1494 FUMONISIN DISRUPTION OF SPHINGOLIPID METABOLISM AND ITS IMPLICATIONS IN HUMAN AND ANIMAL DISEASE

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The structural similarity of the sphingoid base sphinganine (Sa) and fumonisin B_1 (FB_1) led to the hypothesis that fumonisin's mechanism of action involves disruption of sphingolipid metabolism or function. FB_1 competitively inhibits the acylation of Sa and sphingosine (So) by ceramide synthase in cells *in vitro*, animals, plants and fungi and increased Sa concentrations and extent of target tissue injury have been repeatedly correlated in animal experiments. The extent to which sphingoid bases and their 1-phosphate and other metabolites accumulate in cells or tissues depends on the relative activities of the enzymes involved in the biosynthetic and turnover pathways. In rats, FB_1 exposure causes the amounts of free Sa and sphinganine 1-phosphate to increase rapidly in tissues, especially kidney. Once accumulated in tissues, the free sphingoid bases persist and their elevated concentrations therein can be prolonged by the administration of low, sub-threshold doses of FB_1. Furthermore, sphinganine 1-phosphate also accumulates in blood (rat) and might, like sphingosine 1-phosphate, act directly on sphingosine 1-phosphate receptors (S1PR, formerly EDG receptors) to affect critical pathways dependent on S1PR signaling. Disrupted sphingolipid metabolism also leads to imbalances in phosphoglycerolipid metabolism, inhibition of ceramide biosynthesis, and depletion of complex sphingolipids formed from ceramide. The latter contributes to FB_1-induced cytotoxicity and affects lipid raft functions including vitamin transport and cell-cell and cell-substratum contact. Thus, inhibition of ceramide synthase by FB_1 causes a wide spectrum of changes in lipid metabolism that in turn affect multiple lipid-dependent functions in a manner that could account for the cell damage and diseases caused by fumonisins.

1495 FUMONISIN B1-INDUCED INHIBITION OF FOLATE RECEPTOR-MEDIATED VITAMIN UPTAKE

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Folate is an essential nutrient critical for a variety of cellular processes involving one-carbon metabolism. Folate deficiency has been linked to an increase risk of neural tube defects (NTD) in the developing fetus and heart disease and cancer in mature individuals. Adequate folate nutrition as well as competent mechanisms for uptake of this vitamin into cells is required to maintain the necessary levels of intracellular folate. The transport of folate from serum into many tissues with high requirements for this vitamin, including the placenta, is mediated by a high-affinity, glycosylphosphatidylinositol (GPI)-anchored transporter known as the folate receptor. Formerly referred to as the folate binding protein, the folate receptor mediates vitamin uptake into the cell via fluid phase endocytosis. Like other GPI-anchored proteins, the folate receptor resides in membrane microdomains commonly referred to as lipid rafts. These rafts are enriched in cholesterol and sphingolipids and can affect the function of proteins found in them.

In cultured cells, fumonisin B1-induced inhibition of ceramide synthase significantly altered cellular sphingolipid levels and decreased folate receptor-mediated vitamin uptake into the cell. Alterations in both the endocytic trafficking and the amount of the receptor that is available for transport contributed to the inhibition of the folate receptor. In cultured mouse embryos, exposure to fumonisin B1 resulted in a dose-dependent increase in the number of embryos exhibiting NTDs. Concurrent alterations in levels of sphingoid bases were also found. Supplementation of the embryos with high levels of folate during the fumonisin B1 treatment reduced the incidence of NTDs without correcting the sphingolipid abnormalities, suggesting that the mycotoxin influenced neural tube maturation by affecting the function of the folate receptor. These findings provide a conceptual framework to account for how exposure to fumonisin B1 could increase the risk of NTDs by disrupting sphingolipid-dependent lipid rafts and, consequently, folate receptor function.

