s2
M19	3	CASS	FFGNRGP	NTEAFFG	1s1

Legend same as for Tables 2 and 3

**TABLE 5**

**TCR  $\beta$  GENE USAGE**  
**Beryllium Subject: AB**

Isolate #	BV # <sup>(3)</sup>	BV Sequence <sup>(6)</sup>	CDR 3 <sup>(5)</sup>	BJ Sequence <sup>(8)</sup>	BJ # <sup>(7)</sup>
		Experiment LS 931 - 7/2997			
WT4 <sup>(1)</sup>	5	CASS	LEVSGH	<sup>(9)</sup> SGNTIYFG	1s3
M10A <sup>(2)</sup>	13	CASS	YGAE	SGNTIYFG	1s3
M13	13	CASS	YGAE	SGNTIYFG	1s3
M2	13	CASS	YGAE	SGNTIYFG	1s3
M8B	13	CASS	YGAE	SGNTIYFG	1s3
M5A	13	CASS	YGAE	SGNTIYFG	1s3

Legend same as for Tables 2 and 3

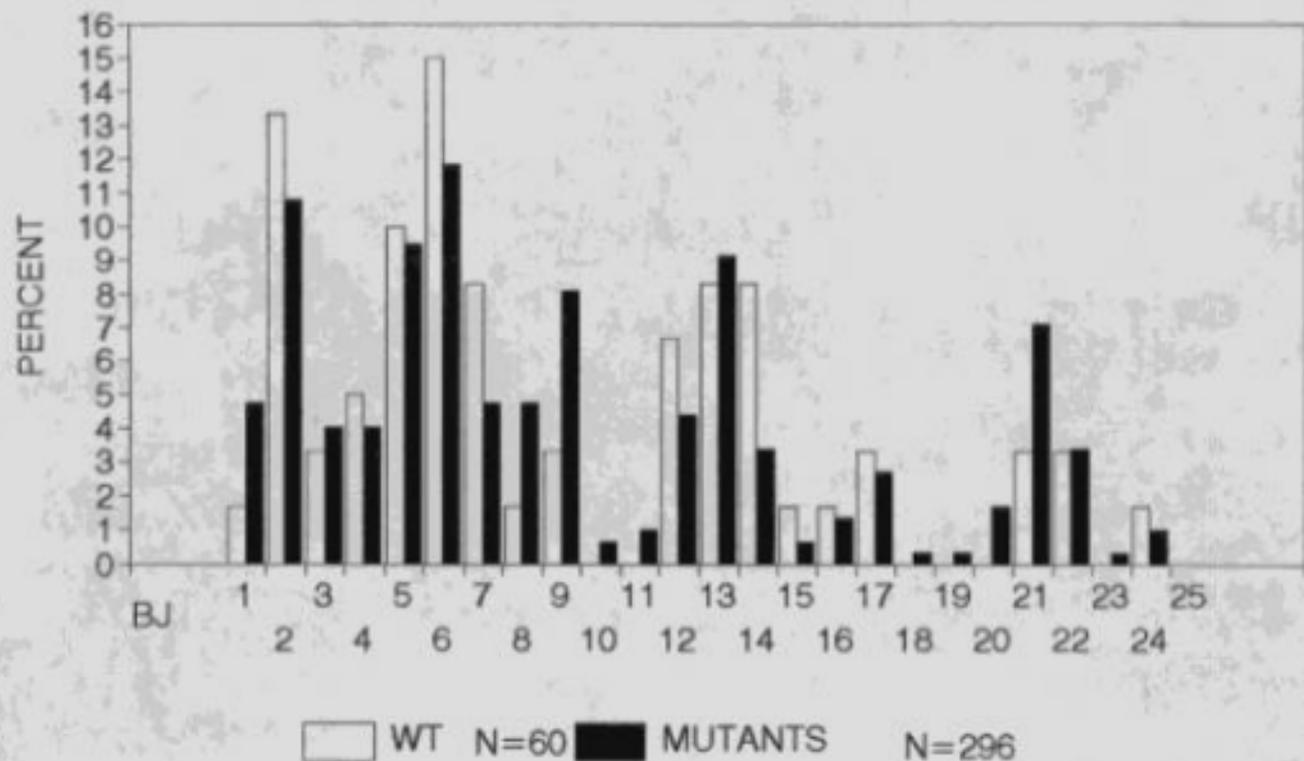
TABLE 6

**TCR  $\beta$  GENE USAGE**  
**Beryllium Subject: AB**

Isolate #	BV # <sup>(1)</sup>	BV Sequence <sup>(2)</sup>	CDR 3 <sup>(3)</sup>	BJ Sequence <sup>(4)</sup>	BJ # <sup>(5)</sup>
<b>Experiment LS 940A - 11/19/97</b>					
M70B	9	CASS	QTT	QETQYFG	2s5
