HLA-DP RESTRICTION OF BERYLLIUM-SPECIFIC CD4⁺ T CELLS BEARING PATHOGENIC T CELL RECEPTOR SEQUENCES IN PATIENTS WITH CHRONIC BERYLLIUM DISEASE. Andrew P. Fontenot, Scott J. Canavera, Lee S. Newman, and Brian L. Kotzin. University of Colorado Health Sciences Center, Denver, CO 80262.

Chronic beryllium disease (CBD) is caused by exposure to beryllium in the workplace and is characterized by granulomatous inflammation and the accumulation of CD4⁺ T cells in the lung. We have previously demonstrated an increased expression of Vβ3⁺ T cells in the lung compared to blood in a subset of CBD patients. Analysis of T cell receptor β-chain (TCRB) genes expressed by these Vβ3⁺ T cells showed clonal expansions and homologous TCRB amino acid sequences that were specific for CBD patients and compartmentalized to the lung. During repeated stimulation of a beryllium-specific T cell line derived from the lung of one CBD patient, a Vβ3⁺ population markedly increased in proportion to other CD4⁺ T cells, and accounted for >90% of the cells after the fourth cycle of stimulation. Junctional region nucleotide sequencing of the Vβ3⁺ cells revealed two related clonal populations expressing the CBD-specific TCR sequences previously defined. This oligoclonal Vβ3⁺ T cell line proliferated only when co-cultured with DPB1*0201 expressing antigen-presenting cells (APCs) and only after the addition of beryllium. APCs from individuals matched with this patient's DR alleles failed to initiate a proliferative response. T cell lines from two other CBD patients were sorted for Vβ3 and CD4 and stimulated using autologous APCs and beryllium. Only APCs matched for the DP alleles were able to stimulate proliferation. Intracellular cytokine staining confirmed the beryllium-specific and HLA-DP restricted nature of these cells with 40-60% of cells releasing interferon-γ following the addition of beryllium. These studies confirm the importance of HLA-DP in the presentation of beryllium to pathogenic T cells and provide further insight into the pathogenesis of this disease.

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MURINE SUSCEPTIBILITY TO BERYLLIUM-INDUCED LUNG INFLAMMATION IS LINKED TO CLASS II GENE LOCI. K. C. Meyer*, Z. Xiang*, A. Cardoni*, L. Kubai†, R. Auerbach*. University of Wisconsin Medical School* and Center for Developmental Biology*, Madison, WI, USA.

Beryllium can induce acute and chronic lung inflammation in animals or humans and causes chronic beryllium disease characterized by granuloma formation in susceptible individuals exposed to Be dusts or metal fragments. We performed experiments involving both acute and chronic exposure of various murine strains which varied at the H-2 MHC loci to determine whether class II MHC genes may be important in the development of beryllium-induced lung inflammation. The following strains (H-2 haplotype) were used; A/J (*a*), C3H (*k*), B10.BR (*k*), B10.A (*a*), B10 (*b*), B10.D2 (*d*), and AQR (*a*). Shorter-term experiments (intratracheal instillation of 5-80 ug beryllium in the form of $BeSO_4$ without previous presensitization, lung pathology at 2 wks) and more long-term experiments with either intraperitoneal, intratracheal, or intranasal presensitization followed by intratracheal instillation with subsequent examination of bronchoalveolar lavage cell (BAL) phenotypes and lung histology at 1, 2, 4, and 8 weeks. Prominent lymphocyte influx into perivascular areas adjacent to bronchial structures were observed in animals with the *a* or *k* haplotypes with some areas of granuloma-like inflammatory change. However, no lymphocyte influx was observed for animals with the *d* haplotype (B10.D2) and relatively little for the *b* haplotype (B10), and no granuloma-like lesions were observed on extensive sectioning of lungs from animals with the *b* or *d* haplotypes. However, *in vitro* spleen cell proliferation in response to beryllium exposure at 4 or 6 months post-sensitization did not vary for the *d* versus the *a* haplotypes. We conclude that H-2 haplotypes play a role in sensitization to beryllium and the induction of lymphocyte influx and lung inflammation in mice.

