

**Research Foci 2: Molecular Basis of Environmental Disease**

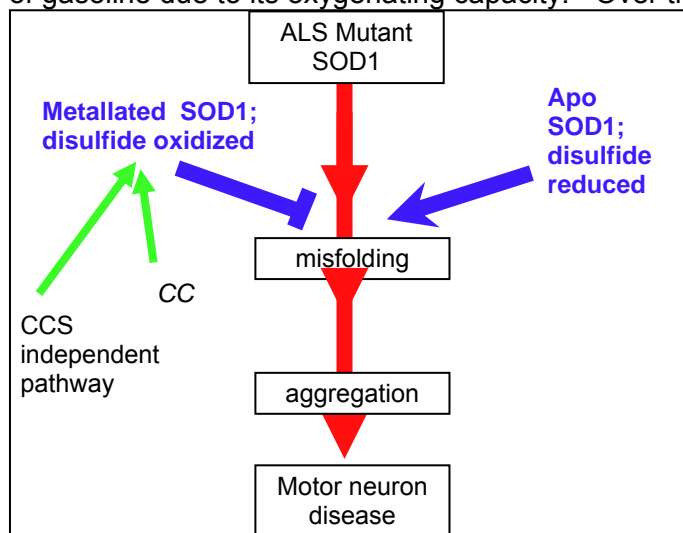
The *Research Foci 2: Molecular Basis of Environmental Disease* is led by Dr. Valeria Culotta, Professor of Toxicology. This group facilitates the investigation of basic mechanistic studies in areas such as oxidative damage including the induction of genes specific to oxidative damage, genetic regulation of superoxide dismutase, mechanisms of lead toxicity, and the study of estrogen and androgen mediated effects in biologic systems. Since it is paramount to this Center that our intervention strategies have a mechanistic rationale, this focus group serves to provide a foundation for our efforts.

**Reactive Oxygen Species (ROS) Biology and Toxicity**

Many members of this focus group have research interests in the area of reactive oxygen toxicity. Dr. Trush has devoted much of his career towards an understanding of ROS production, particularly from the mitochondria, and its role in organic xenobiotic toxicity, a recent example of this work is related to cardiac function (26). Dr. Culotta has been exploiting the simple unicellular eukaryote, yeast, as a model system to clone and characterize genes involved in reactive oxygen toxicity. Much of the work she has accomplished has focused on mitochondrial antioxidant enzymes. This includes an understanding of how the copper/zinc and manganese containing forms of superoxide dismutase (SOD) together provide effective protection against mitochondrial-derived superoxide. In a very recent collaboration with Dr. Walter Watson, one of the new assistant professors (see *Career Development for Environmental Health Investigators* section) he has been investigating the formation of disulfide bonds in oxidative stress. Dr. Watson's work studies the thioredoxins and oxidative stress and the role of thioredoxins in regulating transcription factor activity (27). Dr. Barry Zirkin has had a long-standing interest in the mechanisms of Leydig cell aging in the testes. Over the past funding period, his research has focused on the temporal relationship of testosterone production and aging (28). Dr. Zirkin is also a recent recipient of a MERIT award from NIH. In addition, Dr. Zirkin has been collaborating extensively with Dr. T. Brown in probing androgen receptor function in Leydig cells (29). Dr. T. Brown focuses on the role of androgens in cancer and hyperplasia of the prostate. Much of his work has addressed the transcriptional machinery that plays a critical role in modulating androgen receptor activity and recently, he has reported on the role of Nf-KB in androgen receptor activation(30).

**Metal Homeostasis Biology and Toxicity**

A major theme in this program involves metal homeostasis biology and toxicity. Dr. Culotta has been investigating the mechanisms of metal ion metabolism and metal ion toxicology. In addition to her traditional studies on copper, her attention has been drawn to manganese, a potent neurotoxin and emerging component of gasoline due to its oxygenating capacity. Over the past grant period, this group has identified a new copper trafficking pathway for the superoxide dismutase enzyme that may be relevant to SOD1-linked cases of Lou Gehrig's disease (31). The Cu- and Zn-containing superoxide dismutase 1 (SOD1) largely obtains Cu *in vivo* by means of the action of the Cu chaperone CCS. The proposed mechanism of how copper inserts into SOD1 by the CCS chaperone is shown in the accompanying Figure 6 and was recently reviewed (32). Yet, in the case of mammalian SOD1, a secondary pathway of activation is apparent. Specifically, when human SOD1 is expressed in either yeast or mammalian cells that are null for CCS, the SOD1 enzyme retains a certain degree of activity. This CCS-independent activity is evident with both wild-type and mutant variants of SOD1 that have been associated with familial amyotrophic lateral sclerosis and several follow up studies have suggested the role of this CCS-independent pathway in mutant SOD1 folding relevant to ALS. The possible implications of multiple pathways for SOD1 activation are plausible mechanisms to be