M92	9	CASS	LFTLSTSGGA	NEQYFG	2s1
M36	12	CAIS	ESRTGG	TEAFFG	1s1
M31	12	CAIS	QQS	YEQYFG	2s7
M54	12	CAIS	SSLP	ETQYFG	2s5
M2	12	CAIS	STSGNT	YNEQFFG	2s1
M119	12	CAIS	STSGNT	YNEQFFG	2s1
M41	12	CAIS	STSGNT	YNEQFFG	2s1
M30	12	CAIS	STSGNT	YNEQFFG	2s1
M49	12	CAIS	STSGNT	YNEQFFG	2s1
M33	13	CAS	RTSGR	YNEQFFG	2s1
M19B	13	CAS	RTSGR	YNEQFFG	2s1
M17	13	CASS	YGAE	SGNTIYFG	1s3
M18	13	CASS	YGAE	SGNTIYFG	1s3
M34	13	CASS	YGAE	SGNTIYFG	1s3
M44	13	CASS	YGAE	SGNTIYFG	1s3
M51	13	CASS	YGAE	SGNTIYFG	1s3
M82	13	CASS	YGAE	SGNTIYFG	1s3
M86	13	CASS	YGAE	SGNTIYFG	1s3
M94	13	CASS	YGAE	SGNTIYFG	1s3
M116	13	CASS	YGAE	SGNTIYFG	1s3
M21B	13	CASS	LSATGNVA	GELFFG	2s2
M78	14	CAS	RSPWGG	EQFFG	2s1
M14	14	CAS	RSPWGG	EQFFG	2s1
M28	14	CAS	RSPWGG	EQFFG	2s1
M21A	14	CAS	EQSHS	YEQYFG	2s7
M11	14	CAS	SPQVQGS	YEQYFG	2s7
M99	15	CATS	DFYGLVGV	NEQFFG	2s1

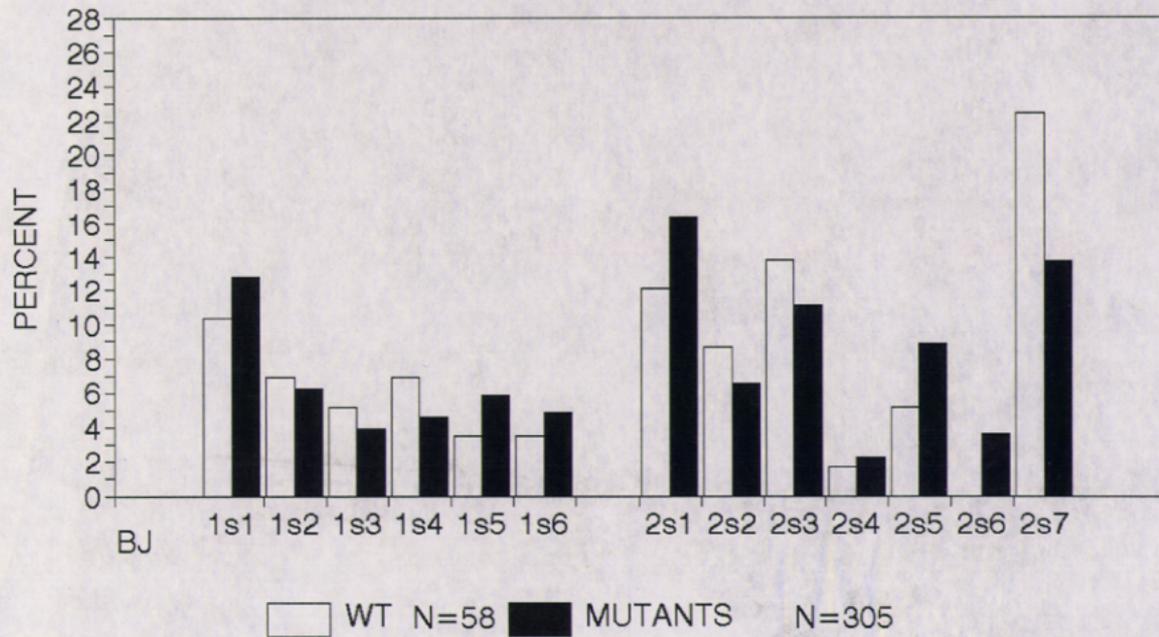
# BV USAGE:

