

## **Interagency Agreement: Immunotoxicity of Workplace Xenobiotics**

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## **Background and Rationale**

Through this Interagency Agreement (IAG) the National Toxicology Program (NTP) provides funding to NIOSH for the performance of collaborative studies that evaluate immunological effects in humans exposed to quantifiable levels of xenobiotics in the workplace. This IAG was established in the early 1990s to determine whether the effects observed in animal studies of immunotoxicity translated to humans. These investigations were stimulated by development and implementation of immune testing batteries developed by NTP scientists in the 1980s that suggested many classes of occupational and environmental chemicals can perturb the immune system. Subsequently, as part of a collaborative effort between the NTP and NIOSH, a comprehensive immunologic test battery was developed and validation studies initiated in the laboratory using human donor blood. Separate tests were developed to help assess hypersensitivity responses to occupational chemicals. Several field studies were conducted to validate the individual testing panels in populations exposed to contaminants in the workplace.

The first field study conducted under the IAG focused on identification of immune suppression and evaluated immune effects in bridge workers exposed to lead-based paints. With an emphasis on the conduct of parallel animal studies for the chemicals being studied in worker populations, the NTP examined the immune system toxicity of lead acetate in B6C3F1 mice. Additional studies to identify immune suppression included the immunologic evaluation of workers exposed to opiates during the manufacture of pharmaceuticals and to the mold *Stachybotrys chartarum* while working at a water-damaged museum. The opiate studies led to the identification of specific IgG antibodies as sensitive markers for exposure. This led to an OSHA recommendation that manufacturers conduct occupational screening using these antibodies for biomonitoring. Subsequently, these efforts were expanded to include allergic (allergy to ovalbumin in poultry plants, latex sensitization in health care workers) and inflammatory diseases. For the latex studies, murine models of latex allergy were developed with routes of exposure relevant to those of human exposure including respiratory (inhalation, intranasal and intratracheal), dermal, and subcutaneous (to mimic surgical exposure), and with antigens relevant to human exposure including latex proteins bound to endotoxin and glove powder. Recent studies have expanded the scope of the IAG to include autoimmune diseases and the IAG has also supported NIEHS epidemiology studies (e.g., the Carolina lupus and agricultural health studies).

The goal of this IAG is to provide support for NTP hazard identification activities targeted toward the prevention of diseases or adverse effects caused by environmental exposure to chemical or physical agents. These cooperative studies contribute to the risk assessment process by identifying agents that produce adverse health effects on the immune system in humans and associated risk factors for immunotoxicity.

Examples of current efforts include the evaluation of unique cohorts of individuals from professions associated with immune-mediated occupational diseases including asthma, contact dermatitis, allergy to mold spores, chronic beryllium disease, allergic rhinitis, silicosis, and latex allergy. These cohorts are being studied for a number of endpoints including impact of genetic polymorphisms on inflammatory disease development and clinical outcomes, and identification of unique immunological biomarkers for disease.

### **Project Highlights**

There are currently 15 individual projects being funded in part by the IAG. NIOSH provides support for these projects primarily in the form of sharing of the costs of supplies. Highlights for three current and one completed project are provided below.

**Investigation of health effects caused by exposure to molds** – NIOSH has an ongoing effort to evaluate the health outcomes of cohorts exposed to different mold antigens. Several of these activities are supported through this IAG.

- As part of a collaborative effort with the NTP and investigators on the Heading off Environmental Asthma in Louisiana (HEAL) study, samples have been received from a cohort of children evaluated for respiratory outcomes and mold exposures following Hurricane Katrina. In total, 243 samples have been analyzed for total IgE and mold mix specific IgE. The 106 mold mix positive serum samples (44%) were further analyzed for 5 Katrina-specific mold IgEs.
- NIOSH is participating in the NIEHS-sponsored Agricultural Health Study. They are evaluating allergic sensitization in a cohort of sera from 700 farmers with or without pesticide exposures. All 700 sera will be screened for total IgE and IgE specific to a mixture of molds. Sera with allergic sensitization to molds will be tested further for IgE specific to potential farm-specific mold allergens. Allergic sensitization and mold reactivity will be correlated with pesticide exposure.
- A cohort of chronic rhinosinusitis patients from the Agricultural Health Study is being evaluated for the presence of allergies to molds. Serum samples from 140 individuals have been analyzed for IgE to perennials, grass, weeds, trees, and indoor molds. As there is currently a lack of information on dry fungal aerosol exposures, aerosols from several organisms are being used to investigate the health consequences of exposures to dry fungal aerosols in a rodent model. Germination (viability) has been suggested to enhance the adverse health effects of fungal exposure.
- Methods for delivering dry fungal aerosol by pharyngeal administration have been developed. Animals are being exposed to two species of *Aspergillus*,

and the extent of lung inflammation and systemic antibody responses are being characterized, which will help provide information on potency and for hazard identification. These studies will contribute critical information with regard to the final design and conduct of the NTP rodent toxicology studies evaluating the toxicity of mold exposures.