**Figure 6: CCS and the CCS independent pathway can protect against misfolding of ALS mutant SOD1**

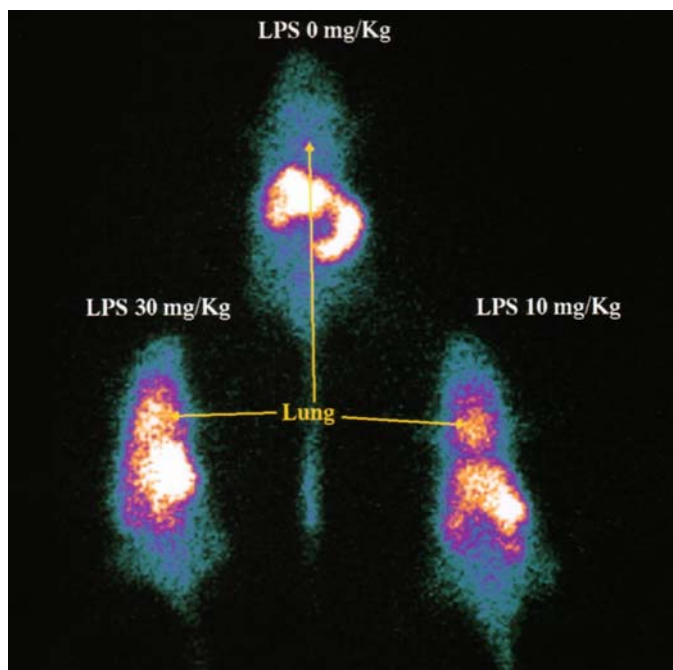
explored in the context of SOD1 evolutionary biology and familial amyotrophic lateral sclerosis.

The Culotta research group has also found that manganese toxicity is influenced by the ACDP family of proteins that have been implicated in urofacial syndrome. Manganese-dependent superoxide dismutase 2 (SOD2) in the mitochondria plays a key role in protection against oxidative stress. The pathway by which SOD2 acquires its manganese catalytic cofactor was explored in the yeast model and it was found that a mitochondrial localization was essential. Specifically, mitochondrial import itself provides the protein unfolding step necessary for manganese insertion into the molecule. (33). In a search for factors that insert manganese into mitochondrial SOD2, the Culotta lab discovered Mtm1p, a mitochondrial carrier protein that is well conserved in yeast and humans and is critical for manganese activation of the enzyme (34). More recent studies have demonstrated that Mtm1p actually plays an important role in preventing metal misincorporation into SOD2: specifically Mtm1p can prevent the non-catalytic iron atom from binding the active site (35). These studies were the first to demonstrate mis-incorporation into the active site of a eukaryotic enzyme *in vivo*. In fact, these findings have relevance to human diseases associated with iron overload. Very recently it was shown that in X-linked sideroblastic anemia, iron rather than manganese inserts into SOD2, and the resulting loss of SOD2 activity leads to profound mitochondrial oxidative stress (Culotta, unpublished). Culotta's current studies on manganese/iron interactions in the mitochondria promise to shed new light into mechanisms of metal toxicity relevant to metal ion mis-incorporation and oxidative stress. Interest in manganese has been heightened by the recognition that widespread exposure in people will possibly occur due to its addition as a gasoline additive.

Another member of this research foci, Dr. Bressler has had a long-standing interest in the transport of lead (Pb) and cadmium (Cd) and the signaling events following exposure to these metals (36). Common scenarios for Pb exposure include occupational, residential, and/or behavioral (hand-to-mouth activity) settings. The main source of Cd exposure for nonsmokers is dietary, through plants or animals that accumulate the metal. Specific cellular importers for Pb and Cd are unlikely as these metals are nonessential and toxic. Accordingly, in the intestine, the operational mechanism is assumed to be inadvertent uptake through pathways intended for essential nutrients such as iron. Results from experimental and epidemiological studies indicated that diets low in iron (Fe) result in increased absorption of Pb and Cd, suggesting common molecular mechanisms of Cd and Pb transport. Indeed, recent mechanistic studies found that the intestinal transporter for nonheme iron, divalent metal transporter 1 (DMT1), mediates the transport of Pb and Cd. DMT1 is regulated, in part, by dietary iron, and chemical species of Cd and Pb that are transported by DMT1 would be made available through digestion and are also found in plasma. Accordingly, the involvement of DMT1 in metal uptake offers a mechanistic explanation for why an iron-deficient diet is a risk factor for Pb and Cd poisoning. It also suggests that diets rich in iron-containing food could be protective against heavy metal poisoning.

Dr. Guilarte has also had a major interest in lead, particularly regarding its effects on the NMDA receptor in mammalian brains (37). The Guilarte group is extensively supported by NIEHS (R01 ES006189, R01 ES007062, R01 ES 010975). This work has been accelerated by the use of the *in vivo* imaging instrumentation (*Facility Core B: In Vitro and In Vivo Imaging*). The ability to visualize quantitatively glutamate carboxypeptidase II (GCPII) levels *in vivo* could advance an understanding of its function in health and disease. In the current study, this team synthesized and evaluated a radiolabeled (iodine-125) analog of N-[N-[(S)-1,3-dicarboxypropyl]carbonyl]-S-3-iodo-L-tyrosine (DCIT), a potent antagonist of GCPII activity. They examined the regional distribution of [125I]DCIT binding in the rodent brain using quantitative autoradiography in order to confirm the validity of this radioligand as a marker of GCPII in the brain. The ultimate goal is to develop an imaging agent for assessing GCPII levels in the living brain. The specific binding of [125I]DCIT to rat brain followed a regional distribution consistent with previous studies describing regional brain GCPII gene expression and activity. The data showed a modest rostrocaudal gradient in which specific binding of [125I]DCIT to GCPII that was lowest in cortical regions, with increasing levels of binding in midbrain structures and high levels of binding in hindbrain and brainstem. Autoradiography of [125I]DCIT in GCPII knockout and wild type mouse brain showed a gene-dose dependency confirming the selectivity of this radioligand for GCPII. It was proposed that [125I]DCIT is a selective radioligand that can be used to quantify brain GCPII levels *in vitro* using quantitative autoradiography.

