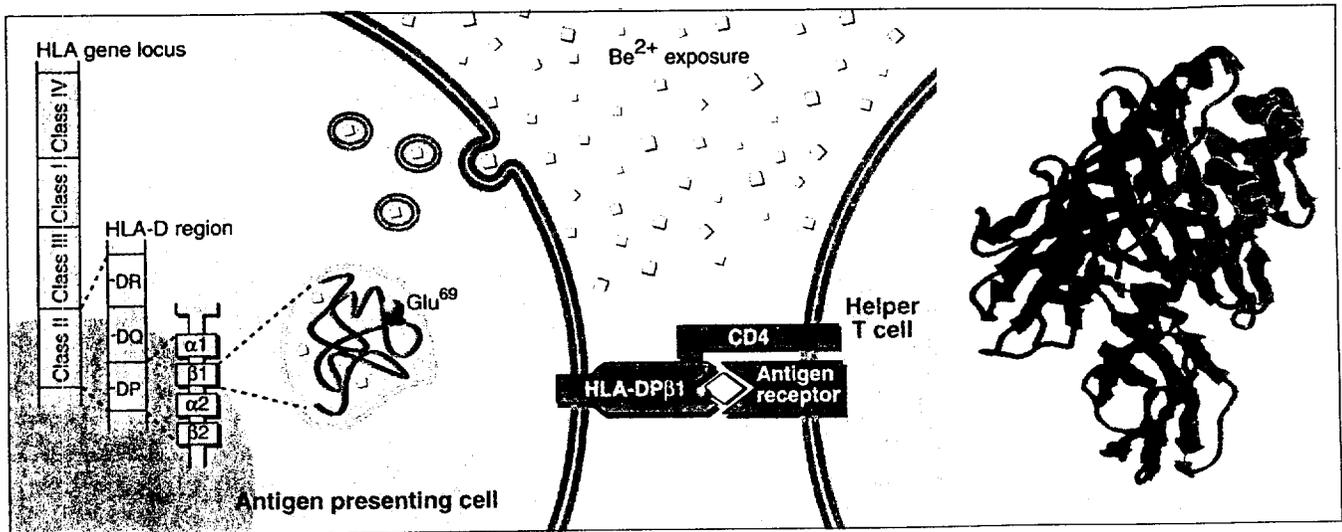


# Beryllium International Research Symposium 2004

January 28 – 30, 2004

Hyatt Regency Denver • Denver, Colorado



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Division of Environmental and Occupational Health Sciences

**NATIONAL  
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Global Leader in Lung, Allergic  
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**Beryllium International Research  
Symposium 2004**

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## Welcome!

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January 28, 2004

Welcome to the Beryllium International Research Symposium 2004. It promises to be a unique opportunity to bring together researchers from the U.S. and Europe who are contributing to our understanding of beryllium and its health effects.

Chronic Beryllium Disease (CBD) and other beryllium-related health conditions continue to be serious problems both in the U.S. and abroad. Cases continue to be reported in both 'old' and new segments of U.S. industry, ranging from alloy manufacture to recycling; metal machining to ceramic production. An increasing number of countries are now reporting disease in North America, Europe, the Middle East, and Asia. Past exposures as well as contemporary exposures are contributing to the persistence of this problem for our workers and patients. Tragically, though we have known about CBD for 60 years, we have yet to eradicate it.

Fortunately, an increasing number of excellent researchers are addressing questions concerning beryllium and its biological and health effects. The goal of this workshop is to bring together many of these scientists—from many disciplines—to share information and seek answers to the most important questions we face.

If our goal is the ultimate elimination of beryllium-related health effects, a number of real-world questions must be addressed using all of the research techniques at our disposal. We must take fundamental and epidemiologic scientific discoveries and find ways to quickly apply that information in a way that helps industry, labor, workers, and patients.

Thank you for joining us in the presentation and discussion of the research, the open exchange of ideas, the development of new collaborations, and for the progress you will make in preventing illness.



Lee Newman, M.D., M.A.

Lisa Maier, M.D., M.S.P.H.

John Martyny, Ph.D., C.I.H.

Division of Environmental and Occupational Health Sciences

National Jewish Medical and Research Center and

University of Colorado Health Sciences Center

# Agenda

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Lee S. Newman, MD, MA

## **CLINICAL RESEARCH ON HUMAN HEALTH EFFECTS OF BERYLLIUM**

**8:15 A.M.** Clinical Overview and Advances in Beryllium Sensitization and Chronic Beryllium Disease Diagnosis and Management  
Milton Rossman, MD

**9:00 A.M.** Cleveland Clinic Experience in CBD Diagnosis and Disease Progression  
Raed Dweik, MD

**9:45 – 10:00 A.M.** BREAK

## **IMPACT OF BERYLLIUM EXPOSURE**

**10:15 A.M.** Exposure and Risk for Sensitization and Disease  
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**11:00 A.M.** Exposure and Cancer Risk  
Wayne Sanderson, PhD, CIH

**11:45 – 1:00 P.M.** GROUP LUNCHEON

**1:00 P.M.** Particles and their Physiochemical Properties  
Mark Hoover, PhD, CHP, CIH

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**1:45 P.M.** Overview of Recent Epidemiologic Studies  
Margaret Mroz, MSPH

**2:30 P.M.** Findings from Medical Surveillance of Beryllium Workers  
Paul Henneberger, MPH, ScD

**3:15 – 3:30 P.M.** BREAK

**3:30 P.M.** Medical Surveillance for Beryllium Disease in a Large Nuclear Weapons Facility Supports Population-based Risk Estimates.  
Tim Takaro, MD, MPH

**4:15 P.M.** The Nevada Test Site: Nuclear Weapons, Rockets, and the Clean-up Fallout  
Lewis Pepper, MD, MPH

**5:00 P.M.** ADJOURN

**7:30 – 8:00 A.M.**

**CONTINENTAL BREAKFAST**

**BASIC MOLECULAR AND GENETIC MECHANISMS**

**8:00 A.M.**

Genetic Factors and Granulomatous Inflammation:  
Lessons from and for sarcoidosis

**Ron du Bois, MD**

*Sponsored by*  **Centocor**

**9:00 A.M.**

Animal Models of Granulomatous Inflammation

**Terry Gordon, PhD**

**9:45 – 10:00 A.M.**

**BREAK**

**10:00 A.M.**

T Cell Recognition of the Beryllium-MHC Complex

**Andrew Fontenot, MD**

**10:45 A.M.**

Cytokine Gene Activation in Chronic Beryllium Disease

**Tristan Barnes, PhD**

**11:15 A.M.**

Taming Inflammatory Mechanisms in the Lung:  
the PPAR gamma Regulatory Pathway

**Barbara Barna, PhD**

**12:00 – 1:00 P.M.**

**GROUP LUNCHEON**

*Sponsored by*  **Centocor**

**1:00 P.M.**

Oxidative Stress and Beryllium

**Brian Day, PhD**

**1:45 P.M.**

Overview of Innate and Acquired Immunity in CBD

**Richard Sawyer, PhD**

**2:30 P.M.**

**Abstract Session**

**5:00 P.M.**

**ADJOURN**

**7:30 – 8:00 A.M.**

**CONTINENTAL BREAKFAST**

**POPULATION BASED GENETIC STUDIES AND GENE AND ENVIRONMENT INTERACTIONS**

**8:00 A.M.**

**Gene-Environment Interactions in Occupational Lung Diseases  
and Applications to CBD  
Anthony Newman Taylor, MD**

**9:00 A.M.**

**Genetic Risk Factors for Beryllium Sensitization, Chronic  
Beryllium Disease, and Severity  
Lisa Maier, MD, MSPH**

**9:45 – 10:00 A.M.**

**BREAK**

**10:00 A.M.**

**Recent Studies of Genetic Susceptibility Factors in CBD  
Cesare Saltini, MD**

**10:45 A.M.**

**Ethical Considerations in Beryllium Research and Genetic  
Testing  
Richard Sharp, PhD**

**11:30 – 12:30 P.M.**

**GROUP LUNCHEON**

**12:30 P.M.**

**Abstract Session**

**2:30 P.M.**

**Symposium Summary  
Lee S. Newman, MD, MA**

**3:15 P.M.**

**Adjourn**

## **Clinical Research on Human Health Effects of Beryllium**

### **Advances in Beryllium Sensitization and Chronic Beryllium Disease Diagnosis and Management**

**Milton D. Rossman, M.D.**

Professor of Medicine  
Hospital of the University of Pennsylvania  
Philadelphia, PA.

Chronic beryllium disease (CBD) is a hypersensitivity granulomatous disease. Originally, this condition was diagnosed by a history of exposure and a clinical condition consistent with a granulomatous process. A major advance in the recognition of CBD was made with the application of lymphocyte proliferation tests to beryllium (BeLPT) was applied to blood and bronchoalveolar lavage specimens. Use of BeLPT aided the diagnosis and confirmed the present of CBD in industries that previously "did not have a CBD problem". Not only is the BeLPT used for diagnosis but has also been useful for screening for disease and for monitoring in the workplace to detect areas of excessive exposure. Screening for CBD with the BeLPT can detect symptomatic CBD that is not apparent on chest radiographs. Treatment regimens have not been standardized, but corticosteroids remain the cornerstone of treatment. Alternative regimens may one day be available, but their effectiveness are currently unknown.

## *Clinical Research on Human Health Effects of Beryllium*

### **Cleveland Clinic Experience in CBD Diagnosis and Disease Progression.**

**Raed A. Dweik, M.D.**

Department of Pulmonary, Allergy and Critical Care Medicine  
The Cleveland Clinic Foundation  
Cleveland, OH

Chronic Beryllium Disease (CBD) is an occupational disorder characterized by granulomatous inflammation of the lungs. It has been traditionally reported in workers in industries where beryllium (Be) is manufactured or processed. In general, about 10% of exposed workers get sensitized as determined by a Be-specific lymphocyte proliferation blood test (Be-LPT) and about 40-50% of sensitized individuals (~5% of all exposed workers) develop CBD as determined by the presence of compatible lung pathology (usually a granuloma). Exposure to beryllium as well as genetic susceptibility are risk factors for developing CBD. Other risk factors for developing the disease or for progression from sensitization to disease are not clear. The correlation of pathologic findings with clinical features and the long term prognosis of these patients are also not clear. Over a 5-year period, we evaluated 209 individuals exposed to beryllium who developed beryllium sensitization (a positive blood BeLPT). All individuals underwent pulmonary function testing and bronchoscopy with transbronchial biopsy (TBBx). CBD was defined by the presence of non-necrotizing granulomas on TBBx. Basic demographic characteristics as well as smoking status and pulmonary function tests were obtained as part of their clinical evaluation. Of the 209 (83% males) evaluated individuals, there were 77 cases of CBD. There were a significantly higher number of non-smokers among patients who had biopsy evidence of CBD than those who did not (Odds Ratio for smokers= 0.63 p=0.009). All 5 African Americans in the cohort had granulomas on TBBx. Pulmonary function abnormalities were similar among CBD and sensitized-biopsy negative individuals [CBD: normal spirometry 79%, obstruction 12%, restriction 9%, and reduced diffusion capacity 23%; sensitized: normal spirometry 72%, obstruction 17%, restriction 11%, and reduced diffusion capacity 16%, p=NS]. Subjects who had evidence of obstruction on spirometry or abnormal diffusion capacity were more likely to be smokers ("current" or "ex" smokers) (p= 0.02). A positive biopsy for granulomas was associated with 5 or greater pieces of tissue taken during the biopsy procedure and the examination of 20 or greater levels of the tissue by the pathologist. Twenty-six patients [14 CBD, 12 sensitized] had pulmonary function tests available over a median of 37 months (inter-quartile range 27 to 56 months) after the initial evaluation. There was no significant difference in lung function loss by spirometry in ml/year between the 2 groups, as well as when adjusted for smoking and duration of follow up but there was a trend towards more decline in the DLCO in the CBD group over the follow-up period.

