Molecular and Cellular Biochemistry **279:** 97–104, 2005.
DOI: 10.1007/s11010-005-8226-z

Leukemic transformation of hematopoietic cells in mice internally exposed to depleted uranium

Alexandra C. Miller,[1] Catherine Bonait-Pellie,[2] Robert F. Merlot,[2] John Michel,[2] Michael Stewart[1] and Paul D. Lison[2]

[1]*Applied Cellular Radiobiology Department, Armed Forces Radiobiology Research, Institute, Bethesda, Maryland, USA;*
[2]*Department of Occupational and Environmental Medicine, University of Paris, Paris, France*

Abstract

Depleted uranium (DU) is a dense heavy metal used in military applications. During military conflicts, US military personnel have been wounded by DU shrapnel. The health effects of embedded DU are unknown. Published data from our laboratory demonstrated that DU exposure *in vitro* can transform immortalized human osteoblast cells (HOS) to the tumorigenic phenotype. Results from our laboratory have also shown that DU is genotoxic and mutagenic in cultured human cells. Internalized DU could be a carcinogenic risk and concurrent alpha particle and heavy metal toxic effects complicate this potential risk. Anecdotal reports have suggested that DU can cause leukemia. To better assess this risk, we have developed an *in vivo* leukemogenesis model. This model involves using murine hematopoietic cells (FDC-P1) that are dependent on stimulation by granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin 3 (IL-3) and injected into mice to produce myeloid leukemia. Although immortalized, these cells are not tumorigenic on subcutaneous inoculation in mice. Intravenous injection of FDC-P1 cells into DU-implanted DBA/2 mice was followed by the development of leukemias in 76% of all mice implanted with DU pellets. In contrast, only 12% of control mice developed leukemia. Karyotypic analysis confirmed that the leukemias originated from FDC-P1 cells. The growth properties of leukemic cells from bone marrow, spleen, and lymph node were assessed and indicate that the FDC-P1 cells had become transformed *in vivo*. The kidney, spleen, bone marrow, muscle, and urine showed significant elevations in tissue uranium levels prior to induction of leukemia. These results demonstrated that a DU altered *in vivo* environment may be involved in the pathogenesis of DU induced leukemia in an animal model. (Mol Cell Biochem **279:** 97–104, 2005)

Key words: DBA, depleted uranium, heavy-metals, internal exposure, leukemia, mice

Abbreviations: DU, depleted uranium; FDC-P1, factor-dependent cells; IL-3, interleukin 3

Introduction

The radioactive heavy metal, depleted uranium (DU) is used as kinetic energy penetrators in military and industrial applications. While the use of DU in these applications has been limited to only a few countries, it has been used in recent military conflicts. Friendly-fire accidents that occurred during the 1991 Gulf War, which resulted in US soldiers with retained DU-fragments, have focused attention on the potential health effects of internalized heavy metals like DU used in military applications. Because of worldwide availability of these munitions, the United States will have to deal with an increased number of casualties from the use of these weapons [1]. Furthermore, aerosolization of DU has lead to unsubstantiated concerns regarding environmental exposure. Since DU munitions are relatively recent additions to the

Address for offprints: A.C. Miller, AFRRI, Applied Cellular Radiobiology Department, 8901 Wisconsin Ave, Bethesda, MD 20889-5603, USA (E-mail: millera@afrri.usuhs.mil)

list of militarily relevant metals, little is known about the health effects of this metal after internalization as embedded shrapnel.

Chemically similar to natural uranium [1], DU is a low specific activity radioactive heavy metal, with a density approximately 1.7-times that of lead (19 g/cm^3 versus 11.35 g/cm^3). DU differs from natural uranium in that it has been depleted of ^{235}U and ^{234}U. As a result, the specific activity of DU is significantly less than natural uranium (0.4 μCi/g versus 0.7 μCi/g, respectively) [2].

Several recent studies have investigated the potential health effects of these militarily relevant heavy metals [1–10]. These *in vitro* and *in vivo* investigations have not only demonstrated the transforming ability [1] and the mutagenicity [2] of DU, but also its neurotoxicity *in vivo* [6]. Studies using neoplastically-transformed human cells and the athymic nude mouse assay, demonstrate the carcinogenic potential of DU [1]. A mechanism by which DU induces cell transformation *in vitro* does appear to involve, at least partially, direct damage to the genetic material manifested as increased DNA breakage or chromosomal aberrations (i.e., micronuclei) [7]. DU has been shown to induce genomic instability in a human cell model [8] further suggesting a carcinogenic potential for DU. Cellular studies have also demonstrated that the alpha particle radiation from DU is responsible for some of the cellular damage induced by DU [9]; chemical damage can occur [7] however and the magnitude of the importance of radiation versus chemical contributions to DU-induced effects is unknown.

There is little available epidemiological evidence among humans with which to conclude that long-term internal exposure to DU is carcinogenic. Therefore, in view of carcinogenesis risk estimates and medical management questions relevant to possible future incidents of DU internalization, an examination of potential carcinogenic effects is necessary to understand the potential carcinogenic effects of these metals. Due to anecdotal reports that DU exposure caused leukemia in exposed military personnel [11], we decided to use a mouse leukemia model to investigate whether internalized DU exposure could cause leukemia.