Over the past two years, the Guilarte group has focused on imaging analysis for localization of the peripheral benzodiazepine receptor (38). The ability to visualize the immune response with radioligands



**Figure 7**

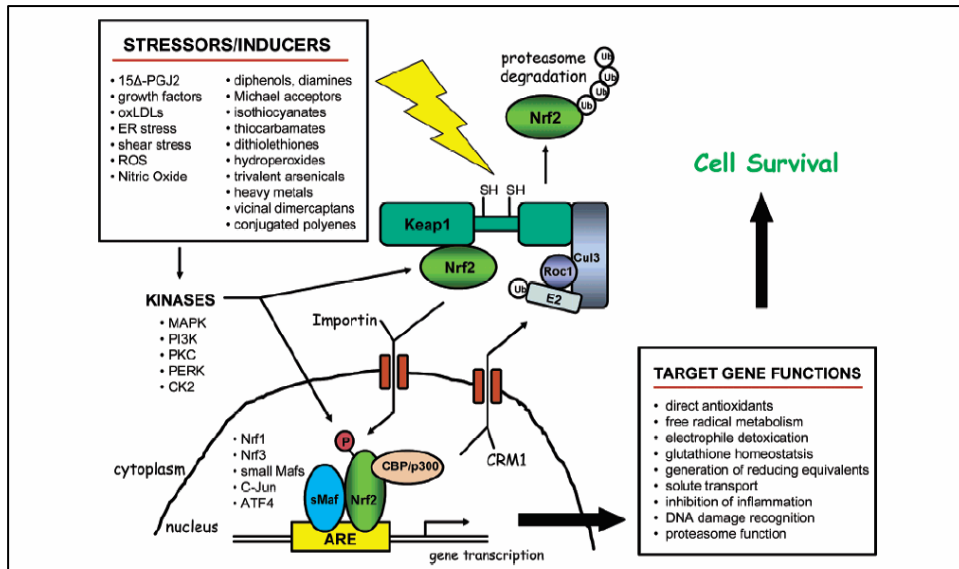
to demonstrate increased [(11)C]-(R)-PK11195 accumulation in the lungs of LPS-treated mice. This study suggests that measuring PBR expression using in vivo imaging techniques may be a useful biomarker to image lung inflammation.

Dr. Silbergeld's laboratory has led a series of studies exploring immunotoxic effects of inorganic mercury and the possibility that this may be modulated by inherent differences in the responsiveness of immune cells related to the age of the donor (39). Cells from lymph nodes, spleen, and thymus, collected from 7- and 10-day-old CD.1 pups, as well as from adult CD.1 mice, were studied in terms of the effects of mercury *in vitro* on responses to Con-A stimulation with respect to proliferation, cytokine production, and cell phenotype. The effects of mercury on proliferation were age and organ dependent, while effects on cytokine production were only age dependent. Effects of mercury were observed only on splenocyte T-cell subpopulations and only in cells from 10-day-old pups and from adults. Mercury had no effect on IFN-gamma and IL-4 production by splenocytes from 7-day-old pups, but significantly decreased release of these cytokines by splenocytes from 10-day-old pups and adults. Hg did not affect IL-4 production by lymph node cells or thymocytes. In lymph node cells Hg affected IFN-gamma production only at 7 days. These data indicate that inherent properties of immune cells at different stages of development may influence the response to immunotoxicants.

#### *Signaling Pathways in Response to Environmental Agents*

As already described in the *Strategic Vision and Impact on Environmental Health* section of the grant, the Nrf2 pathway is a major focus of collaborative research in this research focus group. Drs. Kensler, Biswal, Groopman, Mitzner, Guilarte and Reddy all have efforts underway exploring the multiple effects mediated by this pathway (Figure 8). For example, inflammation and protease/antiprotease imbalance have been postulated to be critical in cigarette smoke-induced (CS-induced) emphysema and oxidative stress has been suspected to play an important role in chronic obstructive pulmonary diseases. Susceptibility of the lung to oxidative injury, such as that originating from inhalation of CS, depends largely on its upregulation of antioxidant systems. Nrf2 is a redox-sensitive basic leucine zipper protein transcription factor that is involved in the regulation of many detoxification and antioxidant genes (see Figure 8). Disruption of the Nrf2 gene in mice led to earlier-onset and more extensive CS-induced emphysema than was found in wild-type littermates. Emphysema in Nrf2-deficient mice exposed to CS for 6 months was associated with more pronounced bronchoalveolar inflammation; with enhanced alveolar expression of 8-oxo-7,8-dihydro-2'-deoxyguanosine, a marker of oxidative stress; and

with an increased number of apoptotic alveolar septal cells--predominantly endothelial and type II epithelial cells--as compared with wild-type mice. Microarray analysis identified the expression of nearly 50 Nrf2-dependent antioxidant and cytoprotective genes in the lung that may work in concert to counteract CS-induced oxidative stress and inflammation. The responsiveness of the Nrf2 pathway may act as a major determinant of susceptibility to tobacco smoke-induced emphysema by upregulating antioxidant defenses and decreasing lung inflammation and alveolar cell apoptosis (40).