## **Impact of Beryllium Exposure**

### **Exposure and Risk for Sensitization and Disease**

**Martyny JW**, Maier LA, Newman LS, Mroz MM, Sackett HM, Silveira L,  
VanDyke MV

The link between exposure to beryllium and the development of a sensitization to beryllium or chronic beryllium disease itself has been the subject of extensive investigation. Both conditions have been found to occur in individuals with beryllium exposures that were well below current OSHA standards ( $2 \text{ ug/m}^3$  as an 8-hr TWA). Exposure parameters of concern have included total beryllium exposure, particle size, metal characteristics, solubility, particle numbers and skin exposure. We report the results of medical screening on a group of 332 beryllium workers that have been screened serially during a period of improving environmental controls. Of that group 7.2% have had repeated abnormal blood tests, with most workers being found to have developed chronic beryllium disease. Since 1995, the rate of abnormal blood tests has been declining among not only those being re-tested but also among newly hired individuals. Prior to 1997, there was a 9.5% rate of sensitization. After 1997, there was only a 1.1% rate of sensitization. In 1995, the company put in place an aggressive exposure monitoring program that set a target of  $0.2 \text{ ug/m}^3$  as the maximum allowable exposure. The company also implemented exposure control systems on most processes and assured that employees followed specific hygiene practices. Exposure monitoring has been conducted on all individuals working at the plant and in both production and non-production areas. Since that change, process exposures and ambient plant exposures have been reduced. This reduction in airborne exposure has corresponded with a reduction in the number and percentage of employees becoming sensitized to beryllium. Exposures to new employees were obtained on a daily basis. Of samples collected, 62% were below the limit of detection. Detectable samples ranged from  $0.009 \text{ ug/m}^3$  to  $0.63 \text{ ug/m}^3$  with only 2.6% of the exposures exceeding the  $0.2 \text{ ug/m}^3$  company goal. Conclusions: Good exposure control can result in a significant decrease in beryllium sensitization and in the rate of chronic beryllium disease within a beryllium machining facility.

(Supported by NIOSH R01CCR815751, ES-11810 and RR00051)

## Impact of Beryllium Exposure

### Exposure and Cancer Risk

*Wayne T. Sanderson*

The University of Iowa

**Background:** Beryllium compounds are toxic to the lungs, causing acute pneumonitis and chronic granulomatous lung disease. Cohort mortality studies have found elevated lung cancer mortality among beryllium processing workers compared to non-exposed populations, but none of these studies evaluated the association between level of beryllium exposure and lung cancer risk. A nested case-control study of lung cancer within a beryllium processing plant was conducted to investigate the relationship between level of beryllium exposure and lung cancer.

**Methods:** Lung cancer cases were identified by mortality follow-up through 1992 of a cohort of male workers at a beryllium alloy production plant in Reading, Pennsylvania. Each of 142 lung cancer cases were age-race matched to five controls using incidence density sampling. Calendar-time specific beryllium exposure estimates were made for every job in the plant and were used to estimate the cumulative, average, and maximum exposures of the cases and controls. Potential exposure to various chemical forms of beryllium and other agents were evaluated for each job, and considered in the case-control analysis. The potential confounding effects of smoking were evaluated using smoking data from a health survey conducted at the plant by the U.S. Public Health Service in 1968.

**Results:** Lung cancer cases had shorter tenures and lower lifetime cumulative beryllium exposures than controls, but higher average and maximum exposures. However, after applying a 20 year lag, all exposure metrics were higher for cases than controls. Odds ratios in analyses lagged 20 years were significantly elevated for those with higher exposure compared to the lowest exposure category. Although categorical analyses did not show monotonically increasing odds ratios, significant positive trends were seen with the log of exposure metrics. Average exposure was a better predictor of lung cancer risk than cumulative exposure. Smoking did not appear to confound exposure-response analyses.

**Conclusion:** The finding of an increased risk of lung cancer among workers with higher beryllium exposures when dose estimates were lagged 10 to 20 years, and lack of evidence for confounding by cigarette smoking, provide further evidence beryllium is a human lung carcinogen. The lack of a linear exposure-response for most beryllium dose metrics must be interpreted with caution. A small number of beryllium air measurements were available during the 1940's and 1950's--when the majority of cases and controls were first employed--reducing the accuracy of job exposure estimates. Workers with the highest exposures often worked for only short periods in the 1940's; exposure misclassification may have affected these workers preferentially, distorting exposure-response relationships.

## Impact of Beryllium Exposure

### **Beryllium Particles and Their Physicochemical Properties**

**Mark D. Hoover, PhD, CHP, CIH**

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention, Morgantown, WV.

More than 50 years ago, Sterner and Eisenbud hypothesized that the development of chronic beryllium disease (CBD) resulted from an immunologic reaction to beryllium ions in the lung. They believed that beryllium compounds were retained in the lung in immunologically significant quantities for very long periods of time and that the immune response depended on not only the rate, but also the quantity of beryllium ions released from the tissue deposit. Work over the last 20 years has substantially improved our understanding of how the physicochemical properties of beryllium particles can influence the risk of CBD. For example, *in vitro* and *in vivo* studies of beryllium oxide (BeO) particles calcined at either 500°C (low-fired) or 1000°C (high-fired) provided a scientific explanation for the long-observed lower risk associated with inhalation of high-fired BeO, as compared to the risk from inhalation of low-fired oxide. The apparent lower toxicity of the high-fired material was associated with its lower specific surface area, and the dissolution and cell toxicity of the two materials were found to be identical when normalized to surface area.

For many years it was also believed that work with copper-beryllium alloys conveyed little risk of CBD. However, recent research has shown this to be false. In fact, it is now recognized that exposure to any chemical form of beryllium is likely to present risk for beryllium sensitization and CBD. Processes that involve high temperatures (e.g., smelting or annealing) can be especially effective at providing opportunities for inhalation exposures, and can also increase exposures to skin. Both routes of exposure may contribute to activation of an immunological response. A universal risk model for beryllium sensitization and CBD is likely to involve a scheme for understanding and quantifying the influence of particle size, surface area, and chemical composition on beryllium bioavailability. Such a model may lead to more protective standards and work practices to appropriately limit beryllium exposures by the inhalation and dermal routes.

# ***Prevention Research: Applied Community-Based Strategies for Beryllium Disease Prevention and Advances in Epidemiologic Research***

## **Epidemiology of Beryllium Sensitization and Chronic Beryllium Disease: Overview of Recent Epidemiologic Studies**

**Margaret M. Mroz, M.S.P.H.**

National Jewish Medical and Research Center

Division of Occupational and Environmental Health Sciences

The epidemiology of beryllium sensitization and chronic beryllium disease dates back to the 1940s when CBD was first identified among workers in beryllium production facilities and among fluorescent light workers. Recent epidemiology has focused on four general areas; 1) population-based prevalence studies defining disease risk, 2) population-based genetic studies, 3) studies of beryllium exposure, and 4) clinical epidemiologic studies. This talk overviews the most recent epidemiologic studies in the medical literature and briefly summarizes the findings and conclusions of the studies in these four different areas. Many of the studies will be discussed in detail by the authors during the rest of the conference.

Population-based studies defining disease risk and work-related risk factors have identified rates of BeS ranging from 1.8% to 11.8% within different occupational sectors using beryllium. These studies have identified process related increased risks and have also identified BeS and CBD occurring among individuals with trivial and bystander exposure. Population-based genetic studies have identified genetic factors involved in the susceptibility of BeS and CBD and also genetic factors involved in disease progression. Studies of gene-environment interactions contribute to a better understanding of disease development. Epidemiologic exposure studies are helping us to define quantitative and qualitative exposure factors related to the development of sensitization and disease. Exposure studies linked with results of medical surveillance can help us to identify areas of disease risk and aid in controlling beryllium exposures. Finally, clinical epidemiologic studies have examined clinical markers related to the development and progression of sensitization and disease, as well as factors related to disease severity.

Future research should focus on defining the exposures associated with the development of BeS and CBD. Genetic and clinical studies will allow us to study the progression of BeS to CBD. Longitudinal clinical studies will allow us to better describe the natural history of CBD identified in early stages. Studies of the gene-environment interactions offer insight into disease mechanism and exposure control. Prevention and treatment of CBD will benefit from ongoing population-based studies.

***Prevention Research: Applied Community-Based Strategies for Beryllium  
Disease Prevention and Advances in Epidemiologic Research***

**Findings from Medical Surveillance of Beryllium Workers**

**Paul K. Henneberger, MPH, ScD**

Division of Respiratory Disease Studies, National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention (CDC), Morgantown, WV.

The primary goal of the beryllium research program at the National Institute for Occupational Safety and Health (NIOSH) is to prevent beryllium sensitization and chronic beryllium disease (CBD). NIOSH has taken a multidisciplinary approach towards achieving this goal. Access to data from medical surveys of exposed workers has made it possible to address several intermediate goals, including: a) measuring the frequency of outcomes among selected worker populations; and b) understanding the exposure-response relationship and the factors that modify it. Surveys of selected worker populations in the 1990s revealed a prevalence of 6 to 10% for beryllium sensitization and 3 to 5% for CBD. The ten-year (1992-2002) cumulative incidence for workers from a ceramics plant was 20% for sensitization and 14% for CBD. Skin exposure may play a role in sensitization, and in one plant, the exposure-response relationship was stronger with measurements of beryllium in settled dust samples than with airborne mass of beryllium. The first population-level survey was conducted at a copper-beryllium alloy facility, and levels of sensitization and CBD were similar to those found at other production facilities. Service center workers, who did little processing of beryllium materials, had the lowest risk for sensitization and CBD. An intervention that addressed both inhalation and skin exposure has appeared to have favorable results. Surveys of exposed workers continue, and future investigations will consider new approaches to characterizing airborne and dermal exposures, and how genetic factors might modify the effect of exposure.

***Prevention Research: Applied Community-Based Strategies for Beryllium Disease Prevention and Advances in Epidemiologic Research***

**Beryllium exposures at former nuclear weapons production facilities: Sentinel cases and surveillance reveals new populations at risk.**

**Takaro TK**

University of Washington Occupational & Environmental Medicine Program

Beryllium is known to cause a chronic immunologically-mediated granulomatous lung disorder at low exposures in susceptible individuals. Workers across the U.S. nuclear weapons complex have been exposed to often unknown levels of beryllium as a result of weapon component and nuclear fuel fabrication, research, development, maintenance and more recently clean up activities at these facilities. For most of these sites, the beryllium hazard was underestimated or ignored until the late 1990s.

The U.S. Department of Energy Former Worker Program (FWP) was established in 1996 to address a congressional mandate to provide a "program for the identification and ongoing medical evaluation of current and former Department of Energy employees who are subject to significant health risks as a result of exposure..." in the course of their work for the Department. Independent hazard evaluations and sentinel cases of chronic beryllium disease in workers without previously known exposure helped to identify 17,450 workers at risk for CBD who were tested with lymphocyte proliferation testing. 38 cases of CBD were found in 165 sensitized individuals who underwent evaluation (23%). Beryllium sensitization rates varied from 0.88% to 2.3%. CBD rates in evaluated individuals range from 0 to 33.3% at the 11 FWP sites.

At the largest site (Hanford), building, job-task and temporal information was combined with sensitization data on an easily visualized map in order to better characterize risk for buildings slated for decontamination and demolition which could pose a risk to remediation workers. 3,269 former production workers were identified as potentially exposed to beryllium at Hanford. A questionnaire assessed occupational exposure was obtained from 786 of these workers along with peripheral blood samples collected for beryllium lymphocyte proliferation testing (BeLPT). The rate of sensitization (2 positive BeLPTs) is over 2.4%. Half of the workers report activities in one or more high risk building (based upon process history) and 76% report some beryllium exposure. Only 15% recall exposure measurements being taken during their tenure. These data were combined geographically with data on buildings where beryllium sensitized and diseased subjects worked. These sentinel health events add important information to beryllium risk estimation at the site.

These data suggest that significant beryllium exposures occurred at U.S. DOE weapons facilities in populations without classic beryllium production tasks i.e. machining, grinding or lapping. Current site remediation workers may also be exposed during decontamination and demolition (D&D) of old facilities where the sensitized and diseased populations worked. Further risk characterization is needed for these populations and for small sub-contractor facilities which have not yet been evaluated by the FWP.

