BERYLLIUM S STIMULATES A LOCAL ANGIOTENSIN SYSTEM IN CHRONIC BERYLLIUM DISEASE GRANULOMAS. LA. Maier*, T Hendry-Hofer* Andrew Fontenot¶, and LS Newman*
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Background: Previous studies indicate that high serum angiotensin converting enzyme (ACE) levels are associated with more severe chronic beryllium disease (CBD), although the source and function of ACE in this granulomatous lung disease are unknown. **Objective**: To test the hypothesis that Be stimulates ACE and its enzymatic product angiotensin II (ATII) during granuloma formation. **Methods**: We enrolled 25 CBD subjects. Of these, 5 underwent Be skin patch testing using aluminum as a negative control. Skin biopsies were performed at intervals between 0 and 35 days. Immunohistochemistry was performed to evaluate CD3,4,8, and 68, ACE and ATII localization in skin granulomas, using morphometry for quantification. The remaining 20 subjects underwent bronchoscopy with bronchoalveloar lavage (BAL). BAL cells were cultured with and without $BeSO_4$ for 0, 24, 72 and 120 hours. ACE and ATII were determined from cell supernatants by ELISA and RIA respectively. **Results**: By 48 hours, CD3,4 and 68 + cells were found in Be skin patch biopsies. ATII staining was noted by 48 hours and continued to increase over time. ACE staining peaked at 96 hours. Double staining revealed colocalization of ACE and CD4 to a greater extent than with CD68, compared to predominant colocalization of CD68 and ATII. BAL cells produced constitutive ACE (peak median 13 ng/ml) and ATII (peak median 33 pg/ml). Be stimulated significant ACE production compared to unstimulated cells (median 22 vs. 11 ng/ml at 120 hours). Be did not stimulate significantly higher ATII production compared to unstimulated cells (median 31 vs. 33 pg/ml). **Conclusions**: We conclude that Be stimulates ACE production from T cells and ATII from monocytes in the skin, while also stimulating significant ACE from BAL cells. This suggests that Be stimulates a local angiotensin system in CBD at sites of pathology. It is possible that this angiotensin system is important in the immune response to beryllium, which will be the topic of future studies.

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Beryllium stimulates activation-induced TNFα pre-mRNA splicing in chronic beryllium disease (CBD). Parsons CE, Sawyer RT, Maier LA, Newman LS. Division of Environmental and Occupational Health Science, National Jewish Medical and Research Center, Division of Pulmonary Science and Critical Care Medicine, University of Colorado Health Sciences Center, Denver, CO.

Beryllium (Be), the etiologic agent of CBD, stimulates bronchoalveolar lavage (BAL) cells from CBD, but not Be-sensitized (BeS), patients to produce TNFα. **Objective:** We tested the hypothesis that Be stimulation could up-regulate CBD/BAL cell TNFα production by the mechanism of activation-induced TNFα pre-mRNA splicing in CBD/BAL, but not BeS/BAL, cells. **Methods:** BAL cells from CBD (n = 17) and BeS (n = 12) patients were cultured in the presence and absence of Be. ELISA was used to measure culture supernatant TNFα levels. TNFα pre- and mature-mRNA levels were measured by RT-PCR. The BeLPT measured Be-stimulated BAL cell proliferation. **Results:** Fresh (0 time) CBD/BAL cells had higher constitutive levels of TNFα pre-mRNA (mean ± SE TNFα/βActin = 0.56 ± 0.26) in comparison to fresh BeS/BAL cells (0.18 ± 0.06). Versus the unstimulated controls in which no significant changes occurred, Be stimulated TNFα pre-mRNA splicing in CBD/BAL cells with a decrease in pre-mRNA (0h = 0.51 ± 0.26; 24h = 0.12 + 0.05), an increase in mature-mRNA (0h = 0.34 ± 0.11; 24h = 0.44 ± 0.14), and an increase in TNFα protein from 0h = 305 ± 299 pg/ml to 24h = 1479 ± 564 pg/ml. Be stimulation did not up-regulate TNFα pre-mRNA splicing or TNFα production in BeS/BAL cells. Anti-HLA-DP mAb (10 μg/ml) blunted peak Be-stimulated CBD/BAL cell proliferation (Be-stimulated BeLPT S.I. = 66 ± 38; Be + anti-HLA-DP S.I. = 15 ± 9), TNFα production (Be-stimulated = 1005 ± 339 pg/ml; Be + anti-HLA-DP = 389 ± 144), and TNFα pre-mRNA splicing (Be-stimulated 4d = 0.18 ± 0.04, 6d = 0.35 ± 0.16; Be + anti-HAL-DP 4d = 0.15 ± 0.02, 6d = 0.17 ± 0.06). **Conclusions:** Be directly up-regulates TNFα protein production in CBD/BAL, but not BeS/BAL, cells by activation-induced TNFα pre-mRNA splicing. Be-stimulated TNFα pre-mRNA splicing and protein production were linked to HLA-DP ligation on CBD/BAL cells, by an unknown mechanism. In CBD, HLA-DP expression and activation-induced TNFα pre-mRNA splicing are associated with the production of Be-stimulated TNFα.