**Figure 8: Nrf2 signaling pathway**

peroxidation, compared to WT cells. Nonetheless, the basal levels of ROS flux and oxidative damage biomarkers in WT and N0 cells were not different. Diquat dibromide (DQ), a non-electrophilic redox cycling bipyridylum herbicide, was used to generate intracellular superoxide anion. Isolated mitochondria from both cell lines exposed to DQ produced equivalent amounts of ROS, indicating a similar cellular capacity to generate ROS. However, N0 cells exposed to DQ for 24-h exhibited markedly decreased cell viability and aconitase activity as well as increased lipid peroxidation and glutathione oxidation, relative to WT cells. 2',7'-Dichlorofluorescein fluorescence was not increased in WT and N0 cells after 30-min of DQ exposure. However, increased levels of ROS were detected in N0 cells but not WT cells after 13-h of DQ treatment. Additionally, total glutathione concentrations increased in WT, but not N0 cells following a 24-h exposure to DQ. DQ exposure resulted in activation of an antioxidant response element-luciferase reporter gene, as well as induction of Nrf2-regulated genes in WT, but not N0 cells. Thus the enhanced sensitivity of N0 cells does not reflect basal differences in antioxidative capacity, but rather an impaired ability to mount an adaptive response to sustained oxidative stress.

Oxidative stress has been postulated to play an important role in the pathogenesis of asthma; although a defect in antioxidant responses has been speculated to exacerbate asthma severity, this has been difficult to demonstrate with certainty. The Nrf2 gene leads to severe allergen-driven airway inflammation and hyperresponsiveness in mice. Enhanced asthmatic response as a result of ovalbumin sensitization and challenge in Nrf2-disrupted mice was associated with more pronounced mucus cell hyperplasia and infiltration of eosinophils into the lungs than seen in wild-type littermates. Nrf2 disruption resulted in an increased expression of the T helper type 2 cytokines interleukin (IL)-4 and IL-13 in bronchoalveolar lavage fluid and in splenocytes after allergen challenge. The enhanced severity of the asthmatic response from disruption of the Nrf2 pathway was a result of a lowered antioxidant status of the lungs caused by lower basal expression, as well as marked attenuation, of the transcriptional induction of multiple antioxidant genes. Recent collaborative studies from Drs. Mitzner and Biswal suggest that the responsiveness of Nrf2-directed antioxidant pathways may act as a major determinant of susceptibility to allergen-mediated asthma (42).

Drs. Kensler, Trush and Biswal collaborated on a study of mouse embryonic fibroblasts derived from Nrf2<sup>-/-</sup> mice (N0) and Nrf2<sup>+/+</sup> mice (WT) to characterize both basal and diquat (DQ)-induced oxidative stress levels and to examine Nrf2 activation during exposure to DQ-generated superoxide anion (41). Microarray analysis revealed that N0 cells have similar constitutive mRNA expression of genes responsible for the direct metabolism of reactive oxygen species but decreased expression of genes responsible for the production of reducing equivalents, repair of oxidized proteins and defense against lipid

The Kelch-like ECH-associated protein 1 (KEAP1) negatively regulates Nrf2 activity by targeting it to proteasomal degradation. Increased expression of cellular antioxidants and xenobiotic detoxification enzymes has been implicated in resistance of tumor cells against chemotherapeutic drugs. In a recent report, a systematic analysis of the KEAP1 genomic locus in lung cancer patients and cell lines revealed deletion, insertion, and missense mutations in functionally important domains of KEAP1 and a very high percentage of loss of heterozygosity at 19p13.2, suggesting that biallelic inactivation of KEAP1 in lung cancer is a common event. Sequencing of KEAP1 in 12 cell lines and 54 non-small-cell lung cancer (NSCLC) samples revealed somatic mutations in KEAP1 in a total of six cell lines and ten tumors at a frequency of 50% and 19%, respectively. All the mutations were within highly conserved amino acid residues located in the Kelch or intervening region domain of the KEAP1 protein, suggesting that these mutations would likely abolish KEAP1 repressor activity. Evaluation of loss of heterozygosity at 19p13.2 revealed allelic losses in 61% of the NSCLC cell lines and 41% of the tumor samples. Decreased KEAP1 activity in cancer cells induced greater nuclear accumulation of NRF2, causing enhanced transcriptional induction of antioxidants, xenobiotic metabolism enzymes, and drug efflux pumps. This is the first study to our knowledge to demonstrate that biallelic inactivation of KEAP1 is a frequent genetic alteration in NSCLC. Loss of KEAP1 function leading to constitutive activation of NRF2-mediated gene expression in cancer suggests that tumor cells manipulate the NRF2 pathway for their survival against chemotherapeutic agents (43).

