



# CEEH Pilot Projects Program Abstracts from Year 4 (April 1, 1998 to March 31, 1999)

## **Developmental neurotoxicity in maternal phenylketonuria** • Lucio G. Costa, PhD, Department of Environmental Health, UW

Phenylketonuria (PKU) is a genetic defect characterized by the absence of a hepatic enzyme that metabolizes phenylalanine (Phe) to tyrosine. Offspring of mothers with untreated PKU display a number of developmental abnormalities, the most notable of which are central nervous system defects (microencephaly and mental retardation). Animal studies suggest that the Phe metabolite phenylacetate (PA) is responsible for the neurotoxicity, while limited evidence in humans suggests that Phe also may play a role. This study proposes to test the hypothesis that PA and/or Phe causes neurotoxicity by inhibiting astrocyte proliferation and/or by inducing apoptosis in glial and neuronal cells. This hypothesis is supported by data indicating that PA can exert these two effects in tumor cells, including gliomas. In these cells, the action of PA has been ascribed to its ability to inhibit isoprenylation of proteins. Because developing glial and neuronal cells share some of the characteristics of tumor cells, it is hypothesized that a similar mechanisms may also account for the neurotoxicity observed in maternal PKU.

## **Functional significance of the cytochrome P450 1A1 m4 polymorphism: Is the variant form differentially active toward 17 $\beta$ -estradiol and/or PAHs?** • David L. Eaton, PhD, Department of Environmental Health

Cytochrome P450 1A1 (CYP1A1) is an important biotransformation enzyme in the metabolism of a variety of environmental carcinogens. A newly described polymorphism of human CYP1A1, termed CYP1A1\*M4, is formed when a mutation in codon 461 results in a change of amino acid from threonine to asparagine. A recent epidemiological study found that the CYP1A1\*M4 allele was associated with a 6-fold increased risk of endometrial cancer. Because other studies have demonstrated that human CYP1A1 is active in the metabolism of estradiol to several less estrogenic, but potentially genotoxic metabolites (catechol estrogens, semiquinones and quinones), we are interested in determining whether the protein product from this variant allele alters enzymatic activity toward estradiol and/or benzo(a)pyrene. To test this hypothesis, we will create the \*M4 mutant cDNA by site-directed mutagenesis of the wild-type CYP1A1 cDNA, express the variant and wild-type proteins in yeast, and determine the catalytic activity of the two enzymes toward estradiol and benzo(a)pyrene. Study results may be of value

in the design of future molecular epidemiology studies of CYP polymorphisms as determinants of susceptibility to a variety of environmentally related diseases, especially smoking-related cancers. The study approach also may lay the groundwork for a program that utilizes yeast and other eukaryotic expression systems to study the functional significance of other human biotransformation enzyme polymorphisms.

**Polymorphisms in biotransformation enzymes and renal cell carcinoma risk •**

Diane C. Farrow, PhD, Department of Epidemiology

The goal of this study is to investigate whether inherited polymorphisms in biotransformation enzymes involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs) are associated with the risk of renal cell carcinoma (RCC). Polymorphisms proposed for analysis include cytochrome p450 1A1 (CYP1A1), microsomal epoxide hydrolase (mEH), NAD(P)H:quinone oxidoreductase (NQO1), and glutathione S-transferase P1 (GST-P1). The work will expand on data already available from an existing population-based case-control study of RCC, including extensive exposure data from telephone interviews, DNA isolated from normal tissue, and GST-M1 and GST-T1 genotypes. Strengths of the proposed study include its population-based design, the existence of extensive interview data on exposures that may confound or modify the associations of interest, and the cost-effectiveness of conducting the proposed assays on already-collected samples. The ultimate goal of this research is to understand observed variability in susceptibility to RCC among individuals with well-characterized environmental exposures. Results should provide a unique pilot dataset that may form the basis for a large study of gene-gene and gene-environment interactions in RCC.

**Effect of ozone on airway inflammation and hyperreactivity in a mouse model of asthma •** William R. Henderson, MD, Department of Allergy and Infectious Diseases

Asthma is characterized by a complex inflammatory response of airway eosinophilia, edema, mucus hypersecretion, bronchial epithelial injury, and hyperreactivity. We have developed a protocol for administration of ovalbumin (OVA) as allergen to induce late-phase allergen-specific pulmonary disease in mice (Henderson et al., J. Exp. Med. 184:1482-1494, 1996; Henderson et al., J. Clin. Invest. 100: 3083-3092, 1997). OVA-treated mice display a disease strikingly similar to allergen-induced human asthma with airway mucus release and eosinophil infiltration and airway hyperreactivity (AHR) to methacholine. Ozone (O<sub>3</sub>) produces adverse respiratory health effects in both animals and humans. Genetic susceptibility to O<sub>3</sub> may be important in O<sub>3</sub>-induction of airway inflammation and AHR. In particular, asthmatics may have enhanced sensitivity to O<sub>3</sub>.