## Beryllium Sensitized Individuals



# BJ USAGE:

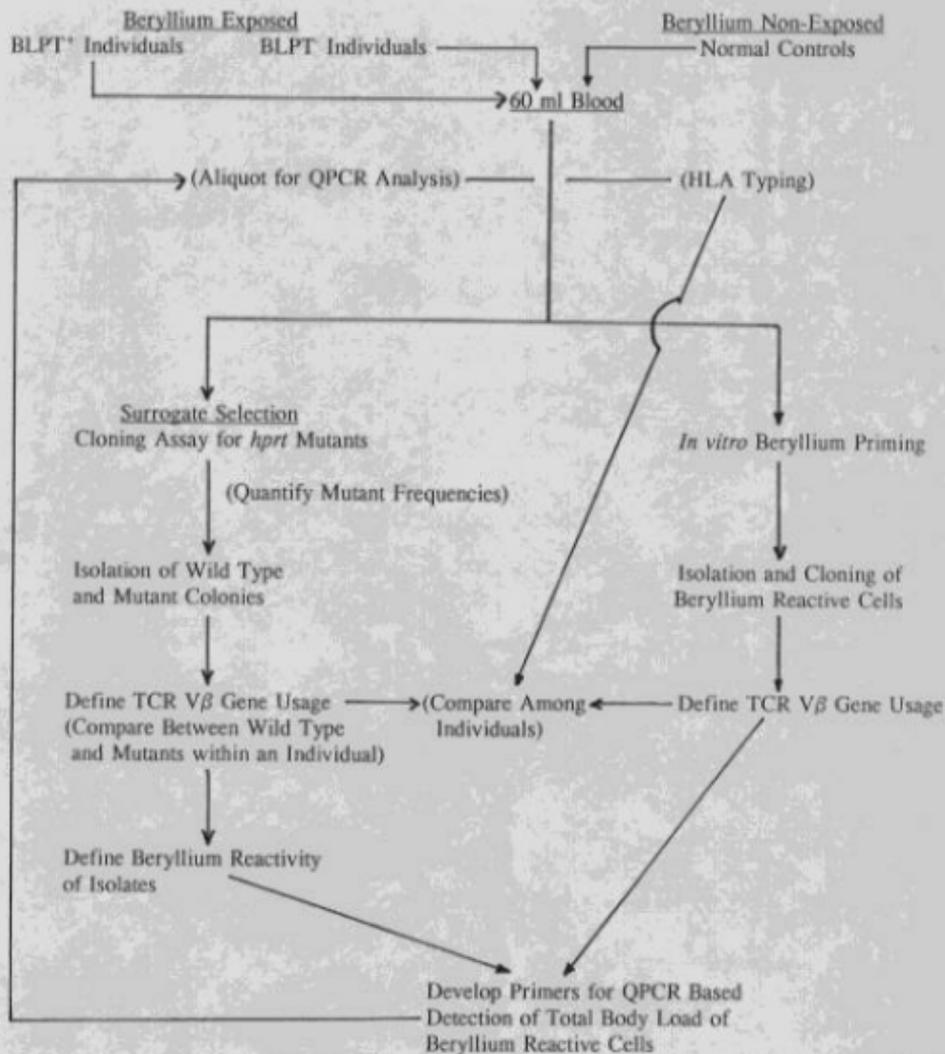
## Beryllium Sensitized Individuals



				STIMULATION INDEX (S.I.) <sup>*</sup>				
				BERYLLIUM RESPONSE IN VITRO				
INDIVIDUAL	SEX	AGE	M.F.x10 <sup>4</sup>	1uM	5uM	10uM	20uM	100uM
G.F.(1)	M	44	16.9	-		-		-
G.F.(2)	M	44	10.1	6.3		5.89		1.63
A.B.(1)	M	48	10	-		-		-
A.B.(2)	M	49	8.8	0.75		0.93		
A.B.(3)	M	49	32.9	0.73	0.53	0.29	0.23	-
J.H.(1a)	M	48	62.5	-		-		-
J.H.(1b)	M	48	87	-		-		-
J.H.(2)	M	48	5.3	18		7.4		0.4
E.S.(1)	F	59	5.8	-		-		-
E.S.(2)	F	59	10.3	0.87		0.66		0.51
E.R.	M	74	0.94	0.84		0.4		0.63
R.F.	M	48	3.7	19.37		4.88		0.45
J.K.	M	-	9.1	1.93		1.78		0.84
J.L.	M	50	15.9	2.06		1.98		1.3
R.Z.	M	48	19	0.62		0.66		0.75
G.L.	F	55	15.1	0.95	0.87	1.07	2.32	-