Collaborative studies from Drs. Guilarte and Kensler explored the role that oxidative stress and the Nrf2 pathway plays in the etiology of Parkinson's disease (PD) in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of PD. Nrf2 knockout and wild-type mice were administered MPTP doses ranging from 20 to 60 mg/kg. Seven days after MPTP administration dopamine transporter (DAT) levels were measured using [(125)I]-RTI-121 quantitative autoradiography as an index of dopamine terminal integrity in the striatum. The results indicate that MPTP administration resulted in a greater loss of DAT levels in the striatum of Nrf2 knockout mice than in wild-type at all MPTP doses tested. Activation of the Nrf2 pathway by oral administration of the Nrf2 inducer 1,2-dithiole-3-thione (D3T) to wild-type mice produced partial protection against MPTP-induced neurotoxicity. The protective effect of D3T was not due to a change in MPTP metabolism since the level of the MPTP metabolite MPP<sup>+</sup> was not significantly different in the D3T treated striatum relative to vehicle control. Administration of D3T to Nrf2 knockout mice did not protect against MPTP neurotoxicity suggesting that the Nrf2 pathway is necessary for the D3T-mediated attenuation of MPTP neurotoxicity. This study demonstrates the significance of activating intrinsic antioxidative mechanisms in an *in vivo* model of neurodegeneration. The *in vivo* activation of the Nrf2 pathway in the brain may be an important strategy to mitigate the effects of oxidative stress in neurodegenerative disorders and neurological disease (44).

### *Lung Pathophysiology*

This research focus group has had years of research collaborations in the area of environmental lung diseases concentrating on the effects of air pollutants and allergens on lung function. These disease effects are often manifested by inflammatory responses, emphysema, asthma, and impaired host defenses. Investigators in this group now have the ability to characterize underlying genetic susceptibility factors that confer increased risk from exposure to these prevalent pollutants. Respiratory illnesses, from lung cancer to emphysema to asthma, affect a large number of people living in urban environments and constitute a major morbidity problem in these populations. The group of researchers in our core bridge experimental and human studies by developing experimental models to explore the mechanisms of action of airborne pollutants. This thrust nicely complements a major thrust of the Center's epidemiologic efforts on the effects of air pollution. Thus, this interactive group of investigators seeks to define quantitatively the responses of the respiratory system to inhaled particles and pollutant gases, to determine the pathophysiologic mechanisms of these inhalants on pulmonary tissues and cells, and then to interpret these relations in the framework of assessment of risk of human exposure to concentrations that are harmful to human health.

Dr. Spannhake's group has for many years worked on factors believed to exacerbate asthmatic symptoms, air pollution and viral infections are considered to be particularly important (45). Although evidence indicates that each of these respiratory insults individually can increase asthma severity in susceptible individuals, we know little about the extent to which exposure to environmental oxidant pollutants can influence the course of respiratory viral infection and its associated inflammation. To investigate the interaction of these two stimuli

within their common epithelial cell targets in the upper and lower respiratory tracks, they infected primary human nasal epithelial cells and cells of the BEAS-2B line grown at the air-liquid interface with human rhinovirus type 16 (RV16) and exposed them to NO<sub>2</sub> (2.0 ppm) or O<sub>3</sub> (0.2 ppm) for 3 hr. Independently, RV16, NO<sub>2</sub>, and O<sub>3</sub> rapidly increased release of the inflammatory cytokine interleukin-8 through oxidant-dependent mechanisms. The combined effect of RV16 and oxidant ranged from 42% to 250% greater than additive for NO<sub>2</sub> and from 41% to 67% for O<sub>3</sub>. We abrogated these effects by treating the cells with the antioxidant N-acetylcysteine. Surface expression of intercellular adhesion molecule 1 (ICAM-1) underwent additive enhancement in response to combined stimulation. These data indicate that oxidant pollutants can amplify the generation of proinflammatory cytokines by RV16-infected cells and suggest that virus-induced inflammation in upper and lower airways may be exacerbated by concurrent exposure to ambient levels of oxidants commonly encountered in the indoor and outdoor environments.

Research work stemming from an EHS pilot grant application 4 years ago, led to an RO1 funding Dr. Wagner to look at how soluble and insoluble particles are cleared from the lung. While most of the insoluble particles on the surface of conducting airways are rapidly cleared (within 24 h) by the mucociliary escalator, a small fraction is retained for extended periods of time. Soluble particles have been less well studied, but recent work from Wagner and Foster has shown that a surprising fraction of soluble particles are also cleared by the cilia. The largest fraction, however, is cleared from the lung by absorption into the bronchial circulation supplying the airways. As the soluble particle used (DPTA) has a size of 80 nanometers, this model has been further developed into one in which ultrafine particle clearance is now being studied. Though it was at first surprising to find such large particles readily passing into the blood, this observation presents a potential mechanism for how ultrafine particles might cause a depressant cardiovascular response. Work on this topic is being independently pursued by Dr. Tankersley, who will extend this clearance work to lungs of aging mice. Since mice do not have a bronchial circulation, it will be of interest to see how their lungs' clearance compares with larger mammals. Work on this research takes advantage of the current NIEHS Center *Facility Core B: In vitro and In vivo Imaging and Analysis*, with particular emphasis on the PET scanner.