***Prevention Research: Applied Community-Based Strategies for Beryllium Disease Prevention and Advances in Epidemiologic Research***

**The Nevada Test Site: Nuclear Weapons, Rockets, and the Clean-up Fallout**

**Lewis Pepper, MD, MPH**

Environmental Health Department  
Boston University School of Public Health

**Background:** From 1951 through 1992, 928 nuclear weapons tests were conducted at the Nevada Test Site. At the peak of underground testing in the mid-1980's, as many as 15,000 workers were employed, about one-third of them construction workers. While beryllium was never manufactured at NTS, it was used in a variety of applications with exposure occurring in a number of settings. Beryllium was ground, machined, modified, cut, lathed, heated, combusted in fuel, dispersed underground during nuclear blasts, and dispersed with explosives above ground at NTS. Since 1996, a U.S. DOE funded medical screening and surveillance program has been conducted in Las Vegas for former NTS workers. In 2000, screening for beryllium-related disease was initiated for a segment of the former worker cohort. In 2001 a case of CBD was diagnosed in a current employee leading to extensive medical screening by the contractor and a DOE report of historic and current beryllium use at the NTS.

**Methods:** A beryllium Needs Assessment was conducted prior to organizing the medical screening program. The nature and extent of beryllium exposure at NTS was derived from several key sources: contractor industrial hygiene data spanning the years 1954-1992; DOE OpenNet citations, obtained online, from DOE-Nevada, and Los Alamos National Laboratories (LANL); interviews with knowledgeable individuals conducted both in person and by telephone; and focus groups conducted with former NTS employees in Las Vegas. Based on the Needs Assessment, all beryllium eligible individuals had a Be-LPT, CXR, and spirometry, in addition to their regular screening tests.

**Results:** Approximately 1000 former NTS workers have been screened for beryllium-related disease by the BUSPH program. The NTS contractor has screened an additional several hundred. Presently there are approximately 25 double positive BeLPT's and three CBD cases in this population. The BeLPT data will be presented by personal, work, and job-task risk factors. The BeLPT predictive value of self-reported job tasks will be presented. Finally, job characteristics of the CBD cases will be discussed.

**Conclusions:** There are multiple sources of beryllium exposure for NTS BeS and CBD cases that corresponds to the NTS historic legacy of its beryllium experience. Current and former NTS workers may be at risk from activity in "cleaned" beryllium locations or in areas in which beryllium exposure was not considered a risk. The NTS experience requires that we reexamine cleanup policies in known former beryllium use facilities. How clean is clean? Some potential explanations for the beryllium outcomes at NTS suggest that tracking and take-home exposure constitutes a real risk to families and friends. Should there be a screening effort directed toward them?

## **Basic Molecular and Genetic Mechanisms**

### **Genetic Factors and Granulomatous Inflammation: Lessons from and for Sarcoidosis**

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**Ron du Bois**

Royal Brompton Hospital &  
Imperial College of Science, Technology & Medicine

“Sarcoidosis is a systemic granulomatous disease of unknown cause...” is the opening phrase of a majority of manuscripts and reviews on sarcoidosis. This begs an important question: how do we know that it is a single disease? Before granulomatous disease in response to beryllium was identified, patients would have been diagnosed as having sarcoidosis (and in cases of a poor occupational history almost certainly still are). Certain other clinical phenotypes such as Löfgren’s syndrome and uveitis also stand out as being different.

With our current approaches to precision high resolution genetics allied to increasingly precise phenotyping, our suspicions that these might be distinct diseases have been sustained. Different patterns of disease and severity of disease are associated with individual genetic predisposing factors. This “dissection” of clinically and now clinically/genetically defined subsets should re-invigorate our approach to the identification of triggers for “sarcoidosis” subsets: a sort or reverse genetics epidemiology strategy. In berylliosis, where the trigger is known, the key questions relate to genetic subsetting that may unravel genetic factors that determine varying disease pattern and outcome in response to different exposures.

This talk will focus on sarcoidosis but also reflect on lessons learned from studies in each disease that complement the other.

## Basic Molecular and Genetic Mechanisms

### **Animal Models of Granulomatous Inflammation.**

**Terry Gordon**

NYU School of Medicine, Tuxedo, NY.

Occupational and environmental exposure to beryllium is associated with severe respiratory diseases such as acute chemical pneumonitis, chronic beryllium disease (CBD), and cancer. A considerable amount of research has examined the pathogenesis of CBD and clearly established that genetics play an important role and a cell-mediated immune response is the underlying cause. The extrapolation of data from animal models to human health is always difficult, yet genetic researchers have strong evidence that animals can be similar to humans in terms of both genomics and functionality. Animal experiments have demonstrated that beryllium-induced lung granulomas occur in strain 2, but not strain 13, guinea pigs and in strain A, but not BALB/c or C57BL/6, mice. A small study with 4 strains of mice has also observed inter-strain differences in the immune response to repeated beryllium inhalation and we have confirmed these inter-strain differences in a pilot study with 5 strains of mice treated with beryllium. Because guinea pigs and mice are genetically homogeneous within a strain (99+% for inbred mice), these observed differences in inter-strain response are likely due to genetic differences. In two of these studies, the murine strains were known to differ at the major histocompatibility complex (MHC), suggesting a role for the immune system as has been demonstrated in human patients with CBD.

The prominent role of the immune system in CBD has been confirmed in a number of animal and human studies and the contribution of multiple genes to granuloma induction in the mammalian lung has been demonstrated for other immunotoxicants. In these experimental studies, genes within the H-2 locus (the MHC equivalent in mice) were linked to the induction of granulomas in the mouse lung. Mice with an H-2<sup>k</sup> haplotype responded strongly to the interstitial lung disease (hypersensitivity pneumonitis) produced by pigeon dropping extract, while mice with an H-2<sup>b</sup> haplotype had only a mild inflammatory response after repeated challenge. Although these results suggested that the H-2 haplotype controls the murine response to the pigeon dropping extract, additional work in mice has suggested that the induction of granulomas is multifactorial with a variety of immune and non-immune genes contributing to the susceptibility. Thus, the interaction of the genetic and immune factors that drive the granulomatous response in animal models is complex in nature.

## *Basic Molecular and Genetic Mechanisms*

### **T Cell Recognition of the Beryllium-MHC Complex.**

**Andrew P. Fontenot.**

University of Colorado Health Sciences Center, Denver, CO 80262.

Chronic beryllium disease (CBD) is caused by beryllium exposure in the workplace and is characterized by the accumulation of beryllium-specific CD4<sup>+</sup> T cells and granulomatous inflammation in the lung. Evidence suggests that these CD4<sup>+</sup> T cells are intimately involved in the development of beryllium-induced disease. For example, large numbers of effector memory beryllium-specific CD4<sup>+</sup> T cells are compartmentalized to the lungs of the patients and produce Th1-type cytokines such as interleukin-2 (IL-2), interferon- $\gamma$  (IFN- $\gamma$ ), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) upon beryllium exposure in culture. Conversely, <1 in 500 peripheral blood CD4<sup>+</sup> T cells release Th1-type cytokines after beryllium exposure in culture. Increased disease susceptibility has been linked to particular HLA-DP alleles, especially those possessing negatively charged residues at certain positions of the peptide-binding pocket. The mechanism for this disease association lies in the ability of certain HLA-DP molecules, with associated peptides, to bind and present beryllium to pathogenic CD4<sup>+</sup> T cells. Despite the recent advances in our understanding of the immunopathogenesis of CBD, a number of important questions remain unanswered. It remains unclear why HLA-DP molecules are so important in beryllium presentation and how beryllium interacts with the MHC/peptide complex that is subsequently recognized by the T cell receptor. Answers to these questions may allow prevention of disease development in susceptible individuals as well as new therapeutic strategies to prevent progression to end-stage lung disease.

## Basic Molecular and Genetic Mechanisms

### **Cytokine Gene Activation in Chronic Beryllium Disease.**

#### **Tristan S. Barnes**

The Robert H. Hollis Laboratory of Environmental and Occupational Health Sciences,  
Division of Environmental and Occupational Health Sciences, Department of Medicine,  
National Jewish Medical and Research Center, Denver, CO 80206

In this presentation we will examine newer studies that focus on the molecular mechanisms by which beryllium (Be) up-regulates TNF $\alpha$  and IFN $\gamma$  gene expression in chronic beryllium disease (CBD).

Be-stimulation of CBD BAL cells has been shown to activate the expression of genes that encode pro-inflammatory cytokines such as TNF $\alpha$  and IFN $\gamma$ . High levels of these cytokines are found in Be-stimulated cell cultures from blood and BAL. High levels of Be-stimulated TNF $\alpha$  in BAL have been associated with increased disease severity. IFN $\gamma$  is known to up-regulate the expression of both macrophage and lymphocyte genes during inflammation and immune response.

The TNF $\alpha$  gene consists of four exons, three introns, a promoter and a 3'-UTR. Transcriptional activation of the promoter results in a pre-mRNA that is spliced to remove introns and the resulting mature mRNA is translated into protein. While the composition and processing of the IFN $\gamma$  gene is much the same, the promoter regions of the two genes are known to contain binding sites for different protein transcription factors.

Studies support models of Be-induced TNF $\alpha$  production that are both dependent and independent of MHC Class II:TCR complex formation. TNF $\alpha$  production by peripheral blood mononuclear cells is HLA-DP-unrestricted, and H36.12j cells, a mouse macrophage hybrid line that lacks HLA-DP expression, up-regulates TNF $\alpha$  expression after Be-exposure. In comparison, studies show that CBD BAL cells up-regulate TNF $\alpha$  expression in a MHC Class II:TCR restricted manner. A recent study using inhibitors of TNF $\alpha$  gene transcription and mRNA splicing suggest that CBD BAL cell TCR activation results in the production of TNF $\alpha$  mRNA and protein in a transcription-dependent manner.

The TNF $\alpha$  gene promoter region contains binding sites for the transcription factors: AP-1, AP-2, CREB, Egr-1, Ets, NFAT, NF-kB and Sp-1. Alternatively, the IFN $\gamma$  gene promoter contains binding sites for the transcription factors: AP-1, AP-2, ATF-1, ATF-2, CREB, NFAT, NF-kB, Oct-1, Sp-1, STAT, and YY1. We recently examined the patterns of transcription factor expression in isolated CBD macrophages and lymphocytes. Electrophoretic mobility shift assay (EMSA) and supershift analysis point to an interesting association between Be-stimulated CBD TNF $\alpha$  and IFN $\gamma$  gene activation and a Be-specific up-regulation in members of the AP-1 transcription family (fos/jun), NF-kB, STAT family members and Yin Yang-1. These data are consistent with multiple mechanisms of Be-dependent cytokine regulation in CBD. (Supported by ES-06538, ES-11810, HL03887 and RR00051 from the National Institutes of Health).

## Basic Molecular and Genetic Mechanisms

### **Taming Inflammatory Mechanisms in the Lung: the PPAR gamma Regulatory Pathway.**

**B.P.Barna**<sup>1</sup>, D.A.Culver<sup>1</sup>, B. Raychaudhuri<sup>1</sup>, T. L. Bonfield<sup>1</sup>, S. Abraham<sup>1</sup>, A. Malur<sup>1</sup>, N. John<sup>1</sup>, C. F. Farver<sup>1,2</sup>, M. S. Kavuru<sup>1</sup>, M.J.Thomassen<sup>1,3</sup>.

Departments of <sup>1</sup>Pulmonary and Critical Care Medicine, <sup>2</sup>Anatomic Pathology, and <sup>3</sup>Cell Biology, Cleveland Clinic Foundation, Cleveland, OH 44195.

**The PPAR family of Transcription Factors.** Knowledge of inflammatory mechanisms in the lung has expanded rapidly yet relatively little is known about homeostatic anti-inflammatory pathways. Recent studies in experimental animals and human disease have revealed that a family of ligand-dependent nuclear transcription factors, the peroxisome proliferator-activated receptors (PPARs) represents a surprising and potent source of anti-inflammatory regulators. PPARs were originally identified as regulators of adipocyte differentiation, lipid and glucose metabolism and comprise three isoforms, PPAR $\alpha$ , PPAR $\gamma$ , and PPAR $\delta$ , each encoded by a separate gene. Two PPAR $\gamma$  isoforms have been found: PPAR $\gamma$ 1, which is widely expressed throughout the body, and PPAR $\gamma$ 2 which is highly expressed in adipose tissue. In the inactivated state, PPARs are complexed to corepressor proteins. Ligand-activated PPARs dissociate from corepressors to form heterodimers with retinoid X receptors (RXR) and via altered conformation, bind transcriptional coactivators. The complex allows the PPAR to bind to specific peroxisome proliferator response elements (PPREs) within the promoter regions of target genes. PPAR $\gamma$  not only transactivates target genes in an agonist-dependent fashion but via transrepression mechanisms, negatively regulates other transcription factors such as NF- $\kappa$ B, AP-1, NFAT, and STAT-1 by direct binding, competition for coactivators, or inhibition of MAP kinase activation.