In these multidisciplinary studies, which involve investigators in the UW departments of medicine, pathology, and environmental health, the objective is to characterize the effect of O<sub>3</sub> on allergic pulmonary inflammation and AHR in a mouse model of asthma. The investigators will utilize inbred mouse strains that are O<sub>3</sub>-susceptible and O<sub>3</sub>-resistant to O<sub>3</sub>-induced inflammation and AHR in this asthma model. They will examine these questions:

- a) Will an allergic diathesis convert an O<sub>3</sub>-resistant mouse strain to O<sub>3</sub>-susceptibility?
- b) Will an allergic diathesis increase O<sub>3</sub>-induced airway inflammation and AHR in O<sub>3</sub>-susceptible mouse strains?

Through this study, investigators hope to define the key molecular mechanisms involved in O<sub>3</sub>-induced augmentation of allergic injury in the lung. Employing a mouse strain of asthma, they hope to better understand the genetic susceptibility to this important environmental agent of respiratory toxicity. The more specific knowledge of these biochemical and immunological changes becomes, the more likely it is that interventions to reduce O<sub>3</sub>-induced inflammation will be found.

**Environment and T-cell dysfunction: A nutritional model in transgenic mice •**  
Warren Ladiges, DVM, Department of Comparative Medicine

The mechanisms by which environmental factors such as foods may affect the immune system in the aging population are not well understood or appreciated. The global theme behind this proposal is to identify food factors that can enhance immunity and thus decrease the incidence of age-related immune dysfunction. We have developed a T-cell receptor transgenic (Tg) mouse model of T-cell dysfunction that phenotypically expresses acute onset of inflammatory joint disease in the digits of the paws 30 days after immunization with bovine collagen. A preliminary experience has shown that N-acetylcysteine (NAC), a thiol compound similar to that found naturally in commonly ingested foods, such as vegetables, lessens the severity of inflammation in this Tg mouse model. The investigators hypothesize that NAC will delay the onset and lessen the severity of T cell-driven joint disease by a selective inhibition of the proinflammatory T helper (Th1) cell cytokine pattern. Investigators will first administer NAC in the drinking water at 3 dosage levels for 5 weeks, beginning the day of immunization, to determine the dose response required to prevent or delay the onset and/or lessen the severity of joint disease. Secondly, Th cytokine patterns, either Th1 or Th2 in NAC-treated or -untreated Tg mice, will be determined by measuring the IL2/IFN $\gamma$  and IL-4, respectively, in supernatants of lymph node T-cell cultures. Thirdly, the role of NF- $\kappa$ B, a transcriptional mediator for cytokine activity in T cells, will be correlated with the ability of NAC to suppress joint disease. Results of this project will be useful in designing studies to evaluate the effects of food or

other environmental factors on T cell dysfunction in Tg mice and, ultimately, in older adults.

**Ecogenetics of nicotine addiction: Search for functional polymorphisms in the gene for the beta2-subunit of the neuronal nicotinic acetylcholine receptor (CHRNA2) •**  
Carl C. Ton, PhD, Department of Medical Genetics

Chronic exposure to the toxic and carcinogenic components of cigarette smoke, in contrast to occupational or environmental exposure to other hazardous xenobiotics, occurs largely through a behavioral choice driven by an individual's addiction to nicotine. The neurochemical basis of this self-injurious psychomotor response appears rooted in the complex interactions between the nicotine agonist and the neuronal nicotinic acetylcholine receptors (nAChR) of the central nervous system. One predominant component of the nAChR, the beta2-polypeptide subunit, appears to play a key role in mediating this addictive response to nicotine. What would be the pharmacogenetic and ecogenetic consequences of functional polymorphisms in this molecule?

This pilot project proposes to develop the preliminary data necessary to address this question in detail by:

- 1) determining the genomic sequences of the gene for the beta2-subunit of the neuronal nicotinic acetylcholine receptor (CHRNA2),
- 2) using this information to develop sequencing primers covering the exons and splice donor/acceptor sites of CHRNA2, and
- 3) performing direct genomic sequencing of these targeted regions in CEPH family progenitors and specimens in the Human Diversity Collection, in order to identify common CHRNA2 variants in the human population.

This work will make it possible to develop rapid PCR- or ligation-based assays for such variants for future large-scale surveys. Knowledge of the ecogenetics and pharmacogenetics of the nAChRs might suggest more effective therapeutic measures to help addicted persons stop smoking.