The research concerned with health problems associated with ozone exposure, especially those concerned with factors underlying susceptibility, continues to be a major focus of this Research Core. The work showing an important role for the Toll-like receptor is being carried out by Drs. Reddy and Kleeberger. The effect of genetic regulation is actively being pursued by Dr. Tankersley. His work has involved the mechanism of the role of inhaled particulates and ozone in a unique mouse genetic model. This work is being paralleled with experiments in culture and animal models by Dr. Spannhake. These studies incorporate the very important interaction of viral infections with airborne pollutants, especially ozone. Dr. Spannhake works largely in cell cultures, and new studies are investigating how ozone affects intracellular adhesion molecules, lipid metabolism and mediator synthesis by the airway epithelium. These experiments are being carried out in a novel culture chamber built by our Instrumentation Core that allows exposure of cultured cells to selected uniform concentrations of ozone. These studies have direct impact on our understanding of environmental asthma, a disease that is actively being studied by Dr. Eggleston in the Baltimore community. Discussions with Dr. Eggleston about potential new biomarkers are ongoing.

A major research effort of this group focuses on an understanding of the basic regulatory mechanisms of airway epithelium and smooth muscle. Much pulmonary disease as well as acute responses to inhaled pollutants involves contraction of airway muscle and subsequent dyspnea. Although diseases like asthma are triggered by allergic and inflammatory stimulation, it is important to recognize that by itself, this would not lead to the salient feature of an asthmatic attack. Without the physiologic end point of airway smooth muscle contraction, asthma might not be the terrible disease that it is. Thus, it is essential to have a solid understanding of the underlying normal physiology of airway smooth muscle and epithelium in order to be able to understand not only asthma, but also the dyspnea and emphysema caused by urban environmental pollutants. It is a major strength of the NIEHS Center that we can readily draw upon the expertise available to analyze the effects of inhaled pollutants on respiratory tissues. Mitzner's work in recent years has been to develop a new imaging method to measure the reactivity of airways in vivo. This work allows analysis and even reconstruction of the airway tree from in vivo HRCT images. This approach uses a method to assess local heterogeneity of the airways in response to a host of challenges and insults. Experimental work has for

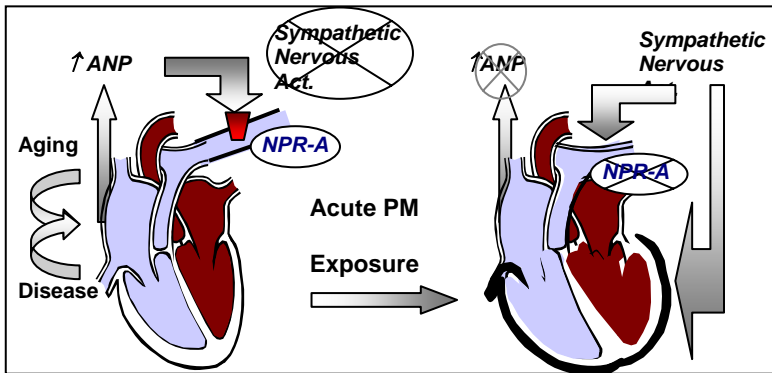
the first time shown the ability to localize the effect of ozone induced inflammation in the airway tree. This intra-programmatic collaborative study with Dr. Brown will continue in the coming year in an effort to understand the heterogeneous response already observed in individual airways in vivo following acute ozone exposure.

Since the epithelium is the first lung tissue attacked by pollutants, knowledge of normal epithelial function is essential. Aberrant cell proliferation and differentiation following toxic injury to bronchial epithelium can lead to the development of various respiratory diseases including lung cancer, but the underlying molecular mechanisms involved in such processes remain enigmatic. The co-director of this Research Core, Dr. Reddy, is working on gene expression and regulation during toxicant induced airway epithelial injury-repair and transformation. Exposure to cigarette smoke (CS) can lead to the development of lung cancer and other respiratory diseases such as emphysema, but the molecular mechanisms underlying these processes remain unclear. Given that activator protein 1 (AP-1) regulates genes involved in both physiological and pathophysiological processes, Dr. Reddy lab is investigated the effects of CS on Jun and Fos family member expression and regulation in pulmonary epithelial cells. Exposure to CS caused a marked upregulation of c-Jun, c-Fos, and Fra-1, but not of Fra-2, Jun-B, and Jun-D expression. Because Fra-1 is overexpressed in various tumors and upregulates genes associated with airway squamous metaplasia and tumor progression, his lab further elucidated the mechanisms that control CS-stimulated fra-1 induction. Currently, his lab is using both in vivo and in vitro models to understand the role and biology of AP-1 family of transcriptional factors and upstream signaling pathways in respiratory pathogenesis. Oxidative stress plays a major role in hyperoxia-induced acute lung injury. Reddy and Steve Kleeberger (who is now at NIEHS) laboratories previously showed that mice lacking the NF-E2-related transcription factor 2 (Nrf2) are more susceptible to hyperoxia than wildtype mice. Nrf2 activates antioxidant response element (ARE)-mediated gene expression involved in cellular protection against toxic insults. Currently, in collaboration with Kleeberger, his lab is investigating the mechanisms that control activation of Nrf2 by hyperoxia. Dr. Reddy's work on basic molecular and cellular mechanisms on bronchial carcinogenesis and oxidant induced lung injury compliments ongoing studies of Dr. Biswal, who is working on chemoprevention against acrolein induced lung cancer development and cigarette smoke promoted respiratory pathogenesis, such as emphysema. In addition, this new Core's interest in potential control of lung tumors is directly related to new studies by Mitzner, Wagner, and Biswal (, which involve a mouse model of lung angiogenesis. A recently funded pilot project is involved with using the Affymetrix gene chips to investigate the temporal and differential expression of genes in a well controlled mouse model. Based on this pilot data, an NIH RO1 was recently funded with a 1.1% priority.