\* x3 is significant

## Flow Diagram of Proposed Research



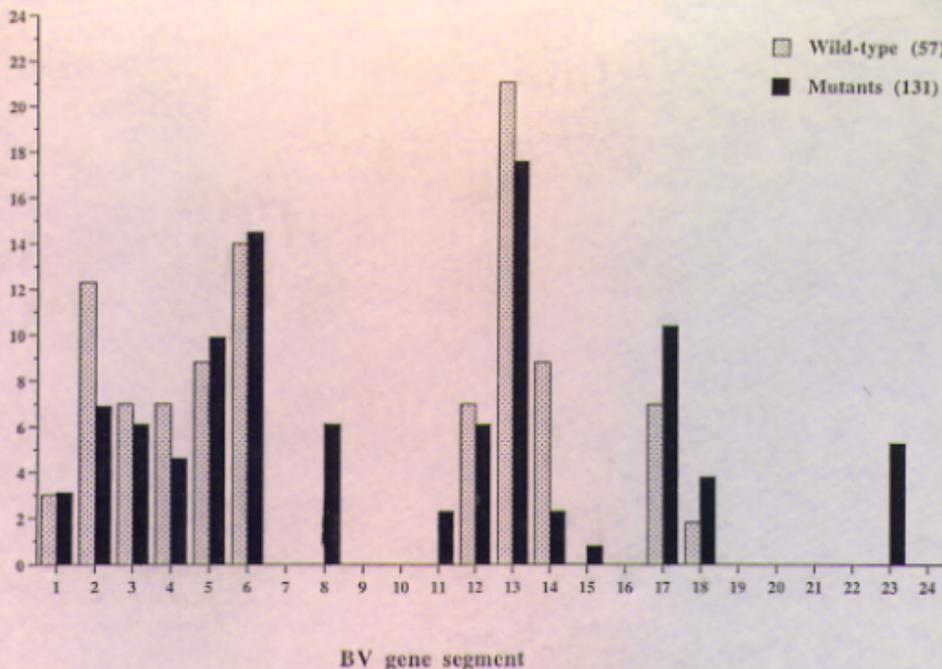
# SURROGATE SELECTION

**METHOD FOR SELECTING IMMUNE-RELEVANT T-CELLS UNDERGOING PROLIFERATION IN VIVO BY SELECTION OF CELLS WHICH HAVE UNDERGONE MUTATION.**

## **RATIONALE**

- 1. Gene mutations occur preferentially in proliferating cell subsets. Reasons for this are:**
  - errors during DNA replication
  - insufficient time to repair DNA damage prior to replication
  - mutations become fixed at replication.
- 2. Individuals with autoimmune diseases will have subpopulations of T-cells proliferating that are relevant to the disease process. These cells are:**
  - responding to autoantigens
  - involved in the immune network
  - present at low frequencies
- 3. The hprt clonal assay selects for cells which have undergone mutation at the X-linked hypoxanthine-guanine phosphoribosyl transferase gene. Dividing autoimmune cells will undergo mutation at this locus and therefore, will become enriched within the hprt mutant fraction. Advantages of this assay are:**
  - selection is antigen independent
  - cells are cloned directly from blood facilitating study
- 4. Mutant and wildtype isolates are available for analysis of:**
  - surface phenotype
  - antigen reactivity
  - T-cell receptor gene usage

Percentage usage



**Figure 1.** TCRBV gene usage in representative normal control RCI. Independent wild-type (hatched bars) and *hprt* mutant (solid bars) T cell isolates were recovered by cloning assay and BV usage ascertained by PCR and sequencing. The number of isolates studied is provided in parenthesis in the figure legend. Specific BV region subfamily member usage could be determined in many instances as the BV consensus primer is located approximately midway within the V gene segment. However, for simplicity, BV regions are broken down by subfamily alone.

## STUDIES OF AUTOIMMUNE DISEASE/TRANSPLANTATION USING SURROGATE SELECTION

Disorder	Findings
Multiple Sclerosis*	Elevated MF values MBP reactive <i>hprt</i> T-cells
Systemic Lupus Erythematosus	Elevated MF values Clinical correlations Mutant and wild type isolates helped B-cells → $\alpha$ DNA abs
Guillain-Barré Syndrome	Elevated MF values CD8 <sup>+</sup> mutants
Chronic Inflammatory Demyelating Polyneuropathy	Elevated MF values
Systemic Sclerosis	Elevated MF values correlated with duration of skin involvement
Mixed Connective Tissue Disease	Elevated MF values
Rheumatoid Arthritis*	No change (Non-significant MF elevation)
Psoriasis*	No quantitative change BV 13.1 predominant among <i>hprt</i> PBL and lesional skin infiltrates
IDDM*	TCR BV usage restriction to 14s1 among mutants
Heart Transplant Recipients	Elevated MF correlates with rejection episodes

\* Some or all of studies done in Vermont.