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INFLUENCE OF MHC CLASS II IN SUSCEPTIBILITY TO BERYLLIUM SENSITIZATION AND CHRONIC BERYLLIUM DISEASE LA Maier*, P Lympany¶, D McGrath¶, R du Bois¶, E Wilcox* and LS Newman* *National Jewish Medical and Research Center, Denver, Colorado, USA. and ¶ National Heart and Lung Institute, London, England, UK.

Background: Exposure to beryllium in the workplace can result in beryllium sensitization (BeS) and chronic beryllium disease (CBD). Previous studies indicate that the presence of a glutamic acid at residue 69 in the HLA-DPB1 gene (Glu69) is associated with CBD. Markers of BeS are unknown to date. **Objective:** The purpose of this study was to test the hypothesis that MHC Class II polymorphisms are important in susceptibility to BeS and CBD. **Methods:** Demographic information was obtained from BeS (n=52), CBD (n =103) and beryllium exposed non-diseased (Be-exp) controls (n=122). Genomic DNA was extracted from peripheral blood. HLA-DPB1,-DRB1 and -DQB1 genotyping was determined by PCR-SSP (sequence-specific primers). **Results:** We found a higher phenotypic frequency of the DPB1 Glu69 gene in CBD (85% , n=82 of 96) and BeS (87%, n=45 of 52) subjects compared to the Be-exp controls (38%, n=44 of 116), associated with odds ratios (OR) of 9.6 (95% confidence interval [CI] 4.7, 20.4) for CBD vs Be-exp and 10.5 (95% CI 4.2, 29.7) for BeS vs Be-exp. Glu69 homozygosity was higher in the CBD subjects (25%, n=24 of 96), while BeS subjects were intermediate (15%, n=8 of 52) and Be-exp lowest (2%, n=2 of 116). The prevalence of DR1, DR3 and DQ5 alleles were reduced in CBD vs Be-exp subjects (OR= 0.28, 95% CI 0.14, 0.56 for DR1, OR=0.19 95% CI 0.08, 0.44 for DR3 and OR=0.41, 95% CI 0.24, 0.71 for DQ5) while DR3 was reduced in CBD vs BeS (OR=0.23, 95% CI 0.09, 0.58). DR17 alleles occurred more commonly in CBD than BeS and Be-exp (OR=1.6, 95% CI 1.4, 1.7 and OR=2.3, 95% CI 2.0,2.5, respectively). **Conclusions:** We conclude that DPB1 Glu69 is a marker of BeS and not specific for CBD. This would suggest that HLA-DPB1 may be important in the immune response to beryllium and subsequent development of BeS, but not CBD per se. Detailed evaluation of amino acid analysis may be important in understanding genetic susceptibility in BeS and CBD.

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BERYLLIUM-STIMULATED TNF-α IS ASSOCIATED WITH THE –308 TNF-α PROMOTER POLYMORPHISM AND WITH CLINICAL SEVERITY IN CHRONIC BERYLLIUM DISEASE. LA Maier*, RT Sawyer*, RA Bauer*, LA Kittle*, D McGrath¶, R du Bois¶, P Lympany¶, E Daniloff*, CS Rose*, and LS Newman* *National Jewish Medical and Research Center, Denver, Colorado, USA and ¶ National Heart and Lung Institute, London, England, UK.