1496 MATERNAL FUMONISIN EXPOSURE AND NEURAL TUBE DEFECTS: MECHANISMS IN A MOUSE MODEL

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An association between maternal exposure to the mycotoxin Fumonisin B1 (FB1) and increased risk for neural tube defects (NTDs) has recently been observed in human populations that rely on corn as a dietary staple. FB1 inhibits the enzyme ceramide synthase, resulting in elevated sphinganine levels, and depletion of downstream glycosphingolipids. The GPI-anchored folate receptor co-localizes with ganglioside GM1 in lipid rafts, and FB1-induced depletion of GM1 compromises folate uptake. Early gestational exposure to FB1 results in NTDs in 80% of inbred LM/Bc mouse fetuses. Daily maternal folate supplementation reduces the incidence of NTDs, while replacement therapy with GM1 restores folate uptake and rescues the phenotype. Embryos with FB1-induced NTDs demonstrate TNF? expression in the ventricular zone of the open neural tube that corresponds to positive staining for inducible nitric oxide synthase (iNOS) and TUNEL (apoptosis). Microarray data indicate altered expression of cytokines, as well as numerous genes involved in sphingolipid metabolism and redox homeostasis. Several genes involved in pterin metabolism are downregulated following FB1 exposure, including key enzymes in the tetrahydrobiopterin (BH4) de novo biosynthesis and salvage pathways. BH4 is an important co-factor for iNOS, and inadequate BH4 levels result in the production of reactive oxygen species (ROS), reactive nitrogen species (RNS), protein nitrosylation, and oxidative stress. FB1-induced activation of cytokines and iNOS, coupled with reduced BH4 levels, results in oxidative stress and increased apoptosis in the developing neural tube. Maternal supplementation with BH4, or folic acid, a structurally related pterin compound, prevents nitrosylation of proteins in the open neural tube, and effectively rescues the phenotype. The results suggest that folate may functionally substitute for BH4, and protect against FB1-induced NTDs through a novel mechanism involving maintenance of redox homeostasis.

1497 MODELS AND MECHANISMS OF OCCUPATIONAL/ENVIRONMENTAL ASTHMA

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Occupational asthma is defined as variable airflow limitation and bronchial hyperresponsiveness due to causes and conditions encountered in a workplace environment. A myriad of substances are responsible for occupational asthma, including low molecular weight chemicals and protein allergens. Though asthma is treated as a single lung disorder, it is increasingly clear that the pathophysiology of asthma may differ substantially depending on the allergen, exposure conditions and environmental triggers. Diagnostic, immunologic and genetic factors associated with disease development are being actively investigated. Animal models for occupational asthma are being developed to define mechanisms of this lung disorder and determine more effective methods for prevention, diagnosis and treatment of occupational asthma. Both toluene diisocyanate and trimellitic anhydride are prototype low molecular weight chemicals that cause asthma in the workplace, and studies defining the effector mechanisms for these allergens are being investigated. Environmental factors such as ozone and particulate air pollution can exacerbate existing asthma and profoundly influence the pathophysiology and clinical outcome. Understanding the interactions of environmental and genetic factors is also important to define potential populations at risk. Continued studies in the workplace and in animal models are needed to more clearly diagnose, define the pathophysiology and triggers, and effectively treat this important public health problem.

1498 PATHOGENESIS OF OCCUPATIONAL ASTHMA

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Occupational asthma (OA) is variable airflow limitation and/or airway hyperresponsiveness due to causes and conditions encountered in a particular work environment. This disorder can be subclassified mechanistically into those OA disorders caused by immunologic and non-immune mechanisms. Non-immunologic OA refers to irritant induced OA or reactive airway dysfunction syndrome (RADS) induced by short-term inhalation of toxic substances. Most attention, however, has been focused on understanding the immunopathogenesis of OA caused by specific workplace respiratory sensitizers. Classic IgE mechanism explains OA caused by organic (i.e., plant or animal derived) occupational allergens that represent 1/3 of new cases. However, the vast majority of new cases are caused by chemicals, and with few exceptions, mechanisms explaining respiratory sensitization have not been elucidated. The acid anhydride class of chemicals is one exception; these agents act as haptens, which readily form allergens by combining with tissue proteins (e.g., human serum albumin) and elicit IgE mediated asthma. The isocyanates are sensitizers and major causes of OA that have been widely investigated. This discussion will focus on postulated allergic and genetic mechanisms of OA caused by chemicals and on the potential roles of in vitro and in vivo tests in the diagnosis of OA.

