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New York University School of Medicine
Project Title: Transgenic Mouse Model of Chronic Beryllium Disease
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Background

Animal models provide powerful tools for dissecting dose-response relationships and pathogenic mechanisms and for testing new treatment paradigms. Mechanistic research on beryllium exposure-disease relationships is severely limited by a general inability to develop a sufficient chronic beryllium disease animal model. Discovery of the Human Leukocyte Antigen (HLA) - DPB1Glu69 genetic susceptibility component of chronic beryllium disease permitted the addition of this human beryllium antigen presentation molecule to an animal genome which may permit development of a better animal model for chronic beryllium disease. Using FVB/N inbred mice, Drs. Rubin and Zhu, successfully produced three strains of HLA-DPB1 Glu 69 transgenic mice. Each mouse strain contains a haplotype of the HLA-DPB1 Glu 69 gene that confers a different magnitude of odds ratio (OR) of risk for chronic beryllium disease: HLA-DPB1*0401 (OR = 0.2), HLA-DPB1*0201 (OR = 15), HLA-DPB1*1701 (OR = 240). In addition, Drs. Rubin and Zhu developed transgenic mice with the human CD4 gene to permit better transmission of signals between T cells and antigen presenting cells. This project has maintained the colonies of these transgenic mice and tested the functionality of the human transgenes.

Aim

The purpose of this project is to provide the scientific community with an animal model which will allow the study of chronic beryllium disease. To achieve this goal we are maintaining the existing HLA-DPB1 Glu 69 and CD4 transgenic mice colonies. Also, to insure that the human transgenes are functional in the 3 transgenic strains with the HLA-DPB1 Glu 69 gene, we have challenged these mice with beryllium using dermal exposure.

Outcomes

1. With one exception, the transgenic mice have been maintained successfully. The exception, unfortunately, has been the instability of the HLA-DPB1*1701 transgene the mice. After the successful completion of the first ear swelling functional assay (manuscript submitted), we determined by routine genotyping of the mice bred over the next 6 months that the HLA-DPB1*1701 gene was unstable. Dr. Zhu shipped us the DNA for the HLA-DPB1*1701 transgene and we have generated new HLA-DPB1*1701 mice in the NYU School of Medicine's Transgenic Mouse Core Facility.

2. To monitor the functional expression of the human transgenes in the mice, the immunologic sensitivity of the 3 HLA-DPB1 Glu 69 transgenic strains has been evaluated with an ear swelling bio-assay which involves skin sensitization and an ear challenge with beryllium sulfate. As reported last year, the HLA-DPB1*1701 transgenic mice, the strain with the highest risk transgene in human workers, had significantly increased ear swelling when sensitized dermally with beryllium and challenged on the ear with beryllium**.** No significant changes were observed in the other (HLA-DPB1*0201 and HLA-DPB1*0401) transgenic strains or the FVB/N background strain for any treatment condition**.** These results suggested that the HLA-DPB1*1701 transgene product was responsible for inducing a beryllium-sensitive phenotype and that this transgenic mouse model will be a useful tool for investigating beryllium sensitization and lung lesions.

3. We successfully repeated this transgenic mouse ear swelling study for confirmatory reasons. In parallel studies (funded by NIEHS), we next tested 7 inbred strains of mice in a similar fashion and observed significant interstrain differences in the ear swelling test, thus suggesting that a genetic host factor controls the sensitization response in our mouse model. To identify candidate genes that might be responsible for the genetic component of skin sensitization to beryllium, 21 different inbred mouse strains were utilized to see if they would exhibit varying hypersensitivity responses to beryllium in the mouse ear swelling test. The SJL/J strain appeared to exhibit the greatest hypersensitivity responses with a 38% increase over the baseline ear thickness in the Be sensitized/Be challenged group compared with a 3% increase

in the control vehicle group. The FVB/N strain, in contrast, did not have significant increases in ear thickness. The data for the 21 strains have been analyzed by haplotype mapping to uncover genes associated with sensitization to beryllium. In collaboration with Drs. Delano and Wiltshire (Novartis), a significant locus was identified on chromosome 3 by haplotype analysis. Vav3, a protein which is thought to orchestrate signaling events downstream of lymphocyte antigen receptors and is involved in lymphocyte development and activation (processes which would be highly important during beryllium sensitization), is a promising candidate gene located within the identified locus.

3. In an *in vivo* study (funded by NIEHS), seven inbred strains of mice were aspirated with 20 to 50 μg of beryllium metal particle or water vehicle monthly. As in the ear swelling test, clear strain differences in beryllium-induced lymphogranulomatus nodules were observed. The transgenic mice will be tested for sensitivity to beryllium-induced lung granulomas in the coming year.

Tarantino LM, Sorrentino C, Zhu Y, Rubin EM, Nadas A, Weston A, Tinkle SS, Gordon T. Genetic Determinants of Sensitization to Beryllium in Mice (submitted).