A major new initiative has begun to investigate the reasons underlying the increased morbidity and mortality associated with increased airborne particulates. Although the epidemiology is quite convincing, the underlying mechanisms are not at all clear. Two years ago we began a NIEHS Center supported informal discussion group to present articles and experimental data related to particle exposures in humans and experimental models. Dr. Tankersley's most recent data shows a potentially important role of Atrial Natriuretic Peptide in the cardiac response to airborne particulate matter. This pilot data has formed the basis of an RO1 application that was recently funded with high priority from NIH.

Inbred mice have been routinely used in studies of genetic effects that determine behavioral variation due to circadian rhythm. In addition to activity patterns (Act), we aimed to characterize variations in the circadian rhythm of deep-body temperature (T(db)) and heart rate (HR) in a specific genetic model of differential cardiorespiratory control. Radiotelemeters were implanted in C3H/HeJ (C3; n = 11) and C57BL/6J (B6; n = 11) inbred strains. Reciprocal first-generation offspring, B6C3F1/J (B6F1; n = 8) and C3B6F1 (C3F1; n = 3) mice, were included to initiate an evaluation of heritable phenotypes. Mice were housed individually in a facility maintained at 23-24 degrees C, and the light-dark cycle was set at 12-h intervals. In each animal, repeated measurements were obtained at 30-min intervals, and the circadian patterns of Act, T(db), and HR were assessed by novel statistical methods that detailed the periodic function for each strain. During the dark phase, B6 mice demonstrated two distinct peaks in Act and T(db) relative to a single early peak for C3 mice. In contrast to the parental strains, B6F1 and C3F1 mice demonstrated intermediate second peaks in Act and T(db). With respect to HR, the C3 strain demonstrated a significantly ( $P < 0.01$ ) greater daily average compared with B6 mice. The circadian rhythm in HR differed significantly from the Act and T(db) patterns in B6

mice (but not in C3 mice); that is, the periodicity in HR for B6 mice preceded the rise and fall in Act and T(db) during both peaks. The B6 phenotype was also observed in F1 mice. In conclusion, these data suggest that the circadian regulation of Act, T(db), and HR vary significantly among C3, B6, and F1 mice. Furthermore, phenotypic differences between C3 and B6 strains can be used to explore the genetic basis for differential circadian regulation of body temperature and HR (46).



**Figure 9 The cardioprotective mechanisms of ANP reduces right ventricular stress by lowering blood volume and by pulmonary vascular dilation. Aging causes a progressive loss of the protective role of ANP making the elderly susceptible to the effects of acute PM exposure on the heart.**

Elderly populations face greater risks of mortality when exposed to changes in environmental stress. The purpose of the following study was to develop an age-dependent susceptibility model that achieved the following three goals: 1) to operationally define homeostasis by assessing the stability and periodicity in physical activity, heart rate (HR), and deep body temperature (T(db)), 2) to specify alterations in activity, HR, and T(db) regulation that signal imminent death, and 3) to test the hypothesis that the decay in homeostasis associated with imminent death incorporates the coincident disintegration of multiple physiological systems. To achieve these goals, the circadian regulation of activity, HR, and T(db) was assessed using radiotelemeters implanted in AKR/J (n = 17) inbred mice at approximately 190 days of age. During a 12:12-h light-dark cycle,

weekly measurements were obtained at 30-min intervals for 48-h periods until each animal's natural death. The average (+/-SE) life span of surgically treated animals did not differ from untreated controls (319 +/- 12 vs. 319 +/- 14 days). Cardiac and thermal stability were characterized by a circadian periodicity, which oscillated around stable daily averages of 640 +/- 14 beats/min in HR and 36.6 +/- 0.1 degrees C in T(db). Stable HR and T(db) responses were compared with extreme conditions 3 days before death, during which a disintegration of circadian periodicity was coincident with a fall in the daily average HR and T(db) of approximately 29 and approximately 13% lower (i.e., 456 +/- 22 beats/min and 31.7 +/- 0.6 degrees C), respectively. The results further suggested that multiple predictors of cardiac and thermal instability in AK mice, including significant bradycardia, hypothermia, and a loss of circadian periodicity, forecast life span 5-6 wk before expiration (47).

Because epidemiology studies consistently identify the elderly at risk for air pollution-related morbidity and mortality, we developed a model of senescent-dependent susceptibility based on indices of physiological aging. In the current study, we hypothesized that heart-rate regulation during PM exposure differs with senescence-dependent susceptibility owing to variation in autonomic nervous control. Heart rate (HR) and heart-rate variability (HRV) parameters were measured from 162 samples of 2-min electrocardiograph (ECG) recordings in age-matched healthy (n = 5) and terminally senescent (n = 3) AKR mice during 3-h exposures to filtered-air (FA, day 1) and carbon black (CB, day 4; <200 µg/m<sup>3</sup>). On day 1, HR was significantly (p <.01) depressed during FA in terminally senescent mice. By day 4, HR was further slowed significantly (p <.01) due to the effects of CB exposure for 3 days. The combined effects of terminal senescence and CB exposure acted to depress HR to an average (+/-SEM) 445 +/- 40 bpm, or approximately 80 bpm lower compared to healthy HR responses. The change in rMSSD, an HRV parameter corresponding to relative influences of parasympathetic tone on HR, was significantly (p <.01) greater on day 1 and day 4 in terminally senescent mice compared to healthy mice. In contrast, the LF/HF ratio, an HRV parameter derived from spectral analysis indicating relative changes in cardiac sympathetic tone, was significantly (p <.01) depressed in terminally senescent mice on day 1. By day 4, significant increases in LF/HF were evident in healthy mice during CB exposure, suggesting that HR regulation was associated with an increase in sympathetic tone. Alternatively, terminally senescent mice appeared to modulate a lower HR without change in LF/HF ratio during CB exposure, suggesting an absence of sympathetic tone. In conclusion, older healthy mice increase cardiac sympathetic tone during PM exposure while terminally senescent mice show a greater PM-induced