Total deletion of the PPAR $\gamma$  gene results in embryonic lethality but the development of conditional PPAR $\gamma$  gene knockout mice has aided PPAR investigation. Naturally occurring PPAR $\gamma$  ligands include arachidonic acid metabolites derived from cyclooxygenase and lipoxygenase pathways, such as 15-deoxy-delta-12,14-prostaglandin J2 (15d-PGJ<sub>2</sub>), and 13-hydroxyoctadecadienoic acid (13-HODE). Phospholipase A1 action on oxidized LDL also yields a natural ligand, hexadecyl azelaoyl phosphatidylcholine (asPC). 15d-PGJ<sub>2</sub> is not highly specific, however, and elicits both PPAR $\gamma$ -dependent and independent responses. Thiazolidinediones (TZDs) are synthetic PPAR $\gamma$  ligands which are in clinical use for treating diabetes (e.g. rosiglitazone {"Avandia"}). Beneficial effects of TZDs have also been reported in experimental autoimmune encephalomyelitis, atherosclerosis, inflammatory bowel disease, experimental arthritis, and asthma.

**PPAR $\gamma$  and Immune Cells.** PPAR $\gamma$  is weakly expressed in monocytes but intensifies during differentiation to macrophages. Cytokines such as IL-4, GM-CSF, and M-CSF which promote macrophage differentiation also enhance monocyte expression of PPAR $\gamma$ . Several pro-inflammatory macrophage target genes including TNF $\alpha$ , IL-6, and IL-12, are

### **Taming Inflammatory Mechanisms in the Lung: the PPAR gamma Regulatory Pathway. (continued)**

down-regulated by PPAR $\gamma$ . PPAR $\gamma$  agonists upregulate expression of CD-36, a lipid scavenger receptor which regulates lipid uptake and metabolism. In quiescent T lymphocytes, PPAR $\gamma$  is weakly expressed but increases with mitogenic stimulation. IL-4 indirectly suppresses IL-2-mediated T-cell proliferation by upregulating macrophage production of the endogenous PPAR $\gamma$  ligand, 13-HODE, which when secreted, can interact with T cell PPAR $\gamma$ . Activation of PPAR $\gamma$  in T cells blocks proliferation, binding of NFAT and NF- $\kappa$ B transcription factors, and production of IFN $\gamma$ .

**PPAR $\gamma$  and Lung Disease.** PPAR $\gamma$  is constitutively expressed and functionally active in alveolar macrophages of healthy individuals (Bonfield et al). By immunohistochemistry, PPAR $\gamma$  has been reported to be increased in bronchial submucosa, airway epithelium and smooth muscle of asthmatics, and to correlate negatively with lung function (Benayoun et al). Pulmonary alveolar proteinosis is characterized by a severe PPAR $\gamma$  deficiency that can be reversed by GM-CSF therapy (Bonfield et al). Reduced PPAR $\gamma$  has also been reported in vascular lesions of patients with pulmonary hypertension (Ameshim et al). Our studies have been the first to address the question of PPAR $\gamma$  in granulomatous lung disease. In pulmonary sarcoidosis, PPAR $\gamma$  mRNA expression and functional activity are deficient in alveolar macrophages while, in contrast, NF- $\kappa$ B activity is strikingly elevated, suggesting that insufficient PPAR $\gamma$  activity may perpetuate chronic inflammatory injury by failing to repress NF- $\kappa$ B (Culver et al). Chronic Beryllium Disease (CBD), like sarcoidosis, is characterized by complex interactions between alveolar macrophages and T lymphocytes leading to granuloma formation and variable degrees of fibrosis. Preliminary studies in CBD also suggest decreased PPAR $\gamma$  expression in alveolar macrophages that is inversely related to production of IFN $\gamma$ . Although mechanisms remain to be elucidated, ongoing studies in patients and in the THP-1 myeloid cell line suggest that IFN $\gamma$ , a hallmark cytokine of both sarcoidosis and CBD, antagonizes PPAR $\gamma$  mRNA expression, activation and phosphorylation. These data suggest that understanding the role of PPAR $\gamma$  in inflammatory granulomatous lung disease may have implications for both diagnosis and treatment.

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## Basic Molecular and Genetic Mechanisms

### **Oxidative Stress and Beryllium**

#### **Brian J. Day**

Division of Environmental and Occupational Health Sciences, Department of Medicine, National Jewish Medical & Research Center, Departments of Medicine and Pharmaceutical Sciences, University of Colorado Health Sciences Center, Denver, CO

Chronic beryllium disease (CBD) is an inflammatory hypersensitivity lung disease that continues to occur in 10% of the estimated 800,000 beryllium-exposed workers in the United States and is characterized by the presence of non-caseating granulomas with accumulation of macrophages and beryllium specific CD4<sup>+</sup> T lymphocytes. Upon beryllium stimulation *in vitro*, these CD4<sup>+</sup> T cells proliferate and produce Th1 cytokines (i.e. TNF- $\alpha$ , IFN- $\gamma$ , and IL-2) at unusually high levels. Oxidative stress is defined as an imbalance between the production of reactive oxygen species and the cell's antioxidant capacity to scavenge them. Currently, very little is known about oxidative stress in CBD and whether oxidative stress is a cause or consequence of CBD. However, oxidative stress has been implicated in other immunologic disorders involving antigen presentation, such as T-cell mediated diabetes. Current research suggests that antioxidants can modulate MHC class II responses such as Th-1 cytokine production and T cell proliferation. It is proposed that inherent differences in either resting antigen presenting cell's antioxidant status or oxidant response to beryllium are critical factors in determining whether people exposed to beryllium go on to develop CBD. This area of research has the potential to further define the etiology of CBD, risk factors, and suggest novel approaches to prevent and treat this disease.

(Supported by ES-012504 from the National Institutes of Health).

## *Basic Molecular and Genetic Mechanisms*

### **Overview of Innate and Acquired Immunity in CBD**

#### **Richard T. Sawyer**

The Robert H. Hollis Laboratory of Environmental and Occupational Health Science, Department of Medicine and Division of Environmental and Occupational Health Sciences, National Jewish Medical and Research Center, Division of Pulmonary Science and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO 80206.

The objective of this talk is to summarize what is known about the host response to beryllium (Be), and to highlight gaps in our knowledge and understanding of the pathogenesis of CBD. During the initial encounter between the host and antigen, the innate immune system provides barriers that minimize antigen exposure and that promote inflammatory processes to eliminate antigen. If antigen escapes the innate immune system, it encounters the acquired immune system and may elicit responses that serve to both eliminate antigen and generate specific, acquired, immunologic memory of antigen exposure. This, in turn, primes the host to generate secondary immune responses to subsequent antigen exposure. With the use of Be in industrial settings, a number of health effects are noted including acute pneumonitis, sensitization to Be, interstitial lung disease and dermatological disease. In some Be exposed workers, this initial encounter can result in elimination of Be. Yet, the lungs of Be exposed workers often contain detectable Be levels, even decades after the last known Be exposure has ceased. Studies show that Be damages the lung epithelial barrier and elicits lung inflammation that can include the emigration of immunocompetent cells to the site of Be exposure. Persistent Be is able to elicit a delayed-type hypersensitivity immune response in the lungs that is restricted by major histocompatibility (MHC) class II molecules and generates Be-antigen specific CD4<sup>+</sup> effector-memory T cells that express the Th1 phenotype. Be antigen triggers the proliferation of these CD4<sup>+</sup> T cells and amplifies production of pro-inflammatory cytokines, resulting in pulmonary interstitial mononuclear cell infiltration and formation of the non-caseating granulomas seen in chronic beryllium disease. Much has been learned about the important role for MHC class II restriction in this disease, the mechanisms of Be-induced pro-inflammatory cytokine gene activation, the structure of antigen-specific responder T cell receptors, and the relationship between an individual worker's genetic background and progression from Be sensitization to CBD. There remain significant gaps in our understanding of the pathogenesis of CBD. (Supported by ES-06538, ES-11810, HL03887 and RR00051 from the National Institutes of Health).

## ***Population Based Genetic Studies and Gene and Environmental Interactions***

### **Gene-environment interactions in occupational lung diseases and applications to CBD**

**Anthony Newman Taylor**

Royal Brompton Hospital &  
Imperial College of Science, Technology & Medicine

The dominant occupational lung diseases of the 19<sup>th</sup> and of much of the 20<sup>th</sup> century were the mineral dust pneumoconioses – pulmonary fibrosis caused by inorganic dusts, retained in the lungs, such as silica, coal and asbestos. The primary determinant of risk of fibrogenesis was cumulative exposure, usually estimated as the product of intensity and duration of exposure. The role, if any, of individual susceptibility was considered small.

For occupational asthma, now the most frequently reported occupational lung disease in UK and W. Europe caused by contemporary exposures at work, the opposite has been the case. In UK an estimated 1500 new cases of occupational asthma are reported annually to a national reporting scheme (SWORD). The majority are the outcome of an acquired hypersensitivity response to proteins (generally IgE associated) such as enzymes (in detergent industry and bakeries) and urinary proteins of rats and mice (in pharmaceutical and university laboratories) and also low molecular weight chemicals (associated with IgE for a minority of causes) such as, platinum salts, acid anhydrides and isocyanates. Much research into occupational asthma has focussed on the contribution of individual susceptibility; indeed it is only in the past decade that studies have been undertaken which have provided consistent evidence of exposure-response relationships for asthma caused by several of these agents.

The host factors identified as determinants for some of the causes of occupational asthma are atopy, cigarette smoking and HLA phenotype. The risk of development of IgE and asthma is increased in atopic individuals for several protein causes of occupational asthma, including laboratory animal urine proteins, latex and enzymes. There is limited evidence that the risk of developing laboratory animal allergy is greater in atopics than non-atopics at lower levels of exposure. Cigarette smoking has been found to increase the risk of sensitisation to some low MW chemicals, such as acid anhydrides and platinum salts, but not others such as isocyanates. A study of platinum refinery workers suggested that smoking and exposure interacted to increase the risk of sensitisation to platinum salts.

## **Gene-environment interactions in occupational lung diseases and applications to CBD (continued)**

Studies of HLA phenotype have found significant associations between IgE production and asthma caused by the acid anhydride trimellitic anhydride (TMA) with HLA DR3, the complex platinum salt, ammonium hexachloroplatinate (AHP) positively with HLA DR3 and inversely with HLA DR6 and rat urine protein (RUP) positively with HLA DR7 and inversely with HLA DR3. The strength of association between HLA DR3 and DR6 with specific IgE to AHP was greater at lower levels of exposure to AHP; a similar variation in strength of association with HLA DR3 and specific IgE (with symptoms) to TMA was suggested in TMA workers.

To date where reported, for enzymes (in the detergent industry), latex (in health care workers) and isocyanates in Toronto, Canada, reduction in the incidence of occupational asthma has been achieved by reduction in exposure to the causal agent, rather than by the identification and exclusion of susceptible individuals. Evidence from genetic studies of platinum refinery and possibly acid anhydride workers, however, would suggest that, if generalisable, as exposure is reduced, genetic susceptibility will be increasingly important in determining the risk of sensitisation and asthma.