- A related project includes work to characterize the role of terrelysin as a marker of *Aspergillus terreus* exposure. Monoclonal antibodies have been developed to a hemolytic extract prepared from *Aspergillus terreus*. These antibodies are being characterized for antigen recognition and their utility as immunodiagnostic reagents for exposure and biological effects.

**Role of Gene Polymorphisms in Inflammatory Disease** - The NIOSH Laboratory for Occupational Genomics serves as a resource for obtaining biological samples from individuals with occupationally related diseases including chemical-induced irritant and allergic contact dermatitis, chronic beryllium disease, pulmonary fibrosis, HIV infection, neurodegenerative disease, occupational asthma and rhinitis, and silicosis. A major emphasis has been placed on genetic risk factors, especially within the major histocompatibility complex (MHC) region of the genome, which is rich in genes involved in cytokine production and antigen-specific immune responses, because a large number of occupational/environmental diseases are associated with chronic inflammation and specific immune responses. An on-going study involves individuals with occupational asthma that is associated with exposure to low-molecular weight agents who have been recruited in occupational pulmonary clinics in the United States, Canada, and Europe. DNA samples from this cohort are being genotyped for MHC region variations (2,360 loci) using Illumina high-density single nucleotide polymorphism (SNP) microarray platforms. Preliminary analyses comparing individuals with asthma to low molecular weight antigens to either those with asthma to high molecular weight allergens or controls showed that over 100 genetic variants are uniquely associated with occupational exposure, several of which were unexpected. The Chronic Beryllium Disease (CBD) project, being conducted in collaboration with the Center for Genomics at CDC, is investigating the contribution of genetic variations in the MHC region to the development of beryllium sensitization and CBD. This study is unique in that the genetic profiles from the individuals in this cohort are being compared with mRNA expression in blood to correlate genotype with phenotype. Data have been collected using high-density analysis of SN microarray (2,360 loci). An additional project has examined the influence of genetic factors associated with the immune system on the development of irritant contact dermatitis. Although preliminary, it appears possible to identify healthcare workers prone to develop dermatitis as a result of multiple hand washing by either their response to a test panel of irritants or the presence of specific genetic variants involved in inflammation.

NIOSH's laboratory of occupational genomics is also investigating the contribution of genetic variability in the immune-inflammatory-antioxidant responses to environmental and occupational skin allergens in the development and/or severity of allergic contact dermatitis (ACD). Subject recruitment and skin testing with a panel of occupational/environmental allergens are planned for approximately 500 individuals

being studied at Dartmouth Medical Center and Case Western Reserve University. Additional aspects of this study will investigate underlying genetic profiles in nickel-sensitized patients, patients sensitized to weak allergens, and patients with reactions to more than 3 of the 70 allergens tested in the standard screening series (polysensitized). Comparisons of their genetic profiles will be made to those from individuals previously enrolled in a study of irritant contact dermatitis in health care workers. Individuals who have no irritant contact dermatitis and no history of allergic skin disease as identified by a questionnaire will be selected as controls.

**Biomarkers of Inflammatory Disease** - Diisocyanates are a leading cause of allergic rhinitis as well as occupational asthma resulting from chemical exposure. Investigators at NIOSH are generating monoclonal antibodies to use in developing specific biomarker assays for toluene diisocyanate (TDI) exposure and asthma in humans. At present, the polyclonal antibodies commonly used as biomarkers for exposure are not good markers of disease or exposure because of inconsistent reactivity. Monoclonal antibodies that recognize both exposure and disease-relevant haptens have been developed and will be used to screen human and animal tissues as well as urine to identify those conjugated proteins that are important for exposure and possibly disease based on haptenization profiles.