1499 USING MURINE MODELS TO UNDERSTAND THE IMMUNOLOGIC BASIS OF TOLUENE DIISOCYANATE ASTHMA

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Diisocyanates are the leading cause of occupational asthma afflicting between 5 and 15% of exposed workers. Despite this prevalence, the immunological basis for this disease remains poorly understood. Whether toluene diisocyanate (TDI) asthma is driven by TH2 responses, if TH1 responses are important, and the role of specific antibodies in disease pathogenesis are a few of the unanswered questions. We have developed a murine model in which mice become sensitized following long-term, low level inhalation exposure to TDI vapor. Sensitized mice display characteristic asthma symptoms upon challenge, including airway hyperreactivity (AHR), airway inflammation and antibody production. In addition, mice show elevated lung expression of TH2 and TH1 cytokines. Genetically modified mouse models and antibody neutralization approaches have suggested important roles for TH2 and TH1 responses as well as antibody production. All disease symptoms were reduced in CD4 and CD8 deficient mice. IL-4 and IL-13 neutralization showed moderate effects on all disease endpoints whereas combined depletion of both cytokines ablated disease. Mice deficient in IFNγ showed a marked decrease in AHR but only minor effects on lung pathology, cytokine production and antibody levels. These findings show that both TH2 and TH1 responses are important. The ability of serum to transfer disease and attenuation of disease in Fc receptor deficient mice underscore the importance of antibodies in TDI asthma. This model also demonstrated an important role for pro-inflammatory cytokines. Prevention of TNF or IL-1 signalling reduced inflammation and AHR. Importantly, inhibition of IL-1 prevented TDI-induced increases in adhesion molecules required for recruitment of inflammatory cells to the lung. Therefore, it is likely that pro-inflammatory cytokines are early mediators of disease and may be involved in the initial recruitment of inflammatory cells to the lung during the effector phase of the disease. In summary, these studies demonstrate distinct and overlapping roles for TH1, TH2 and antibody responses and pro-inflammatory cytokines in TDI asthma.

1500 DIFFERENTIAL GENE EXPRESSION AND EFFECTOR MECHANISMS IN MURINE MODELS OF TRIMELLITIC ANHYDRIDE AND OVALBUMIN-INDUCED ASTHMA

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Numerous allergens cause occupational asthma including trimellitic anhydride (TMA), a small molecular weight chemical, and ovalbumin, a reference protein allergen. Heterogeneity in asthma has long been recognized and many clinical variations have been described relating to occupational exposure, age of onset, gender, association with atopy, type of airway inflammation, etc. Different allergens may also contribute to the heterogeneity in asthma by setting into motion unique effector pathways leading to asthma symptoms. To determine if different effector mechanisms are responsible for asthma depending on the allergen, mice were sensitized with TMA or ovalbumin, followed by intratracheal challenge with TMA conjugated to mouse serum albumin or ovalbumin, respectively, to elicit the allergic phenotype. The same significant increase in lung eosinophilia was seen after challenge with either allergen. Using Affymetrix gene arrays, a subset of genes expressed in common for both allergens reflects a common effector pathway for asthma, regardless of the allergen. However, distinct transcriptional signatures were also noted in lungs of mice challenged with ovalbumin versus mice challenged with TMA, with the same eosinophil infiltration. In particular, differences in arginase and chemokine gene expression with the two allergens are being investigated, and suggest alternate signaling pathways and cellular involvement may contribute to asthma heterogeneity with different allergens.

1501 THE ROLE OF OXIDATIVE STRESS IN THE EXACERBATION OF ASTHMA BY PARTICULATE AIR POLLUTION

A. E. Nel. *Medicine, UCLA, Los Angeles, CA.* Sponsor: J. Regal.

Diesel exhaust particles (DEP) and ambient particulate matter (PM) induce reactive oxygen species (ROS)and oxidative stress which can lead to a number of adverse health effects, including exacerbation of asthma. Previous studies have demonstrated that the impact on asthma includes an adjuvant effect, an increase in already existing airway inflammation, and exacerbation of airway hyperreactivity. In order to explore the mechanistic link between oxidative stress and airway inflammation, we made use of the strengths of proteomics to do comprehensive discovery of the biology of oxidative stress. ROS generation by pro-oxidative PM chemicals induce a hierarchical oxidative stress response, in which a protective antioxidant defense responses upgrade to pro-inflammatory and cytotoxic effects as the level of oxidative stress increases. These responses are relevant to asthma as follows: The antioxidant defense pathway is mediated by the transciption factor, Nrf2, and leads to the expression of phase II enzymes. Weakening or polymorphisms of this mechanism could determine who is susceptible to pro-oxidative effects of PM. At the level of pro-inflammatory effects, PM perturb signaling cascades that impact cytokine and chemokine production, as well as change the function of dendritic cells (DC). The functional impact on DC change their role as antigen presenting cells, which can explain the role of PM as adjuvants in the immune system. The role of PM-induced oxidative stress provides a logical avenue for treatment of some asthmatics.