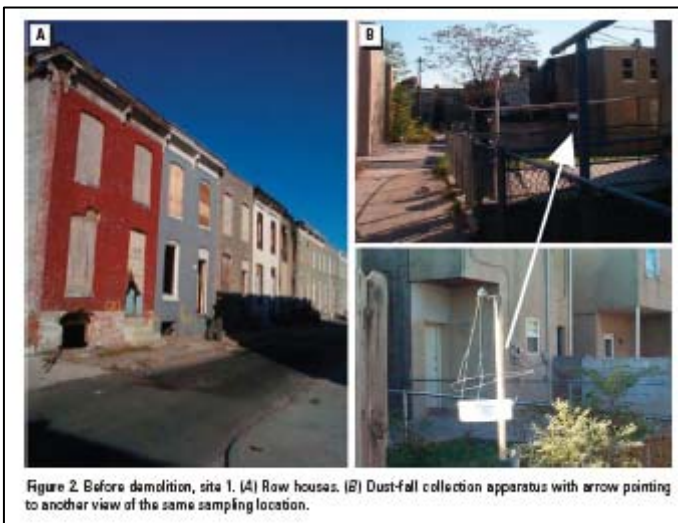
parasympathetic tone in regulating HR. The significance of the current results suggest that PM-induced HR regulatory changes may ultimately depend on the degree of physiological aging (48).

### **Research Foci 3: Public Health Interventions and Prevention**

The *Public Health Interventions and Prevention Research Focus* group, led by Dr. Paul Strickland, fosters the development and validation of biomarkers for exposure, effect, and susceptibility in experimental and human investigations. These tools are applied in studies of disease etiology and evaluation of preventive interventions in at-risk populations. Investigating the etiology of many diseases of current interest has proved challenging for conventional epidemiologic approaches. The temporal sequence of causation and disease can be particularly difficult to investigate. In some diseases, such as cancer, multiple etiological agents act at different points during a multi-stage progression from the earliest stages to diagnosis. Further, intrinsic susceptibility factors may greatly affect individual risk and the time course of disease development. Extensive research on biomarkers linking exposure and susceptibility to risk of disease is now in progress. Validation of biomarkers requires studies in both experimental systems and in exposed human populations. This need forms the basis of a number of collaborations between members of this group and the other research foci in the Center. Thus, researches among the faculty in this program serves to bridge and complement the disciplines of epidemiology, toxicology, and exposure assessment in order to develop tools for identifying individuals at-risk for the development of disease. It is the ultimate objective of the Core investigators to translate their scientific findings into prevention strategies in community settings, especially in urban populations and some examples of this work is found in the following sections that relate to the translation to risk assessment.

#### *Demolition and Community Air Quality Impacts*

Reports about current residential demolition practices received from residents and plans for large-scale urban redevelopment in East Baltimore provided impetus for a collaborative study with the COEC to assess community concerns and develop approaches to addressing them. Dr. Farfel and Lees working with the COEC developed the study regarding residents' experiences with demolition and gut rehabilitation of older housing performed as part of urban redevelopment. Issues examined included; lack of notification and awareness about protective measures, concerns about environmental and safety hazards, psychosocial impact from displacement, disruption in daily life, and inattention to community concerns and recommendations to improve redevelopment practices, including ideas to control neighborhood exposure to environmental hazards potentially exacerbated by residential demolition and gut rehabilitation. The findings from focus groups substantiated and deepened an understanding of earlier anecdotal reports of residents' concerns and emphasized the need for including community perceptions and ideas in addressing environmental and psychosocial issues related to urban redevelopment (49).



**Figure 10**

removal to 61  $\mu\text{g Pb/m}^2/\text{hr}$  (440  $\mu\text{g Pb/m}^2$  per typical work day). Lead concentrations in dust fall also increased

Demolition of older housing for urban redevelopment purposes benefits communities by removing housing with lead paint and dust hazards and by creating spaces for lead paint-free housing and other community resources. However, given the evident health concerns from contaminated dusts, a study was conducted to assess changes, if any, in ambient dust lead levels associated with demolition of blocks of older lead-containing row houses in Baltimore. The results of dust-fall samples collected from fixed locations within 10 m of three demolition sites were characterized to determine dust lead changes on streets, sidewalks, and residential floors within 100 m of the demolition sites (see accompanying Figures). Geometric mean (GM) lead dust-fall rate increased by greater than 40-fold during

demolition to 410  $\mu\text{g Pb/m}^2/\text{hr}$  (2,700  $\mu\text{g Pb/m}^2$  per typical work day) and by more than 6-fold during debris