

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

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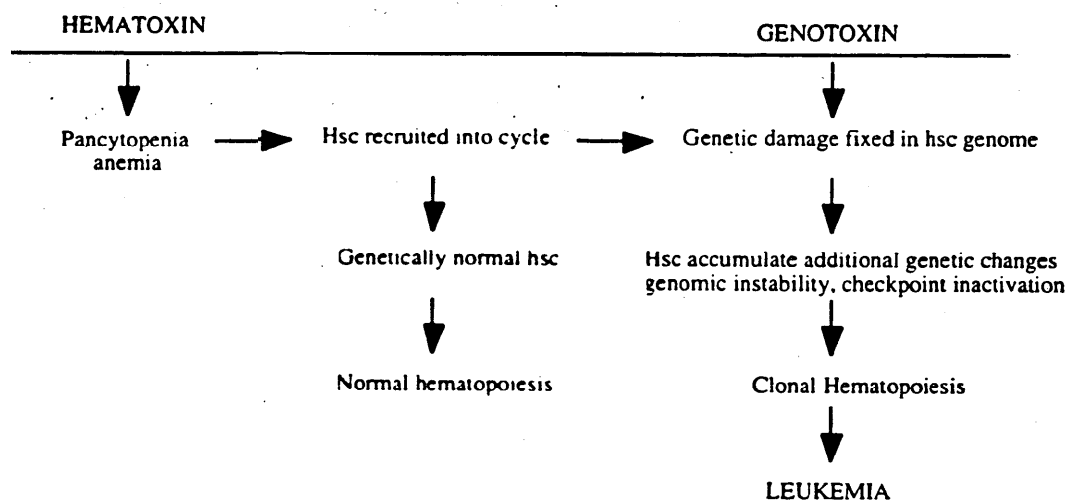
1.0 OBJECTIVE

Occupational or environmental exposure to organic ligands, solvents, fuel hydrocarbons, and polychlorinated biphenyls is linked to increased risk of developing leukemia, a blood cancer. The long term health effects of exposure to complex mixtures of chemicals and radionuclides are of particular concern because their biologic effects may synergize to increase risk of malignancy. Increased understanding of steps in the progression pathway of a normal cell to a cancer cell is important for biomonitoring, risk assessment and intervention in exposed individuals.

2.0 APPROACH

Leukemias are characterized by multiple genetic aberrations. Accumulation of multiple genomic changes may reflect genomic instability in the affected cells. Thus agents that induce DNA damage or genomic instability may increase accumulation of genomic alterations, thereby predisposing cells to transformation. However, not all DNA damaging agents predispose to transformation. Other factors such as genetic susceptibility, cell and tissue response to genotoxicity and cytotoxicity, DNA repair, etc. will impact malignant progression. We proposed a progression model (Figure 1) of environmentally-induced leukemia that can be evaluated using mouse models.

Figure 1 Model of Environmentally-Induced Leukemia



Critical components of this model include induction of genomic changes in stem cells (the population responsible for maintaining lifetime blood cell production), accumulation of additional genetic changes in stem cell progeny, and cell cycle check point inactivation.

Importantly, the model postulates that mixtures of chemicals that kill blood cells and are genotoxic will synergize to increase the frequency of cells with genomic changes, and 'thus increase the risk of transformation. Although stem cells are normally quiescent, they are recruited into cycle following hemotoxic insult, thereby facilitating generation of progeny with altered genomes. Chemicals that increase the rate of genomic instability in hemopoietic stem cells (hsc) are likely to increase the frequency of hsc and progeny with altered genomes.

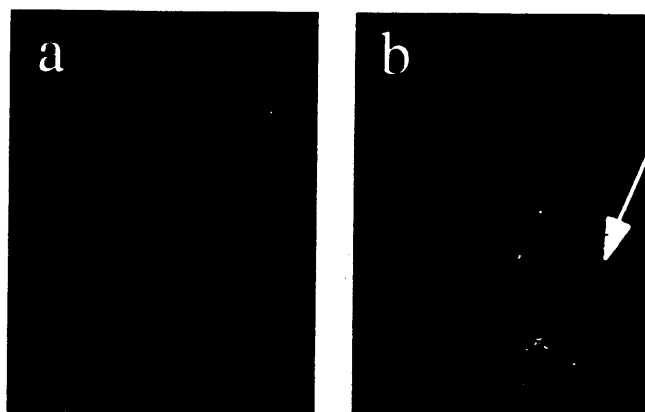
3.0 RESULTS TO DATE

This model assumes that the genetic alterations in leukemic blast cells will be present in stem cells, which are precursors to blast cells in normal hematopoiesis. Indeed, we demonstrated that stem cells in marrow aspirates from patients with leukemia carry similar genomic aberrations as the leukemic cells. We next determined whether exposure to radiation induces genomic changes in stem cells in mice. In these studies, we isolated stem cell populations from bone marrow using cell sorting from mice that were exposed to radiation one year prior to harvest. Genomic damage was measured using fluorescence *in situ* hybridization to detect translocations, stable structural chromosomal aberrations in the isolated stem cell populations and lymphocytes. We demonstrated that few stem cells from mice which do not develop radiation-induced leukemia carry translocations. Studies are underway to measure translocations in stem cells of mice that are genetically susceptible to radiation and benzene-induced leukemia.