Background: Beryllium-antigen stimulates the production of TNF-α from bronchoalveolar lavage (BAL) cells in the granulomatous disorder chronic beryllium disease (CBD). **Objective:** The purpose of this study was to test the hypothesis that high levels of beryllium (Be)-stimulated TNF-α might be related to polymorphisms in the TNF-α promoter region and with clinical markers of disease severity in CBD. **Methods:** Demographic and clinical information were obtained from CBD patients (n = 20). TNF-α levels were measured in Be-stimulated CBD bronchoalveolar lavage (BAL) cell culture supernatant by ELISA. *A priori*, we categorized CBD subjects as either high or low TNF-α producers using a cut off of 1500 pg/ml. The TNF-α promoter sequence, +64 to -1045, was determined by direct sequencing. HLA-DPB1 and -DRB1 genotyping was determined by PCR-SSP (sequence-specific primers). **Results:** There was a significant association between the high expression levels of Be-stimulated BAL cell TNF-α and TNF2 alleles (OR 13.5 for high vs. low TNF-α producers, 95% Confidence Interval 1.2, 152.2), Hispanic ethnicity, presence of a known susceptibility factor for CBD, HLA-DPB1 Glu69, and absence of HLA-DR4. Be-stimulated CBD BAL cell TNF-α levels correlated with markers of disease severity, including chest radiograph, beryllium lymphocyte proliferation test (BeLPT), and spirometry. We found no novel TNF-α promoter polymorphisms. **Conclusions:** We conclude that the presence of the TNF2 A allele at –308 in the TNF-α promoter region is a functional polymorphism, associated with a high level of Be-antigen-stimulated BAL cell TNF-α, and that these high TNF-α levels indicate disease severity in CBD.

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POPULATION-BASED RISK OF BERYLLIUM DISEASE AT A BERYLLIUM COPPER ALLOY PLANT

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RATIONALE: Case reports of beryllium disease exist in persons exposed only to beryllium copper alloys with less than 2% beryllium content, but population-based risk in alloy plants remains unexplored. **METHODS:** At an alloy processing facility we screened 152 volunteers in a workforce of 184 (82.6%), using an interviewer-administered questionnaire and split-sample blood beryllium lymphocyte proliferation testing (BeLPT) for beryllium sensitization. Workers with confirmed abnormal BeLPTs, at both testing laboratories or at a single laboratory, were offered clinical evaluation, including bronchoalveolar lavage and transbronchial lung biopsy for demonstration of lung granulomas. **RESULTS:** Eight workers (5.3%) had abnormal BeLPT results from two laboratories; four of whom (50%) were then determined to have beryllium disease, for a disease prevalence of 2.6% among the tested workers. An additional nine workers (5.9%) had two abnormal BeLPT results from a single laboratory; none of these workers was found to have beryllium disease. **CONCLUSIONS:** The 2.6% beryllium disease rate at this alloy plant is lower than rates observed at beryllium metal (4.2%) and ceramics (5.3%) plants, utilizing the same split-sampling screening methodology for beryllium sensitization. Comparable sensitization rates occurred at these three plants, despite substantial differences in historical beryllium exposures, but differences in abnormal test rates in the two laboratories may reflect quality performance issues.

CDC, Brush Wellman, Inc.

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BERYLLIUM SENSITIZATION AND DISEASE AMONG WORKERS IN A CERAMICS PLANT

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Rationale: Workers in a beryllium ceramics plant were screened for beryllium sensitization and disease in 1998, six years after a similar plant-wide screening in 1992. **Methods:** All current workers were invited to complete a questionnaire, to provide blood for the beryllium lymphocyte proliferation test (BeLPT), and to receive diagnostic follow-up in the event of a positive BeLPT. Beryllium disease was defined as a positive bronchoalveolar lavage (BAL) BeLPT and/or characteristic granulomas on lung biopsy. **Results:** With the exclusion of one person who had a BeLPT+ finding in the 1992 screening, there were 150 participants. Fourteen (9.3%) were BeLPT+, and follow-up revealed 8 (5.3%) had beryllium disease. The proportion sensitized was about the same for both the 74 short-term workers (7/74=9.5%) hired since the 1992 survey and the 76 long-term workers (7/76=9.2%) hired before the 1992 survey. The 7 sensitized short-term workers had been hired within the past two years and only one had beryllium disease. The 7 sensitized long-term workers had been hired at least 8 years earlier and all had beryllium disease. Machinists had been at higher risk for sensitization in the 1992 survey, and this was still evident in the current study among long-term workers (6/39=15%) but not short-term workers (2/36=6%). **Conclusions:** The findings suggest that beryllium sensitization can occur after a short period of exposure, but beryllium disease requires a longer latency or period of exposure. The apparent improvement among machinists might be due to exposure control efforts implemented after the 1992 survey.

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