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***Population Based Genetic Studies and Gene and  
Environmental Interactions***

**Genetic Risk Factors for Beryllium Sensitization, Chronic Beryllium Disease, and Severity**

Lisa A. Maier, MD MSPH

The low frequency of chronic beryllium disease (CBD) and beryllium sensitization (BeS), findings of parent-child and sibling pairs with disease and animal models of disease restricted by genetic differences indicate a genetic susceptibility for CBD and BeS. It is likely that CBD and BeS are multigenetic. The majority of the studies to date have focused on the Class HLA-D genes as potential risk factors. In a study, Richeldi *et al* reported an increased prevalence of HLA DPB1 with a glutamic acid at amino acid position 69(Glu69) in cases of CBD (97%) compared to Beryllium-exposed non-diseased (Be-non-diseased) controls (30%). These results were later confirmed by additional studies, although the frequency of the Glu69 variant was slightly lower. Three of four more recent studies indicate that Glu69 is not specific for CBD, but instead is a marker of sensitization, as subjects with BeS have a similar prevalence of Glu69 as do those with CBD. Other aspects of the Glu69 gene, such as the presence of non-0201 alleles and homozygosity have been evaluated with mixed results. There is mounting evidence that the non-0201 alleles are expressed at higher levels in cases of CBD and possibly BeS. This has potential implications for antigen binding. A recent study by our group revealed that Glu69 is associated with markers of disease severity, including a lower FVC, DLCO, PaO<sub>2</sub> at rest, maximum workload on exercise testing, and a higher actual-alveolar gradient at rest, confirming the functional significance of this gene. Despite the increased frequency of Glu69 in BeS and CBD subjects, this marker has low positive predictive value for disease.

***Population Based Genetic Studies and Gene and  
Environmental Interactions***

**Recent Studies of Genetic Susceptibility Factors in CBD**

**Cesare Saltini, Massimo Amicosante, Floriana Berretta**

University of Roma "Tor Vergata"

Via Montpellier 1, Roma, Italy, 00141

Email: saltini@med.uniroma2.it

Susceptibility to berylliosis has been associated with the supratypic variant HLA-DPbGlu69. To define role of the HLA-DP gene coding for glutamate at position beta69 soluble HLA-DP molecules carrying either Glu69 or Lys69 were produced in *Drosophila melanogaster* for structural analysis.

Using molecular modeling we have predicted a detailed structure of the HLA-DP2 molecule carrying Glu69 complexed with CLIP and compared it with a mutated form carrying Lys at b69 demonstrating major differences in the shape and charge distribution of pocket 4 and of the nearby pocket 6. Pocket 4 of HLA-DPGlu69 showed high affinity for positively-charged, aromatic, and polar residues while aliphatic residues were disfavored. Pocket 4 of the HLA-DPLys69 mutant showed a reduced aminoacid selectivity with aromatic residues most preferred. Pocket 6 of HLA-DP2 showed high affinity for aromatic residues, which was increased in the DP2K69 mutant and extended to arginine. Thus, the HLA-DPb69 polymorphism plays a fundamental role in the peptide binding selectivity of HLA-DP. Furthermore, as the Lys to Glu substitution represents the major polymorphic change in the pocket 4 area of HLA-DP, it could represent a supertype among HLA-DP molecules significantly contributing to the selection of epitopes presented in the context of this HLA isotype.

Be binding assays using the invariant chain-derived peptide CLIP as competitor were carried out to assess the role of Glu69 in beryllium binding. BeSO<sub>4</sub> was capable of displacing CLIP from the HLA-DP2Glu69 (IC<sub>50</sub>%; 4.5mM of BeSO<sub>4</sub> at pH 5.0 and 5.5 mM of BeSO<sub>4</sub> at pH 7.5) but not from the HLA-DP2Lys69 molecule (IC<sub>50</sub>%; 480 mM of BeSO<sub>4</sub> at pH5.0 and 220 mM of BeSO<sub>4</sub> at pH 7.5) demonstrating a direct interaction of Be with the HLA-DPGlu69 molecule, in the absence of antigen processing.

Molecular modelling was used to define an interaction model between beryllium and HLA-DP molecule. To this end, beryllium has been modelled together with HLA-DP in all the HLA-DP polymorphic positions resulted associated with beryllium-hypersensitivity, including His9, Leu11, Val36, Asp55, Glu56, Glu69 and Ile76. The co-presence of Gln13, Glu14, Arg27 and Tyr28 together with Glu69 determines a particular electron-donor rich micro-environment in the pocket 4 of the HLA-DP molecules within unique conditions for beryllium coordination. In fact, when Be is modelled in pocket 4 of the HLA-DP molecule, close to Glu69, the internal free energy of the HLA-DP/Be complex is the one presenting the highest stability respect to the internal free energies obtained when Be is positioned close to the other HLA-DP polymorphic positions

## **Recent Studies of Genetic Susceptibility Factors in CBD (continued)**

associated with Be-hypersensitivity. Furthermore, the presence of Be in pocket 4 of the HLA-DP determines critical changes in the HLA-DP structure suggesting that these modifications themselves may be responsible for the activation of Be induced T-cell stimulation mediated on HLA-DP. Finally, this analysis suggests that, as the internal free energy obtained with Be in pocket 4 is lower than that of some peptides presenting medium-low affinity for HLA-DP, such as CLIP, but is higher than that of HLA-DP high affinity peptides, it may be possible that certain peptides could be used as competitors in Be-stimulated T-cell activation.

***Population Based Genetic Studies and Gene and  
Environmental Interactions***

**Ethical Considerations in Beryllium Research and Genetic Testing**

**Richard Sharp**

*Abstract available at symposium*

ELSI Program - Human Genome - James Watson

Workplace Testing

- Model Genetic Privacy Act

- Executive Order applicable to federal employees (Clinton 10-2000)

Case 1 Burlington Northern

Feb 12, 2001

Case 2 Chronic Beryllium Disease

BW

## **Conference Summary: Research Directions in Beryllium Disease**

**Lee S. Newman, M.D., M.A., FCCP**

Head, Division of Environmental and Occupational Health Sciences, and Professor of  
Medicine and Preventive Medicine

National Jewish Medical and Research Center

This conference summary will emphasize the new discoveries being made by researchers investigating beryllium's health effects. I will highlight promising directions for future research that emerge from this information exchange. New information was presented at this conference concerning: exposures, host factors, genetics, immunopathogenesis, immunotoxicology, utility of animal models, lessons from medical surveillance and from other workplace interventions, and future directions for possible therapeutic interventions. We thank all of the participants for contributing to the ultimate goal: prevention and eradication of beryllium-related disorders.

## **Submitted Abstracts: In order of Presentation**

**Thursday, January 29<sup>th</sup>:**

### **Beryllium Chemical Speciation in Elemental Human Biological Fluids**

**M. Sutton, S. R. Burastero**

**Abstract:** The understanding of beryllium chemistry in human body fluids is important for understanding the prevention and treatment of chronic beryllium disease. Thermodynamic modeling has traditionally been used to study environmental contaminant migration and rarely in the examination of metal (particularly beryllium) toxicology. In this work, a chemical thermodynamic speciation code (MINTEQA2) has been used to model and understand the chemistry of beryllium in simulated human biological fluids such as intracellular, interstitial, and plasma fluids, a number of airway surface fluids for patients with lung conditions, saliva, sweat, urine, bile, gastric juice, and pancreatic fluid. The results show that predicted beryllium solubility and speciation vary markedly between each simulated biological fluid. Formation of beryllium hydroxide and/or phosphate was observed in most of the modeled fluids, and results support the postulation that beryllium absorption in the gastrointestinal tract may be limited by the formation of beryllium phosphate solids. It is also postulated that beryllium is potentially 13% less soluble in the airway surface fluid of a patient with asthma when compared to a "normal" case. The results of this work, supported by experimental validation, can aid in the understanding of beryllium toxicology. Our results can potentially be applied to assessing the feasibility of biological monitoring or chelation treatment of beryllium body burden.

This work was performed under the auspices of the U.S. Department of Energy by the University of California Lawrence Livermore National Laboratory under contract No. W-7405-Eng-48. UCRL-JC-150385. Published in Chem. Res. Toxicol. 2003, 16, 1145-1154.

## **Pilot Study of Beryllium-Specific Chelators in Mice**

**M. Sutton, M. L. Chiarappa-Zucca, S. C. Szechenyi, J. Perkins, S. R. Burastero**

**Abstract:** It has been documented that many Chronic Beryllium Disease (CBD) patients have evidence of Be in their parenchymal lung tissue [1]. It has also been suggested that the presence of retained Be in the lung may enhance the progression of CBD due to the slow dissolution of beryllium particles trapped in the lungs and the subsequent increased immunologic activity, lymphocytic infiltration and granuloma formation. This may help explain an increase in severity and prevalence of CBD in higher exposure environments [2]. In theory, if selective and effective chelators were able to remove retained beryllium in individuals with high body burden, the immunologic challenge would be lessened and the risk of CBD progression could be reduced. The use of metal chelators to remove a toxic body burden is a common medical practice. Traditionally, the development of new chelators has largely been empirical and nonspecific, leading to side effects such as the depletion of the body's essential minerals.

Modern chemical thermodynamic and molecular modeling and laboratory experiments have led to a better understanding of the chemistry of metal-specific chelators and has potential application for the development of more selective and effective chelators [3]. One such chelator, a diphosphonic acid derivative, has shown to be a promising candidate for pilot investigation. In this work, we administered an effective and selective beryllium chelator to mice that were exposed to beryllium (1.5 ug Be / 200 ul, intra-peritoneal, 30 g mouse). Some of the mice were administered with a previously studied chelator as a reference, while other mice were administered with a diphosphonic acid chelator at concentrations capable of potentially chelating the entire beryllium dose. Urine and feces samples were taken at 24 and 48 hours, and organs were excised after 48 hours. Samples were digested in concentrated nitric acid, diluted and analyzed for beryllium by inductively coupled mass spectrometry (ICP-MS). No toxicology data is available for the diphosphonic acid and LD-50 values could only be estimated by comparing known chemical analogues. Our primary experimental goal was to assure the absence of detrimental effects in the mouse resulting from chelation therapy.

No detrimental effects were observed in any mice within the scope of the experiment up to 48 hours after chelation. In addition, the results indicate that overall beryllium urinary excretion from the mouse was increased, and the beryllium burden in the liver, spleen and plasma was reduced on administering a single dose of diphosphonic acid chelation therapy. Future studies will include varying beryllium administering methods, additional chelator-metal combinations, different chelation dosing schedules, larger sample sizes and greater tissue/organ sampling.

## **Pilot Study of Beryllium-Specific Chelators in Mice (continued)**

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## **Mutagenesis, Comutagenesis and Proteins Affected by Beryllium: a Bacterial Mutation Assay System**

**Kirsten J. Taylor-McCabe, Zaolin Wang, Nancy N. Sauer and Babetta L. Marrone**

**Abstract:** Inhalation of beryllium dust or fumes is well known to produce human toxicity. The most widely studied effect of beryllium on human health is Chronic Beryllium Disease (CBD), a granulomatous lung disease. Beryllium also appears to be a human lung carcinogen. However, the effects of beryllium as measured in various mutagenicity assays have been contradictory. In this study, the mutagenicity of beryllium sulfate and beryllium chloride and the comutagenicity with a known mutagen (1-methyl-3-nitro-1-nitrosoguanidine, MNNG) were studied using a novel forward mutation detecting system developed in *E.coli*. Using this detection system, both beryllium compounds showed a weak mutagenicity at all dose points but no clear dose response relationship was observed. Both compounds were found to enhance the mutagenicity of MNNG up to 3.5X over MNNG alone. In a parallel study, proteomics was been used to provide insight into biochemical pathways involved in beryllium toxicity. 2D electrophoreses and PDQuest techniques were used to separate and analyze proteins in untreated versus MNNG treated versus MNNG and beryllium sulphate co-treated *E.coli* cells. Proteins showing differential expression were identified by MS-MALDI-TOF and ProFound software. The proteins identified help understand the mechanism of beryllium action as a co-mutagen and its effects on cellular mechanisms in general. The data collected can provide a starting point for further, more detailed investigation of mammalian proteins regulated by beryllium. Many of the identified proteins have human homologs, which will be studied to discern if they are involved in the human immune response to CBD and/or beryllium's carcinogenic effects in humans.

Supported by a Laboratory Directed Research and Development grant.

## **Beryllium mediated immune response in primary cells from healthy individuals**

**Anu Chaudhary, Nancy N. Sauer, Goutam Gupta**

**Abstract:** The effect of beryllium (Be) exposure has been extensively studied in patients with chronic beryllium disease (CBD). CBD patients carry mutated MHC class II alleles and show a hyperproliferation of T cells upon Be exposure. The exact mechanism of Be-induced T cell proliferation in these patients is not clearly understood. It is also not known how the inflammatory and suppressive cytokines maintain a balance in healthy individuals and how this balance is lost in CBD patients.