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A FLOW CYTOMETRIC TEST FOR BERYLLIUM SENSITIVITY. Milovanova, T., Liang, J., Yuan G., Rossman MD, Pulm. Allergy and Critical Care Division, Dept. of Med. Univ. Of PA, Phila. PA.

Testing for beryllium hypersensitivity is used in industry to screen current and former beryllium workers for chronic beryllium disease. Tritiated thymidine incorporation is the standard test used to demonstrate beryllium hypersensitivity. However, surveys have shown that 30-70% of individuals with positive blood samples will have negative lung responses. Since a positive blood test usually means that a patient will undergo a bronchoscopy, a better screening test of beryllium hypersensitivity would be useful. We utilized CSFE staining and flow cytometry to identify CD3+ cells that specifically proliferated after beryllium stimulation. Cells were stimulated with PHA, Candida, 100 μM BeSO4 and 10 μM BeSO4. Cells were cultured for 7 days and the proportion of divided cells and proliferating fraction of original cells determined. Eight non-beryllium exposed individuals and 14 beryllium exposed individuals that were positive in blood using tritiated thymidine uptake were studied. While both controls and beryllium positive individuals were positive by both techniques to PHA, fewer positive responses to antigen stimulation (either Candida or Be 100/Be 10) were detected using flow. These results suggest that while flow cytometry may be able to identify the specific cell types that are proliferating, the technique appears to be less sensitive that tritiated thymidine incorporation. Whether the specificity of the response measured by flow cytometry is useful clinically, remains to be determined.

This abstract is funded by: Univ. of Pa. Beryllium Fund

BERYLLIUM SENSITIZATION AND RESPIRATORY DISEASE IN FORMER WORKERS AT A LARGE NUCLEAR WEAPONS SITE

Tim K. Takaro, Katherine Ertell, John Dement[1], Kathleen Omri, Knut Ringen[2], Laura S.Welch[3], Elaine Faustman, Scott Barnhart
Consortium for Risk Evaluation with Stakeholder Participation (CRESP) Univ. of WA.,1) Duke Univ, 2)Ctr. to Protect Workers'Rights, 3) Washington Hospital Ctr.

INTRODUCTION: Beryllium is an important hazard for nuclear weapons workers, due to its genetically mediated immunologic effect on the lungs even at very low doses which leads to chronic beryllium disease (CBD). Exposure and disease findings in former workers can provide risk information for current workers involved in decontamination and demolition (D & D) of old production facilities.

METHODS: As part of a U.S. Department of Energy screening program for beryllium disease, 1367 former Hanford workers received the lymphocyte proliferation test for beryllium (BeLPT), an ATS respiratory symptom and exposure questionnaire, spirometry and B-read chest radiograph. From this cohort, two sex/age/ smoking matched controls with exposure but not sensitization were selected for comparison.

RESULTS: Nineteen workers (1.4%) had two positive BeLPTs (sensitized). None of these had ILO profusion scores ≥ 1/0. Comparing spirometry in cases and controls, there was no significant difference in rates of restrictive, mixed or obstructive defects. No cases of pulmonary CBD were noted. An estimate of person-years of exposure and sensitization status was used to produce a risk map for buildings slated for D & D at the Site.

CONCLUSIONS: A screening program at a large nuclear weapons site shows beryllium sensitization in former workers but not associated fibrotic lung disease to date. Sensitization and exposure data can be used to help characterize risk at such a site.