The model postulates that combinations of agents that induce hematotoxicity with those that are genotoxic will recruit stem cells to proliferate and thus induce genetic damage to be propagated in stem cell progeny. To test this hypothesis, we treated mice with 5-fluorouracil (5-FU), a cancer chemotherapeutic reported to recruit cells into cycle. 5-FU treated and untreated controls were then irradiated and translocations measured 3 months thereafter. We postulated that mice that received radiation + 5-FU would carry more translocations than those without stem cell recruitment. Although the frequency of lymphocytes with chromosome 11 translocations was increased compared to chromosome 2, the lymphocyte translocation frequency was similar with both treatments. Subsequent measurements of the fraction of proliferating stem cells in 5-FU treatment mice revealed that stem cells were not recruited into cycle using our treatment regime. A different drug-radiation schedule to recruit stem cells was developed and is now being evaluated in our laboratory.

We postulated that benzene and its metabolites will induce translocations in hematopoietic cells in a dose-dependent manner. We measured translocation frequency using fluorescence *in situ* hybridization to paint individual chromosomes and fluorescence microscopy to quantify the number of cells in which the painted chromosomes translocated to an unpainted chromosome.

An example of a chromosome painting in an unexposed cell and a cell exposed to benzene is shown in Figure 2a and b, respectively.

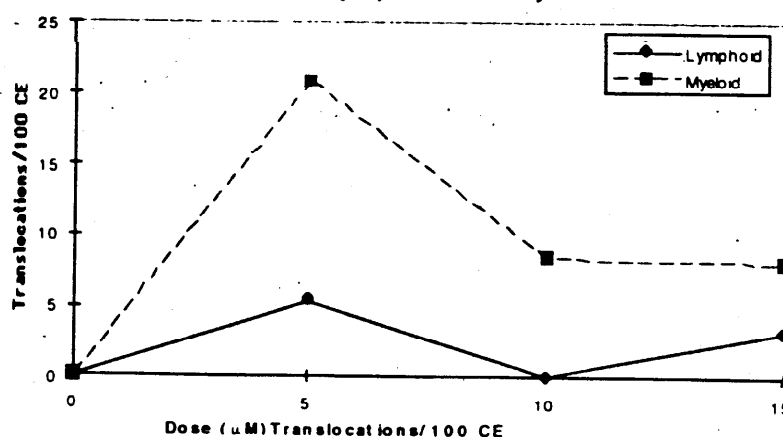


- a. Normal cell with chromosome 2 (red) and 11 (green).
- b. Benzene-exposed cell showing a chromosome 2 translocation

Figure 2. Translocations Detection using Chromosome Painting

Exposure to an active benzene metabolite induces translocations in hemopoietic cells (Figure 3). Myeloid cells show more translocations than lymphoid cells. Myeloid cells show more translocations than lymphoid cells. The translocation frequency appears to be maximum at dose of 5 μ M. Higher doses result in cell death.

Figure 3. 4-Hydroquinone Induces Translocations in Lymphoid and Myeloid Cells



Interestingly, benzene exposure is associated with myeloid, rather than lymphoid leukemias, thus it is intriguing to speculate that cells in the myeloid lineage may accumulate more DNA damage than other cell types.

Assays for cell cycle checkpoint inactivation in hemopoietic stem-cells are under development. Initial plans to use an enzyme inhibitor to assess checkpoint inactivation and genomic instability were not fruitful with hemopoietic cells. Thus, we turned to selective pressure using gamma interferon and TGF-B to isolate cells with a survival advantage and unstable genomes. These agents have been implicated recently in leukemia and myelodysplasia, a pre-leukemic condition in humans. These assays appear promising.

4.0 FUTURE WORK

Studies to be carried out during the next year will focus on evaluation of genomic instability and' checkpoint inactivation induced by benzene metabolites $\pm$ radiation using the aforementioned assays in exposed animals. Animals treated with benzene/radiation earlier in the project should be developing leukemias within- the forthcoming year and thus provide suitable systems to investigate regions of the genome that are altered in environmentally-induced leukemias.

5.0 SIGNIFICANCE

These studies will establish proof-of-principle whether agents associated with leukemias induce genomic changes in hemopoietic stem cells. Furthermore, we will determine whether exposure to two agents (benzene and trichloroethylene) common in mixed waste at DOE sites can synergize to increase the frequency of genetically damaged cells. These data will be useful to establish mechanisms that may lead to increased risk of leukemia in exposed individuals.