To address these issues, we have initiated cellular and biochemical studies to identify Be-responsive cytokines and other cellular markers that help maintain a balance in healthy individuals. We have established an immune cell model derived from a mixture of peripheral blood mononuclear cells (PBMC) and dendritic cells (DC). In this article, we demonstrate that pro-inflammatory cytokine IL6 shows decreased release whereas suppressive cytokine IL10 shows enhanced release after 5-10 hours of BeSO<sub>4</sub> treatment. Furthermore, the Be-specific pattern of IL6 and IL10 release is dependent upon induction of threonine phosphorylation of a 45 kDa cytosolic protein (p45), as early as 90 minutes after Be treatment. Pharmacological inhibition of phosphatidylinositol 3' kinase (PI3'K) by wortmannin and p38 mitogen activated protein kinase (MAPK) by SB203580 reveal that PI3'K mediates Be-specific p45 phosphorylation and IL6 release, whereas p38 MAPK regulates the release of IL6 and IL10 and the phosphorylation of p45 independent of metal-salt treatment. While the IL10 and IL6 release pathways are uncoupled in these cells, they are linked to phosphorylation of p45. These findings suggest that the balance between IL10 and IL6 release and the correlated p45 phosphorylation are important components of the Be-mediated immune response in healthy individuals. Furthermore, while these studies have been performed using soluble Be salts, preliminary data on the cellular effects of Be oxide particulates using this cell model will also be presented.

## **Regulation of chemokine expression in beryllium-stimulated human PBMCs and dendritic cells**

**Elizabeth Hong-Geller (1), Paige E. Pardington (1), Robert L. Cary (1), Nancy Sauer (2), and Goutam Gupta (1),**  
Biosciences (1) and Chemistry (2) Divisions, Los Alamos National Laboratory, Los Alamos, NM

**Abstract:** Beryllium exposure elicits a delayed-type hypersensitivity reaction in susceptible individuals and is thought to mediate a specific immune response by promoting the migration of blood lymphocytes to the lung, leading to the onset of lung inflammation and chronic beryllium disease (CBD). To further investigate beryllium effects in lymphocytes, we have performed microarray analyses to look for genes that are transcriptionally regulated in response to beryllium treatment in a normal human cell model of peripheral blood mononuclear cells and dendritic cells. In cells treated with 100mM BeSO<sub>4</sub> for 10 hrs, we identified a number of upregulated chemokines, including GRO $\alpha$ , GRO $\gamma$ , MIP1 $\alpha$ , MIP1 $\beta$ , RANTES and the inflammatory cytokine IL-1 $\beta$ , compared to cells treated with Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>. Chemokines are chemotactic cytokines that are secreted to set up a gradient to attract migratory T lymphocytes to sites of inflammation. Using RT-PCR, we show that the chemokine mRNA levels in Be-stimulated cells generally begin to increase compared to Al-stimulated cells within 3-6 hrs. GRO $\alpha$  and IL-1 $\beta$  were also released at higher levels in Be-stimulated cells compared to Al-stimulated cells, as measured by ELISA. Interestingly, the mRNA and released protein levels in Be-stimulated cells for some of the chemokines are decreased compared to unstimulated cells, suggesting that beryllium treatment may also have inhibitory effects. Modulation of chemokine expression appears to be a general effect in response to beryllium, since we were able to detect changes in chemokine expression in two additional donor PBMCs and dendritic cell systems. However, the pattern and extent of chemokine release differed between the three donor cells, indicating donor variability in the cellular response to beryllium. We also demonstrate modulation of AML1 and Sp1 transcription factor binding to MIP1 $\alpha$  and GRO $\alpha$  enhancer elements, respectively, using EMSAs, indicating that beryllium activates nuclear signaling. Finally, we are examining the role of another upregulated gene from our microarray analysis, CD44, a cell surface receptor that has been implicated in mediating leukocyte rolling towards inflamed tissues. Taken together, these results suggest that beryllium-stimulated PBMC/DCs exhibit a complex innate immune response and may synergize chemokine and CD44 function to direct immune activity at sites of inflammation.

## **TNF- $\alpha$ Production by Chronic Beryllium Disease CD4+ Lung T Cells is Controlled by a Transcription- and T Cell Receptor-Dependent Process**

**Parsons CE, Sawyer RT, Maier LA, Fontenot AP, Gillespie MM, Gottschall EB, Newman LS**

**Abstract:** Beryllium presentation to CD4+ T cells from patients with chronic beryllium disease (CBD) results in T cell proliferation and production of Th-1 type cytokines. Genetic susceptibility to this granulomatous disorder is associated with particular HLA-DP alleles. Hypothesis: We tested the hypothesis that Be-antigen presentation via HLA-DP resulted in the up-regulation of TNF- $\alpha$  expression in CD4+ T cells, in a transcriptionally dependent manner. Methods: CBD bronchoalveolar lavage (BAL) CD4+ T cells up-regulated expression of TNF- $\alpha$  as determined by flow cytometry and intracellular cytokine staining. Intracellular, Be-induced CD4+ T cell TNF- $\alpha$  expression was blocked by incubation in the presence of 30 mg/ml of anti-HLA-DP B7.21, but not anti-DR, monoclonal antibody (mAb). Real-time PCR analysis was used to determine the levels of TNF- $\alpha$  pre-mRNA expression, the kinetics of TNF- $\alpha$  pre-mRNA splicing into mature-mRNA, and to compare TNF- $\alpha$  mRNA levels in unstimulated to Be-stimulated (10 mM BeSO<sub>4</sub>) CBD BAL cells from 15 subjects. As a positive control, CBD BAL cells were also stimulated with 1 mg/ml of LPS (*E. coli* 0111). Results: In comparison to the unstimulated control, Be exposure lead to higher levels of mature TNF- $\alpha$  mRNA with maximal levels observed at 24h. Incubation in the presence of pentoxifylline (PTX; 1 mg/ml), a transcription blocking agent, significantly reduced ( $p < 0.05$ ) the levels of Be-stimulated TNF- $\alpha$  pre-mRNA in both Be-exposed and LPS-stimulated CBD BAL cells. Incubation in the presence of 2-Aminopurine (2AP; 10 mM), a TNF- $\alpha$  mRNA splicing inhibitor, resulted in a significant increase ( $p < 0.05$ ) in the levels of Be-stimulated TNF- $\alpha$  pre-mRNA and a significant decrease ( $p < 0.05$ ) in the levels of TNF- $\alpha$  mature mRNA in CBD BAL cells that were Be exposed and LPS-stimulated. Conclusions: The data show that the ligation of the HLA-DP/Be-antigen to the TCR results in up-regulation of TNF- $\alpha$  production in CBD CD4+ BAL T cells, and that this increased production is the result of increased transcription of the TNF- $\alpha$  gene.

(Supported by ES-06538, ES-11810, HL03887 and RR00051 from the National Institutes of Health).

**Friday, January 30<sup>th</sup>:**

## **Opportunities for Integrated Beryllium Health and Safety Research**

David Weitzman, Department of Energy

Chronic Beryllium Disease (CBD) is caused by an immune system response to beryllium particles embedded in lungs. This response can cause progressive damage to the lungs, which, in turn, can result in disability and shortening of life. It appears that 3-5% of the population is susceptible to this disease. The United States Department of Energy (DOE) has used large quantities of beryllium since its inception as the Atomic Energy Commission and anticipates continuing to use it well into the future. Unfortunately, some workers who worked with beryllium for DOE have contracted CBD and new cases still are occurring. This presentation will provide a summary of DOE's beryllium disease experience with both former and current workers. DOE has aggressively addressed this disease but is concerned that gaps in beryllium health and safety technology may hinder further efforts.

DOE has categorized the technology gaps into Exposures, Controls, and Health Effects. The Exposures category consists of improving sampling methods and using the improved methods to better characterize exposures. Controls consist of developing innovative controls and observing the impact of lower exposures on disease rates. Health Effects consists of achieving better understanding of the disease process and improving diagnostics. Pursuing research in all three categories requires developing basic research tools and capabilities such as establishing a library of tissues samples taken from beryllium diseased individuals.

DOE has inventoried current research activities and will coordinate its efforts with other agencies and the private sector to assure that the highest priorities are addressed first and that redundancy is avoided. This presentation will present a summary of the number of contemporary research efforts and the funds expended as determined by the inventory in the three categories of technology gaps described above. It also will present current estimates of the amount of funding that will be required to support the needed research. Enlisting the participation of the research community in pursuing this research program presents challenges. Past government solicitations for research applications often were not responded to. DOE is addressing the reasons for this apparent lack of interest which it believes were primarily the lack of access to basic tools and materials needed to conduct the research and uncertainty over the stability of funding.

## Distinct TAP1 And TAP2 Polymorphisms are Associated with Chronic Beryllium Disease and Sarcoidosis

L.A. Maier\*, H. Sato¶, J. Nicholson\*, D. McGrath¶, L. Silveira\*, P. Lympany¶, K. Welsh¶, R.M du Bois¶, CS Rose\* and LS Newman\* and the ACCESS Group  
\*NJMRC, Denver, CO and ¶ICST&M, London, UK

**Background:** There is growing evidence to support a genetic predisposition to the granulomatous lung diseases chronic beryllium disease (CBD) and sarcoidosis. Since these diseases may be clinically and pathologically indistinguishable, we have hypothesized that there are genetic variants, which predispose to these and other granulomatous lung diseases.

**Objective:** To test the hypothesis that the same variants of the antigen-processing genes TAP1 and TAP2 are found at higher rates in CBD and sarcoidosis compared to controls.

**Methods:** Following informed consent, demographic information and genomic DNA were obtained from CBD subjects (n = 94). DNA was obtained from sarcoidosis cases (n=198) and matched controls (n=198) enrolled in ACCESS (A Case Control Etiologic Study of Sarcoidosis). The TAP1 variants at amino acid positions 333 and 637 and TAP2 variants at positions 379, 565, and 665, which resulted in amino acid substitutions were determined by sequence-specific primers-PCR.

**Results:** CBD cases displayed a significantly lower frequency of homozygosity for aspartic acid at TAP1 637 (53.8%) and isoleucine at TAP2 379 (52.7%) compared to the sarcoidosis cases (74.8%, p=0.002 for TAP1 637 and 68.7%, p=0.003 for TAP2 379) and controls (71.2% p=0.01 for TAP1 637 and 65.77%, p=0.02 for TAP2 379). The sarcoidosis cases demonstrated an increased frequency of homozygosity for an alanine at TAP2 565 (90.4%) compared to CBD cases (77.0%, p=0.005) and controls (80%, p=0.02). No significant differences were found in TAP1 333 and TAP2 665 between either group of cases or controls.

**Conclusions:** Variants of the antigen-processing genes TAP1 and TAP2 are significantly associated with CBD and sarcoidosis compared to controls. However, contrary to our hypothesis, there are no TAP genes shared by both of these granulomatous lung diseases. Future studies should compare other potentially common genetic variants between these two clinically similar diseases.

P01 ES011810, K08 HL03887, R01 ES06358, M01 RR00051, NHLBI HR 56066

## Chronic Beryllium Disease and Glutathione Biosynthesis Genes

Bekris, L, Viernes, H, Farin, F, Maier, L, Newman, L, Takaro, T, Kavanagh, T.