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NEWLY DIAGNOSED CHRONIC BERYLLIUM DISEASE (CBD) IN FORMER BERYLLIUM WORKERS. Rossman MD, Rosenman KD, Reilly MJ, Bush A, Hertzberg V, Regovich J, Aronchick J, Parker J, Rice C., Michigan State, East Lansing, MI, Univ. Of PA, Phila. PA, Emory Univ., Atlanta, GA, NIOSH, Morgantown, WV, Univ. of Cinn. Cinn, OH

In a survey of two former beryllium (Be) processing plants, 1,464 individuals participated in medical screening including chest x-ray, spirometry and blood Be proliferation testing (LPT). 48 cases of definite CBD (evidence of granuloma (by biopsy) and positive Be LPT) were diagnosed. 31(64%) of these cases were newly identified cases of CBD. All cases had complete PFTs, chest radiograph, bronchoscopy and standardized examination. The 31 newly identified cases of CBD had a mean exposure of 9.6 years and the average year of last exposure was 1974. 16 were ever smokers and 15 never smokers. No respiratory symptoms were reported in only 6 (19%), 21 complained of cough, and 11 of shortness of breath. Chest examination was normal in 25 (81%), 3 had rales and 3 had wheezing. Chest x-rays had interstitial changes in only 10 (32%). The vital capacity was reduced (< 80% of predicted) in 11, the total lung capacity was reduced in 4, the FEV1.0 was reduced in 17 (55%) and the Dlco was reduced in 15 (48%). A restrictive pattern was observed in 7 and obstructive in 8. Blood LPT to beryllium was 19.0 +/- 3.6 and BAL LPT to beryllium was 107.7 +/- 21.8. Negative BAL LPT was observed in 7 (4 smokers and 3 non-smokers). These results demonstrate 64% of definite CBD was unrecognized in this population, both obstructive and restrictive patterns were seen on pulmonary function and equal numbers of smokers and non-smokers were represented.

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JOB-RELATED RISK OF BERYLLIUM DISEASE AT A BERYLLIUM COPPER ALLOY FACILITY Schuler CR[1], Deubner DC[2], McCawley M[1], Berakis MT[1], Kent MS[2], Henneberger PK[1], Kreiss K[1]. [1]CDC/NIOSH, Morgantown, WV;[2]Brush Wellman, Inc., Elmore, OH. We examined the relationship between work processes and sensitization to beryllium and chronic beryllium disease (CBD) at a beryllium copper alloy plant. At the time of the survey 185 persons were employed, and 83% (153) participated. Prevalence of sensitization with abnormal results from 2 different testing labs was 7% (10/153), and 6 of the sensitized were found to have CBD (4%). An additional 9 had multiple abnormal results from a single testing lab; none was diagnosed with CBD. Possible problems at the latter lab during the survey period made interpretation of results difficult; prevalence of sensitization may have been as high as 12%. Results herein for sensitization refer to workers with abnormal results from 2 labs. Workers who had CBD were more likely to have worked at jobs in the rod & wire production area of the plant; these jobs included: pickling & annealing (10% ever worked, p=.05), bull blocks (10%, p=.06), and straightening/point & chamfer (13%, p= .01). Sensitized workers were also more likely to have worked at the rod & wire process bull blocks (14%, p=.07). No jobs in strip operations or the non-production categories of administration, mechanical maintenance, or support services conferred greater risk for either CBD or sensitization. Most had worked multiple jobs, both within and between the major production areas of rod & wire and strip, reducing the utility of job- and area-level categories. Beryllium air samples for the period 1987-1999 indicate that almost all (97%) samples were below the OSHA permissible exposure limit of 2 $\mu g/m^3$, and most (79%) were less than or equal to .1 $\mu g/m^3$. Samples greater than 2 $\mu g/m^3$ represented 7.4% of all samples taken from pickling and annealing in rod & wire mill, compared to 1.7% of all samples from the rest of the plant (p<.001). In summary, much of the risk for sensitization and CBD at this facility appears to reside in 1 of the 2 main production areas.

This abstract is funded by: CDC

[illegible] WORKERS AT A LARGE NUCLEAR WEAPONS SITE

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ABSTRACTS

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