1502 GENETIC SUSCEPTIBILITY AND OZONE EXACERBATION OF ALLERGIC ASTHMA

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Asthma morbidity and mortality has increased in industrialized countries at an alarmingly high rate for the last 20 years. Rates are disproportionately high in urban environments and in children, but the causes are unknown. It is clear that genetic background is an important determinant of asthma, and it is a complex quantitative disease (i.e., multigenic). The complexity stems from the number of subphenotypes that characterize the disease, including airways hyperresponsiveness, inflammation, and allergic status. A gene or set of genes may confer susceptibility or predisposition to each of these phenotypes. Asthmatics are also known to be particularly susceptible to the exacerbating effects of air pollutants such as ozone and particulate matter (PM2.5 and ultrafine). However, the mechanisms through which air pollutants exacerbate asthma and asthma subphenotypes are not clearly understood. An emerging concept is that genes that predispose to inflammation and injury induced by air pollutants are also important determinants of asthma. Candidate genes that are critical in inflammation (e.g., tumor necrosis factor alpha, Tnf), innate immunity (e.g., toll-like receptor 4, Tlr4), and antioxidant defense (e.g., glutathione S-transferase M1, Gstm1; NF-E2 related factor 2, Nrf2) pathways are currently being tested in animal models and human populations. Understanding the interaction of environmental and genetic factors important in asthma will lead to improved evaluation of risk of a specific genotype(s) for a given environmental exposure, and a potential means for intervention to prevent asthma and asthma exacerbation.

1503 OBESITY AS A MODULATOR OF CHEMICAL TOXICITY

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Obesity as a Modulator of Chemical Toxicity
In the last few decades the overweight and obese in the US have grown from a relatively minor subpopulation to ~60% of all adults. Even more alarming is the dramatic increase in the number of overweight and obese children. This obesity epidemic is linked to increases in the incidences in cardiovascular disease, diabetes mellitus and certain forms of cancer. Adipose tissue is no longer considered a static depot of excess energy reserves but an endocrine-active tissue. Increases in adipose tissue, particularly abdominal fat lead to features of insulin resistance syndrome, also referred to as metabolic syndrome X. This syndrome consists of a clustering of metabolic abnormalities that increase the risk for type 2 diabetes and cardiovascular disease. Adipose tissue also secretes a number of cytokines leading to a low inflammatory state in the obese. Additionally, obesity is associated with higher levels of insulin, insulin-like growth factor I and oxidative stress that are mechanistically linked to carcinogenesis. Given that chemical toxicity is often dependent on the same factors that are at increased levels in the obese, obesity may lead to an environment of increased sensitivity to chemical-induced toxicity. In this symposium, the physiological, biochemical and molecular mechanisms that lead to metabolic syndrome X and changes in growth factors and cytokine secretion in the obese will be discussed. A number of examples in rodent models will be given, in which obesity clearly alters chemical toxicity in a number of tissues. The information presented in this symposium will highlight the fact that the obese should be evaluated as a sensitive population.

1504 OBESITY AS A MODIFIER OF CHEMICAL TOXICITY - HISTORICAL PERSPECTIVE

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Over the past decades, adults classified as overweight or obese in the US have grown to approximately 60% of the population. This is now considered by many to be an epidemic. Obesity decreases life span, in part through its association with increased liver, kidney, and cardiovascular diseases, diabetes mellitus, and certain cancers. Adipose tissue, once considered a relatively static energy depot, is now recognized as an endocrine-active tissue. Excess abdominal fat correlates with insulin resistance syndrome or metabolic syndrome X, a clustering of metabolic abnormalities that increase the risk for type 2 diabetes and cardiovascular disease. Adipose tissues secrete cytokines that contribute to a chronic low-grade inflammatory state. Obesity

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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, and poster sessions of the 45th Annual Meeting of the Society of Toxicology, held at the San Diego Convention Center, San Diego, March 5–9, 2006.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 500.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 534.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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