Beryllium exposure can lead to a specific cell-mediated immune response to beryllium, called beryllium sensitization. Individuals with beryllium sensitization can progress to a chronic granulomatous lung disease called chronic beryllium disease (CBD). CBD is associated with the production of inflammatory cytokines such as, TNF-alpha. The TNF-alpha -308 A variant is associated with beryllium-stimulated TNF-alpha production and more severe CBD. HLA-DPB1 Glu69 is thought to play a role in the development of the antigen-specific, cell-mediated immune response to beryllium. The immune response involved in beryllium sensitization and CBD is probably a complicated multi-genetic process. Thus, investigation into a multitude of genes with probable associations with CBD and/or immune pathology is required. CBD patients have high lung glutathione (GSH) levels and GSH levels have been associated with immune modulation. The aim of this investigation was to determine whether gene polymorphisms of glutathione biosynthesis enzymes play a role in the development of CBD. We examined associations between CBD and gene polymorphisms of the rate limiting enzyme in the synthesis of GSH, glutamate cysteine ligase (GCL). GCL consists of a catalytic subunit (GCLC) and modifier subunit (GCLM). Specifically, we genotyped the following study groups; CBD patients, beryllium sensitized subjects, subjects exposed to beryllium without beryllium sensitivity or CBD, and non-beryllium exposed control subjects (Seattle controls). Subjects were genotyped for the GCLC GAG trinucleotide repeat (GCLC TNR), the GCLC -129 C/T single nucleotide polymorphism (SNP), and the GCLM -588 C/T SNP. Results indicate that there is a significant difference between CBD patients and non-beryllium exposed control subjects (Seattle controls) for the GCLC TNR genotype 7/7 ( $\chi^2 = 6.5$ , p-value = 0.01, OR = 4.5, CI=1.36 to 14.85) that decreases slightly, but is maintained, when comparing beryllium exposed individuals that are not beryllium sensitized and do not have CBD ( $\chi^2 = 3.7$ , p-value = 0.05, OR = 3.2, CI=0.96 to 10.72). There was a slightly protective effect with the GCLM -588 C/C SNP ( $\chi^2 = 3.27$ , p-value = 0.07, OR = 0.36, CI=0.12 to 1.11). No significant association was found for any of the GCLC -129 SNP alleles or the GCLM -588 C/T or T/T SNP alleles when comparing CBD and beryllium exposed subjects or beryllium sensitized subjects. In conclusion, this study suggests that GSH may play an important role in CBD pathogenesis. Unfortunately, multi-gene investigations such as these are hindered by small study groups. Interestingly, associations with CBD were found for both GCL subunit genes suggesting that GSH biosynthesis modulation is important in CBD. Future studies may include investigations into the role of GSH related genes in the progression of beryllium sensitization to disease and disease severity.

**Brush Wellman's medical surveillance (1992-2003) in collaboration with the NJMRC, NIOSH: Lessons learned**

**David C. Deubner**

**Abstract:** For over a decade Brush Wellman Inc (BWI) has conducted an intense program of medical surveillance in collaboration with the National Jewish Medical and Research Center, 1992-1996 (Kreiss, Newman and others) and the National Institute for Occupational Safety and Health (NIOSH), 1998-2003 (Kreiss, Henneberger, Schuler and others). The productivity of this surveillance has been enhanced by the extensive investment BWI made over the years in monitoring beryllium exposure in its facilities, by the linked genetic research by Saltini, Richeldi and others and NIOSH (Weston, McCanlies and others) and by supportive NIOSH basic research (Tinkle and others).

Reported last year was the enhanced preventive model adopted by BWI and the evidence for its effectiveness. This year's report summarizes the data as it relates to beryllium air level and rates of sensitization and CBD as well as some of the questions regarding the natural history of BeBLPT positivity (abnormality) and CBD raised by epidemiological patterns for BeBLPT and CBD.

## **Beryllium Lymphocyte Proliferation Test Surveillance Identifies Clinically Significant Beryllium Disease**

**M. Mroz, L. Newman, L. Maier, M. Strand, L. Silveira, J. Murphy, L. Jui, M. Bausch.**

Division of Environmental and Occupational Health Sciences  
National Jewish Medical and Research Center, Denver, CO

The blood Beryllium Lymphocyte Proliferation Test (BeLPT) is the most sensitive and specific surveillance tool available for detecting beryllium sensitization (BeS) and chronic beryllium disease (CBD). **HYPOTHESIS:** Workplace BeLPT surveillance identifies clinically significant CBD. **METHODS:** We examined demographics, industry, exposure latency, chest x-ray, pulmonary function, DLco, exercise tolerance, arterial blood gases, and treatment in 130 BeS and 119 CBD surveillance-identified cases diagnosed 1982-2001. We modeled outcome variables over time based on latency from first exposure to date of diagnosis, adjusting for age, smoking, race, gender, height. **RESULTS:** At baseline visit, surveillance-identified CBD and BeS cases did not differ in demographics, duration of exposure, or latency from first or last exposure. They did not differ in x-ray profusion, pulmonary function, DLco, VO<sub>2</sub> maximum, A-a gradient at rest or exercise. BeS were more likely than CBD to be current smokers (24% v. 11%,  $p < 0.01$ ), and to have worked with beryllium metal/alloy (96% v. 86%,  $p < 0.002$ ). After a mean 1.3 yr (2.1 SD) follow-up, 26% of surveillance-identified CBD cases developed clinical abnormalities requiring oral immunosuppressive therapy. This clinically-severe CBD group showed a significantly greater annual rate of deterioration in FVC, FEV<sub>1</sub>, and A-a gradient compared to mild CBD and BeS groups, with more pronounced deterioration in those with longer exposure latency. **CONCLUSIONS:** A sizeable subset of CBD cases identified through workplace medical surveillance develop clinically significant disease. Surveillance with the blood BeLPT is warranted to detect, monitor, and treat those at risk of pulmonary impairment.

## ATTENDEE LIST

(as of 1/22/04)

### Beryllium International Research Symposium 2004

January 28 – 30, 2004

(\*Presenters)

Surender Ahir, PhD  
OSHA  
200 Constitution Ave., NW  
N-3718  
Washington, DC 20210  
PH: (202) 693-2280  
FAX: (202) 693-1678  
Email: ahir.surender@dol.gov

Lisa Barker  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room G305  
Denver, CO 80226  
PH: (303) 398-1723  
FAX: (303) 398-1452  
Email: barkerl@njc.org

Barbara Barna, PhD\*  
Cleveland Clinic Foundation  
450 Old Reservoir Rd.  
Berea, OH 44017  
PH: (440) 243-6177  
FAX: (440) 243-3362  
Email: mutchka@prodigy.net

Tristan Barnes, PhD\*  
National Jewish Medical & Research  
Ctr.  
1400 Jackson St.  
Room K721  
Denver, CO 80206  
PH: (303) 398-1249  
Email: barnest@njc.org

Michelle Bausch  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
G306  
Denver, CO 80206  
PH: (303) 398-1374  
Email: bauschm@njc.org

Desiree Bennett, MD  
Honeywell FM&T  
PO Box 419159  
Kansas City, MO 64141-6159  
PH: (816) 997-3200  
FAX: (816) 997-5768  
Email: dbennett@kcp.com

Gregory Burrows, PhD  
Oregon Health & Science University  
3181 SW Sam Jackson Park Rd.  
UHS-46  
Portland, OR 97221  
PH: (503) 494-4345  
FAX: (503) 494-7289  
Email: ggb@ohsu.edu

Beth Canono  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room K1004  
Denver, CO 80206  
PH: (303) 398-1688  
Email: canonob@njc.org

Anu Chaudhary, PhD  
Los Alamos National Laboratory  
P.O. Box 1663  
MS M888  
Los Alamos, NM 87544  
PH: (505) 665-4592  
FAX: (505) 665-3024  
Email: anu@lanl.gov

Marina Chiarappa-Zucca, MS  
Lawrence Livermore National  
Laboratory  
PO Box 808; L-178  
Livermore, CA 94550  
PH: (925) 423-2144  
FAX: (925) 424-3255  
Email: chiarappazucca1@llnl.gov

Brian Day, PhD\*  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room K706  
Denver, CO 80206  
PH: 303-398-1121  
Email: dayb@njc.org

Kay Dellinger, MD, MPH  
US Dept. of Labor - OSHA  
200 Constitution Ave. NW  
Room N3653  
Washington, DC 20210  
PH: (202) 693-2323  
FAX: (202) 693-1647  
Email: dellinger.kay@dol.gov

David Deubner, MD  
Brush Wellman, Inc.  
14710 W. Portage River S. Rd.  
Elmore, OH 43416-9502  
PH: (419) 862-4391  
FAX: (419) 862-4277  
Email:  
david\_deubner@brushwellman.com

Karen Dockstader  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
K720  
Denver, CO 80206  
PH: (303) 398-1782  
Email: dockstaderk@njc.org

Ron du Bois, MD\*  
Royal Brompton Hospital  
1b Manresa Road  
London, SW3 6LR  
PH: 44 2073518328  
FAX: 44 2073518336  
Email: r.dubois@rbh.nthames.nhs.uk

Raed Dweik, MD\*  
The Cleveland Clinic Foundation  
9500 Euclid Avenue, Desk A90  
Cleveland, OH 44195  
PH: (216) 445-5763  
FAX: (216) 445-8160  
Email: dweikr@ccf.org

Kathy Ertell  
Battelle  
PO Box 999  
MSIN P7-75  
Richland, WA 99352  
PH: (509) 372-0996  
FAX: (509) 376-7885  
Email: Katherine.Ertell@pnl.gov

Susan Esmond, PA-C  
National Jewish Medical & Research Ctr  
2918 Champa St.  
Denver, CO 80205  
PH: (303) 298-0197  
Email: EsmondS@njc.org

Andrew Fontenot, MD\*  
UCHSC  
4200 E. Ninth Ave.; B164  
Denver, CO 80262  
PH: (303) 315-1133  
FAX: (303) 315-7642  
Email: [andrew.fontenot@uchsc.edu](mailto:andrew.fontenot@uchsc.edu)

Shirley Fry, MB BCH, MPH  
Central Beryllium IRB  
Oak Ridge Associated Universities  
P.O. Box 117  
Oak Ridge, TN 37831-0117  
PH: (865) 576-1725  
FAX: (865) 576-9557  
Email: [rjmsaf@aol.com](mailto:rjmsaf@aol.com)

George Fulton  
Lawrence Livermore National  
Laboratory  
PO Box 808  
MC L379  
Livermore, CA 94551  
PH: (925) 424-5162  
FAX: (925) 422-5176  
Email: [fulton3@llnl.gov](mailto:fulton3@llnl.gov)

May Gillespie  
National Jewish Medical & Research  
Ctr.  
1400 Jackson St.  
Denver, CO 80206  
PH: (303) 398-1167  
Email: [gillespiem@njc.org](mailto:gillespiem@njc.org)

Craig Glazer, MD  
University of Texas  
5323 Harry Hines Blvd.  
Dallas, TX 75390-9034  
PH: (214) 648-6350  
FAX: (214) 648-2283  
Email:  
[craig.glazer@utsouthwestern.edu](mailto:craig.glazer@utsouthwestern.edu)

Terry Gordon, PhD\*  
NYU School of Medicine  
57 Old Forge Rd.  
Tuxedo, NY 10987  
PH: (845) 731-3536  
FAX: (845) 351-5472  
Email: [gordont@env.med.nyu.edu](mailto:gordont@env.med.nyu.edu)

Charles Gordon  
200 Constitution Ave. NW  
Rm. S-4004, SOL-OSH  
Washington, DC 20210  
PH: (202) 693-5449  
FAX: (202) 693-5466  
Email: [gordon-charles@dol.gov](mailto:gordon-charles@dol.gov)

Ken Groves  
S2-Sevorg Services, LLC  
HC75 Box 1200  
Rutherford, NM 87551  
PH: (505) 588-9064  
FAX: (505) 588-9064  
Email: [sevorgservices@yahoo.com](mailto:sevorgservices@yahoo.com)

Gordon Hall, MD  
Falconbridge Ltd.  
Ste 800, 207 Queen Quay West  
Toronto, ON M5J 1A7  
PH: (416) 982-7073  
FAX: (416) 982-7074  
Email: [gord.hall@toronto.norfac.com](mailto:gord.hall@toronto.norfac.com)

Deanna K. Harkins, MD, MPH  
USA CHPPM  
5158 Blackhawk Rd., #E-1570  
APG, MD 21010-5403  
PH: (410) 436-1012  
FAX: (410) 436-4117  
Email:  
[Deanna.Harkins@apg.amedd.army.mil](mailto:Deanna.Harkins@apg.amedd.army.mil)

James Harris, MD  
Northwest Occupational Health  
Associates  
9370 SW Greenburg Rd.  
Ste. 101  
Portland, OR 97223  
PH: (503) 246-7030

Paul Henneberger\*  
CDC/NIOSH  
1095 Willowdale Rd.  
Morgantown, WV 26505  
PH: (304) 285-6161  
FAX: (304) 285-5820  
Email: pkh0@cdc.gov

Elton Hewitt, BS, CHE  
Fluor Hanford  
PO Box 1000  
MS: A023  
Richland, WA 99352  
PH: (509) 372-3081  
FAX: (509) 373-0242  
Email: elton\_r\_hewitt@rl.gov