To further current understanding of the pathobiology of respiratory disease induced by diisocyanates NIOSH is using whole-genome techniques to identify major biological pathways that may serve as targets for biomarker development, disease diagnosis, and/or treatment of allergic rhinitis. To fulfill this objective, NIOSH has initiated collaborations with extramural partners to collect nasal mucosal tissue from workers that responded positively to challenge with diisocyanates. Nasal cytology will be performed on one sample to examine the cellular inflammation that characterizes this disease. Whole-genome microarrays will then be performed on a second sample to investigate the underlying biology and identify potential molecular biomarkers and targets for therapeutic intervention. Similar studies in diverse worker populations are required to develop biomarkers that may be useful for disease surveillance and diagnosis in geographically and ethnically distinct populations.

**Epidemiology and mechanisms of latex allergy** – A considerable level of effort in the early projects supported by the IAG focused on latex allergies. Latex allergies have been recognized as an important public health problem, and while there is decreasing prevalence of latex allergy in the general population, individuals sensitized via occupational exposure, such as health care and custodial workers, continue to have significant risk for developing hypersensitivity responses to latex. As part of an NTP/NIOSH collaboration begun in 1992, a number of field and laboratory studies have examined the relative contributions of skin versus respiratory exposure in the development of sensitization, role of specific allergens, success of intervention strategies, and the utility of animal models toward furthering our understanding of the natural history of latex allergy. This effort was instrumental in identifying the major allergens present in latex gloves and provided critical information for developing non-allergic gloves. For example, we found that mercaptobenzothiazole (MBT) and zinc

dialkyldithiocarbamates (specifically, diethyldithiocarbamates, ZDEC) were commonly found in latex medical and non-medical gloves and were responsible for significant rates of allergic contact dermatitis. Studies into the chemical mechanisms of these latex allergens was completed and published in fiscal year 2008 (Oct 1 – Sept. 30, 2008). Although no longer a major effort in the IAG, this early work has stimulated the development of a new project that will use both *in vivo* and *in vitro* methods to distinguish prohaptens from haptens, a critical determinant in whether a compound may be a potential sensitizer. An analytical screening method developed under the IAG to measure allergenic chemical accelerators (thiocarbamates, thiurams, and mercaptobenzothiazole) in latex and nitrile gloves has passed final “round robin” testing through the American Society for Testing and Materials (ASTM) and been submitted for acceptance as an ASTM standard method. The recognition of this method as an international standard by the ASTM will provide industry with a validated method for rapidly determining the presence of allergenic chemical accelerators in gloves during the manufacturing process.

### **Significance and Expected Outcomes**

The tasks performed under this IAG rely on the expertise of both NIOSH and the NTP to address important questions related to potential health effects, particularly those related to the immune system in humans, following exposure to materials found in the environment or used in the workplace. As part of a NTP/NIOSH collaboration, field and laboratory studies have examined the relative contributions of skin versus respiratory exposure in the development of sensitization, role of specific allergens, success of intervention strategies and the utility of animal models for understanding the role of the immune system in occupational and environmental diseases including our understanding of the disease processes, genetic risk factors, hazard/potency identification, development of biomarkers of human exposure and disease, as well as incidence data. The NTP and NIOSH select the cohorts jointly based on the probability of demonstrating xenobiotic-induced changes in immune function as predicted from animal toxicology studies and on the ability to conduct accurate and reliable exposure assessments. Attempts are made to: (1) examine populations whose exposure can be mimicked in rodent studies and (2) examine populations in which women, minorities, and a variety of age groups are represented. When possible, the NTP and/or NIOSH conduct parallel immunotoxicological animal studies on the chemicals being studied in the exposed populations to evaluate the relevance of the animal data relative to the findings in humans. In addition, where these chemicals have already been examined in experimental animal models, the NTP and NIOSH take advantage of new and more sophisticated methodologies to maintain the studies as “state of the art” evaluations. These studies have directly contributed to worker safety by improving protective practices and providing better screening tools. For example, IAG-supported studies demonstrated that pulmonary sensitizers such as isocyanates, anhydrides and beryllium could also produce pulmonary sensitization following dermal exposure. As a result of these findings protective practices have been revised and individuals working with these materials wear both gloves and respiratory protection. Identification of opiate-specific IgG antibodies as sensitive markers for exposure in the IAG-supported

studies of pharmaceutical workers led to an OSHA recommendation that manufacturers conduct occupational screening using these antibodies for biomonitoring. Thus, the collaborations conducted through this IAG benefit the NTP and NIOSH by allowing the translation of research into measures that, through the regulatory decision making process, improve worker protection and health.

Selected publications funded in part by NIEHS-NIOSH interagency agreement #Y1-ES-0001 "Immunotoxicity of Workplace Xenobiotics"

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