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Environmentally-induced Malignancies: An In Vivo Model to Evaluate the Health Impact of Chemicals in Mixed Waste

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Research Objective

Increased risk of malignancy following exposure to genotoxic agents in the environment is a major public concern. Exposure to radiation, benzene, and organic solvents is associated with an increased risk of leukemia; however the mechanism of leukemogenesis is unknown. We postulate that chemical(s) that increase the rate of genomic instability and induce hematotoxicity will promote accumulation of genetically-damaged hematopoietic stem cells (hsc), and thus contribute towards development of environmentally-induced hematologic malignancy. We will use molecular and cellular approaches to establish the relationship between hematoxicity, genomic instability and production of genetically aberrant hsc and progeny in mice exposed to radiation, benzene and trichloroethylene (TCE). The goals of this project are to

1) Determine whether recruitment of hsc into cycle by agents that induce hematotoxicity (i.e., pancytopenia, anemia) facilitates fixation of genetic damage in hsc exposed to environmental genotoxins in vivo.
2) Determine whether environmental genotoxins with leukemogenic potential disrupt hsc genomic integrity by inactivating cell cycle checkpoints.
3) Determine whether low dose exposures to agents that induce chronic pancytopenia/anemia and/or cyclic hemopoiesis increase fixation of genetic damage in hsc.

Increased understanding of the relationship between genotoxicity, hematotoxicity and genomic instability will a) lend insight into mechanisms underlying environmental-induction of leukemic progression, b) facilitate development of a rationale to identify chemical combinations which synergize to increase or decrease leukemogenic potential, and c) provide opportunities to optimize approaches for biomonitoring and risk assessment.

Research Progress and Implications

This report summarizes work after 1.5 year of a 3 year project. Accomplishments to-date include demonstration that the cycling status of hemopoietic stem cells at the time of genotoxin exposure alters the frequency and persistence of genetically damaged hemopoietic stem cells and associated progeny (Aim #1), development of assays to measure genomic instability in hemopoietic stem cells and associated progenitors (Aim #2) and quantification of genomically aberrant hsc and progeny following radiation, benzene and TCE exposures (Aim #2). Mice with varying susceptibility to leukemogenesis have been exposed to combinations of radiation, benzene and TCE to allow assessment of the cellular and genomic perturbations associated with leukemogenesis.

Hsc are quiescent cells in the marrow responsible for lifetime maintenance of blood cell production. Hsc can be activated and recruited into cycle by regulatory cytokines or reduced numbers of circulating cells. Genetic damage in quiescent hsc may be more readily repaired, thus preventing propagation of genetically damaged progeny. We postulated that recruitment of hsc into cycle by agents that induce hematotoxicity (i.e., pancytopenia, anemia) will increase the frequency of hsc with genetic alterations and consequently result in elevated levels of genetically aberrant hematopoietic cells that persist during lifetime hematopoiesis (Aim #1). We tested this hypothesis by recruiting stem cells into cycle using 5-fluorouracil prior to radiation and benzene exposure, and then measured the frequency of genetically aberrant hsc and circulating cells 6, 12 and 15 months later. Our data demonstrate that radiation and benzene induce genomic changes (translocations and aneusomies) that persist for

extended time periods after exposure. However, at 12-15 months post exposure, hsc that were in S-phase at the time of genotoxin exposure generated fewer genetically aberrant progeny than hsc that were not replicating DNA. The frequency of aberrant cells appears to be dependent upon the degree of cytopenia. We now postulate that persistent pancytopenia places a high demand on hsc replication and differentiation, and that differentiation of these cells to replace hematopoiesis results in eventual (i.e. by 6-12 months) elimination of the genetically damaged cells. New experiments to recruit hsc into cycle in the absence of cytotoxicity are underway to confirm this hypothesis.

These data have major implication for risk assessment. They demonstrate that persistence of genetically damaged blood cells is highly dependent upon the cycling status of hsc at the time of exposure. Thus, exposure to mixtures of chemicals and/or radiation that affect the cycling status of hsc are likely to have different long term health effects than single agent exposures. Importantly, in some situations, particularly those associated with hematotoxicity, high dose exposures may result in fewer genetically damaged cells than low dose exposures. The dose-response relationship of this phenomena needs further exploration.

Development of malignancy is associated with accumulation of multiple genetic alterations that result in dysregulated cell growth and differentiation. It has been suggested that multiple genomic aberrations arise because a cell becomes genomically unstable. Thus, we postulated that exposures to mixtures of genotoxins that induce genomic instability will result in accumulation of genetically aberrant cells, and in some cases cell cycle checkpoint inactivation (Aim #2). Conventional assays of genomic instability rely solely on in vitro exposures and have been applied primarily to epithelial cells. However, regulation of hematopoiesis is dependent upon complex interactions between hemopoietic cells and their microenvironment, thus, we desired to measure genomic instability after exposures in vivo. We have developed a two-step assay that measures the ability of hematopoietic stem cells to amplify regions of their genome and proliferate under selective pressure. Thus, induction of genomic instability in hsc in vivo can now be assessed using selective pressure cell culture conditions. We have verified that this assay is capable of detecting hsc with checkpoint inactivation that have lost one or both alleles of p53, a cell cycle checkpoint regulator. Evaluation of genomic instability in hsc induced by combinations of radiation, benzene, and TCE exposures in vivo are in progress and represent the first analyses of genomic instability in a rare cell subpopulation involved in leukemic progression.

The development of leukemia is a time-dependent process that requires not only the induction of genomic alterations in cells at risk of clonal expansion, but also the selective propagation of cells with genomic aberrations. We postulated exposure to low doses of chemicals and radiation in complex mixtures would increase the risk of leukemogenesis, and that the development of leukemia would be associated with genomic instability, hsc recruitment and accumulation of genetically aberrant cells (Aim #3). These studies are in the early stages. Mice with different genetic susceptibilities to development of leukemia have been exposed to radiation, benzene and TCE. Leukemia is expected to develop at 7-9 months post exposure. Thus, we are in the process of characterizing the early cellular and genetic changes that occur in hsc in animals exposed to these combinations of genotoxins.

Planned Activities

During the forthcoming 1.5 years we will work to complete studies in each of the aims. We will establish the *dose-dependence* of hsc recruitment, hematotoxicity, genotoxin exposure dose, and frequency of genetically aberrant cells. Although dose-response relationships were not part of the original experimental design, our unexpected findings that genotoxin exposure of hsc cycling in response to induced hematopoietic stress result may result in persistence of fewer genetically damaged blood cells warrants further investigation. The dose-dependence of our observations has major implications for risk assessment and health effects. These studies should be completed and submitted for publication by the end of 1998. We will also complete our studies to establish whether in vivo exposures to benzene, TCE and radiation induce genomic instability in hsc. These studies should be completed and submitted for publication by March of 1999. Finally, we will continue to characterize the genomic and cellular changes in mice exposed to mixtures of benzene, TCE and radiation as they develop leukemia during the next year.