Elizabeth Hong-Geller, MD  
Los Alamos National Laboratory  
Biosciences Division  
HRL1  
Los Alamos, NM 87545  
PH: (505) 665-2465  
FAX: (505) 665-3024  
Email: ehong@lanl.gov

Mark Hoover, PhD, CIH\*  
CDC/NIOSH  
MS: H-2800  
1095 Willowdale Rd.  
Morgantown, WV 26505-2888  
PH: (304) 285-6374  
FAX: (304) 285-5820  
Email: mh Hoover1@cdc.gov

Janele Horner, BS  
DynCorp of Colo, Inc.  
10808 Hwy 93  
Unit B; Bldg T13  
Golden, CO 80403-8200  
PH: (303) 966-7266  
FAX: (303) 966-2873  
Email: janele.horner@rfets.gov

Debra Hurst, RN  
BWXT Y12 LLC  
Bear Creek Rd.  
Oak Ridge, TN 37831  
PH: (865) 574-3897  
FAX: (865) 576-5406  
Email: 2dh@y12.doe.gov

Ann Immele  
HAMMER  
812 S. Penn St.  
Kennewick, WA 99336  
PH: (509) 735-3981  
Email: margaret\_a\_ann\_immele@rl.gov

Robert Immele  
PNNL  
812 S. Penn St.  
Kennewick, WA 99336  
PH: (509) 735-3981  
Email: bob.immele@pnl.gov

Sarah Jerome  
Centocor  
1209 Mesa Tr.  
Keller, TX 76248  
PH: (817) 337-5591  
Email: sjerome2@cntus.jnj.com

Wendy Johnson  
Rocky Flats - Alpha Group  
1790 S. Cole St.  
Lakewood, CO 80228  
PH: (303) 966-7674  
FAX: (303) 966-8482  
Email: wendy.johnson@rfets.gov

Larry Jones  
National Nuclear Security  
Administration  
7352 Lancelot Dr.  
Knoxville, TN 37931  
PH: (865) 576-1853  
FAX: (865) 576-8010  
Email: joneslr@yso.doe.gov

Alexas Jonth  
National Jewish Medical & Research  
Ctr.  
1400 Jackson St.  
K720  
Denver, CO 80206  
PH: (303) 398-1782  
Email: maierlab@njc.org

Cathy Kelley  
Centocor  
900 Kerry Ct.  
Palatine, IL 60067  
PH: (847) 202-4954  
Email: ckelley1@cntus.jnj.com

Marc Kolanz, CIH  
Brush Wellman, Inc  
17876 St Clair Ave.  
Cleveland, OH 44110  
PH: (216) 383-6848  
FAX: (216) 383-4091  
Email:  
marc\_kolanz@brushwellman.com

Sylvain LaPare, PhD  
Noranda Inc. / Falconbridge LTD  
2250 Alfred-Nobel  
Ville St. Laurent, PQ H4S 2C9  
PH: (514) 745-9374  
FAX: (514) 745-5559  
Email:  
sylvain.lapare@montreal.norfalc.com

Richard Lapointe, BS  
Alcan, Inc.  
1188 Sherbrooke West  
Montreal, PQ H3A 3G2  
PH: (514) 848-1257  
FAX: (514) 848-1502  
Email: lapointeri@alcan.com

Loren Lewis, MD  
Advanced Medicine  
4630 S. 4650 W.  
West Haven, UT 84401  
PH: (801) 731-1655  
FAX: (801) 731-1348  
Email: loren.lewis@hill.af.mil

Lony Lim, PhD  
Specialty Laboratories  
2211 Michigan Ave.  
Santa Monica, CA 90404  
PH: (310) 828-6543  
Email: llim@specialtylabs.com

Barbara Lohf, RN  
Sandia National Laboratories  
7011 East Ave.  
MS-9112  
Livermore, CA 94550  
PH: (925) 294-2520  
Email: blohf@sandia.gov

Gary Love  
US Department of Energy  
PO Box 2001  
Oak Ridge, TN 37831  
PH: (865) 576-2861  
FAX: (865) 576-0392  
Email: lovegl@oro.doe.gov

Lisa Maier, MD\*  
National Jewish Medical & Research Ctr  
1400 Jackson Street  
Room G216  
Denver, CO 80206  
PH: (303) 398-1983  
Email: maierl@njc.org

Maria Martinez  
US Dept. of Labor - OSHA  
420 Madison Ave.  
Ste. 600  
Toledo, OH 43604  
PH: (419) 259-7542  
FAX: (419) 259-6355  
Email: martinez.maria@dol.gov

John Martyny, PhD, CIH\*  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room G219  
Denver, CO 80206  
PH: (303) 398-1520  
Email: martynyj@njc.org

Bonnie McCafferty, MD  
Federal Occupational Health  
10159 E. Fair Cir.  
Englewood, CO 80111  
PH: (303) 236-0076  
FAX: (303) 236-3440  
Email: bmccafferty@psc.gov

John McInerney, MD  
Rocky Flats  
10808 Hwy. 93; Unit B  
T130C, RFETS  
Golden, CO 80403-8200  
PH: (303) 966-8377  
FAX: (303) 966-2873  
Email: john.mcinerney@rfets.gov

Marek Mikulski, MPH  
Univ. of Iowa - College of Public Health  
2115 Westlawn  
Iowa City, IA 52242  
PH: (319) 384-4296  
FAX: (319) 353-5649  
Email: marek-mikulski@uiowa.edu

Jenelle Morris  
Kaiser Hill Company, LLC  
10808 Hwy. 93  
Unit B, Bldg T103B  
Golden, CO 80403-8200  
PH: (303) 966-5105  
FAX: (303) 966-3407  
Email: jenelle.morris@rfets.gov

Margaret Mroz, MSPH\*  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room G305  
Denver, CO 80206  
PH: (303) 398-1730  
Email: mrozm@njc.org

Lee Newman, MD, MA\*  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Room G212  
Denver, CO 80206  
PH: (303) 398-1983  
Email: newmanl@njc.org

Anthony Newman-Taylor\*  
Imperial College School of Medicine  
1b Manresa Road  
London, SW3 6LR  
PH: 442073518328  
FAX: 442073518336  
Email: a.newmant@rbh.nthames.nhs.uk

Charles Parsons  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Rm. K720  
Denver, CO 80206  
PH: (303) 398-1782  
Email: newmanlab@njc.org

Lewis Pepper, MD\*  
Boston Univ School of Public Health  
715 Albany St.  
T2E  
Boston, MA 02118  
PH: (617) 638-4620  
FAX: (617) 638-4857  
Email: lpepper@bu.edu

Skottie Phillips  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
G306  
Denver, CO 80206  
PH: (303) 398-1679

Linda Piccinini, PhD  
Centocor  
308 Bent Rd.  
Wyncote, PA 19095  
PH: (215) 885-0010  
FAX: (215) 885-6191  
Email: lpiccini@cntus.jnj.com

Maurice Poulin, MD  
National Public Health Inst. of Quebec  
550 Bonaventure  
Trois-Rivieres, PQ G9A 2B5  
PH: (819) 693-3955  
FAX: (819) 373-1627  
Email: Maurice\_Poulin@ssss.gouv.qc.ca

Merrie Rockwell, DO  
Sandia National Laboratories  
PO Box 5800  
MS 1015, Org. 03335  
Albuquerque, NM 87185  
PH: (505) 845-9025  
FAX: (505) 845-8866  
Email: mhrockw@sandia.gov

Kenneth Rosenman, MD  
Michigan State University  
117 West Fee  
East Lansing, MI 48824  
PH: (517) 353-1846  
FAX: (517) 432-3606  
Email: Rosenman@msu.edu

Milton Rossman, MD\*  
Univ. of Pennsylvania Med. Ctr.  
851 BRBII/III  
421 Curie Blvd.  
Philadelphia, PA 19104  
PH: (215) 573-9890  
FAX: (215) 573-4469  
Email: rossmanm@mail.med.upenn.edu

Holly Sackett, MSPH  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Denver, CO 80206  
PH: (303) 398-1592  
Email: sacketth@njc.org

Cesare Saltini, MD\*  
University of Roma Tor Vergata  
IRCCS  
via Portuense 292  
Rome, 00149  
PH: 39 0655170461  
FAX: 39 065582237  
Email: saltini@med.uniroma2.it

Qussai Samak, PhD  
CSN  
1601, de Lorimier  
Montreal, PQ K2K 4M5  
PH: (514) 529-4956  
FAX: (514) 529-4949  
Email: qussai.samak@csn.qc.ca

Wayne Sanderson, PhD, CIH\*  
University of Iowa  
100 Oakdale Campus #134IREH  
Iowa City, IA 52242  
PH: (319) 335-4207  
FAX: (319) 335-4225  
Email: wayne-sanderson@uiowa.edu

Richard Sawyer, PhD\*  
National Jewish Medical & Research Ctr  
1400 Jackson St., K721  
Denver, CO 80206  
PH: (303) 398-1295 3032  
FAX: (303) 270-2353  
Email: sawyerr@njc.org

Richard Sharp, PhD\*  
Baylor College of Medicine  
One Baylor Plaza  
Houston, TX 77030  
PH: (713) 798-3507  
FAX: (713) 798-5678  
Email: rsharp@bcm.tmc.edu

Lori Silveira  
National Jewish Medical & Research Ctr  
1400 Jackson St.  
Denver, CO 80206

Dennis Smith  
TMSS, Inc.  
73 Spyglass Dr.  
Littleton, CO 80123  
PH: (303) 933-8875  
FAX: (303) 979-8595  
Email: tmssinc@comcast.net

Aleksandr Stefaniak  
Los Alamos National Laboratory  
MS: K553  
Los Alamos, NM 87545  
PH: (505) 667-7783  
Email: abs@lanl.gov

Mark Sutton, PhD  
Lawrence Livermore Nat'l Laboratory  
PO Box 808  
L-231, LLNL  
Livermore, CA 94551  
PH: (925) 424-2137  
FAX: (925) 422-3160  
Email: sutton18@llnl.gov

Tim Takaro, MD, MPH\*  
University of Washington  
4225 Roosevelt Way NE  
Suite 100  
Seattle, WA 98105  
PH: (206) 616-7458  
FAX: (206) 616-4875  
Email: ttakaro@u.washington.edu

Kirsten Taylor-McCabe, PhD  
Los Alamos National Laboratory  
TA 43, Bldg 1, M888  
Los Alamos, NM 87544  
PH: (505) 667-2536  
FAX: (505) 665-3024  
Email: kjmccab@lanl.gov

Daniel Teitelbaum, MD  
Medical Toxicology  
155 N. Madison St.  
Denver, CO 80206  
PH: (303) 355-2625  
FAX: (303) 355-3361  
Email: toxdoc@ix.netcom.com

Mary Jane Thomassen, PhD  
Cleveland Clinic Foundation  
9500 Euclid Ave.; Desk A90  
Cleveland, OH 44195  
PH: (216) 444-4429  
FAX: (216) 444-5172  
Email: thomasm@ccf.org

Gilles Trahan, MD  
Alcan, Inc.  
1100 Boul Saint-Sacrement  
Shawinigan, PQ G9N 6W4  
PH: (819) 539-0765  
FAX: (819) 539-0849  
Email: gilles.trahan@alcan.com

Tonya Warwick, PAC  
BWXT-Y12 LLC  
Bear Creek Rd.  
Oak Ridge, TN 37831  
PH: (865) 574-1576  
FAX: (865) 576-5406  
Email: tow@y12.doe.gov

David Weitzman  
US Department of Energy  
1000 Independence Ave., SW  
Washington, DC 20585-0270  
PH: (301) 903-5401  
FAX: (301) 903-7773  
Email: david.weitzman@eh.doe.gov

Robert Weinman, MD, MSA  
Alcan, Inc.  
1188 Sherbrooke St. W.  
Montreal, PQ H3A 3G2  
PH: (514) 848-8492  
FAX: (514) 848-1568  
Email: robert.weinman@alcan.com

Judy Yeater  
Kaiser Hill Company, LLC  
Rocky Flats Plant  
10808 Hwy 93; #B Bldg T130B  
Golden, CO 80403-8200  
PH: (303) 966-5748  
FAX: (303) 966-7183  
Email: judy.yeater@rfets.gov