Since there are no published models to study the effects of heavy metals on leukemia induction, we decided to use a radiation-induced leukemia model developed by Duhrsen and Metcalf [12–15]. Their murine model for development of myeloid leukemia allows for an analysis of the effects of heavy metal or radiation exposure on the host and analysis of the direct mutagenic effects on the hematopoietic cells. Proliferation *in vitro* of the murine hematopoietic cell line FDC-P1 is dependent on stimulation by granulocyte-macrophage colony stimulating factor (GM-CSF) or interleukin 3 (IL-3). Intravenous injection of the non-tumorigenic FDC-P1 cells into DBA/2 mice was followed by development of leukemia in 100% of animals receiving 100–350 cGy whole body radiation prior to cell injection [12–14]. We chose to use a similar protocol by injecting FDC-P1 cells into animals that had been implanted with DU pellets to determine if leukemia would develop.

Materials and methods

Mice and heavy metal exposure

Mice used were 2 to 3 month old DBA/2 males and were housed at the AFRRI AALAC approved animal care facility. Mice were surgically implanted with sterilized DU pellets within the gastrocnemius muscle under anesthesia with ketamine hydrochloride (40 mg/kg). All animal procedures have been previously described [2, 5–6]. All surgically implanted animals (10–30 animals/group) received either 2 pellets DU (1 per leg) + 6 pellets tantalum (TA) (3 per leg), 6 pellets DU (3 per leg) + 2 pellets TA (1 per leg), or 8 pellets DU (4 per leg) + No TA. A set of mice served as non-surgical controls. Intravenous injections of FDC-P1 hematopoietic cells [1×10^6 cells) were administered in the tail veins at 60 days (2 months) post-pellet implantation.

Factor-dependent cells

The factor dependent hematopoietic cells (passage 1) FDC-P1 and kindly provided by Dr. Scott Boswell, University of Indiana, Indianapolis, Ind., were originally derived from long-term bone marrow cultures of DBA/2 mice. These cells are dependent on multi-colony stimulation factor (multi-CSF or interleukin 3 IL-3) for growth *in vitro* and are non-tumorigenic on subcutaneous injection. The frequency of spontaneous transformation has been estimated to be less than 10^{-9}. Cells were maintained in suspension cultures in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS); the cells were stimulated with 10% conditioned medium from WEHI-3B cells.

Necropsies

Mice were monitored daily and at specific times after pellet implantation, and after subsequent FDC-P1 cell injection, they were euthanized. At necropsy, organ enlargement was recorded and organ samples were taken for histology. Bone marrow cells, obtained by femoral flushing, and cell suspensions from enlarged lymph nodes and spleens were used for further analysis. Chromosomes were prepared as previously described [3].

Agar culture

Agar cultures were established using 35mm Petri dishes containing DMEM with a final concentration of 20% FCS and 0.3% agar. Lymph node cells were cultured at 10^2 and 10^6 cells per dish and stimulated with IL-3 obtained from cultured WEHI cells. Colony formation was scored after 7 days incubation at 37 °C in a fully humidified atmosphere of 10% CO_2 in air.

Generation of continuous cell lines from leukemia clones

Cloned leukemic cell lines were initiated from distinct colonies removed from stimulated agar cultures of lymph node cells containing fewer than 50 cells. For cultures from each animal, between 10 and 20 colonies were sequentially isolated using a cloning loop and single colonies were transferred to individual wells on a 24-well trays containing 1 ml of DMEM with 10% FCS. Colonies from stimulated dishes were expanded in media containing the same stimulus; they were later assessed for IL-3 independent growth. Established cell lines were maintained in 2-ml cultures on 12-well trays in DMEM + 10% FCS and passaged twice weekly.

Transplantation experiments

Cell obtained from enlarged lymph nodes (10^6 cells) were injected subcutaneously into the left flank of 10 DBA/2 male mice. Tumor development was monitored 3× weekly by palpation. When moribund, mice were euthanized and analyzed similarly to animals developing primary tumors.

Uranium content assessment

Uranium determinations in wet-ashed tissues were performed by inductively coupled plasma mass spectroscopy (ICPMS) as previously described [16–17]. Sample preparation involved dry-ashing the tissue sample at 450 °C followed by wet-ashing with a series of additions of concentrated nitric acid and 30% hydrogen peroxide. The ash was dissolved in 1 M nitric acid, and the intensity of ^{235}U and ^{238}U ions were measured by ICP-MS.

Results

Development of leukemia in DU-implanted DBA/2 mice injected with FDC-P1 cells

Previous studies by Duhrsen and Metcalf showed that radiation could induce leukemia in mice injected with FDC-P1 cells [12–15]. We speculated that their model would be a good model to investigate whether DU could induce leukemia. A pilot study was done to determine if internalized DU could cause development of leukemia in this DBA mouse model. Twenty-five DBA/2 male mice were implanted with DU pellets (high dose = 6 pellets/mouse]. At 60 days post-pellet implantation, the mice were intravenously injected with 10^6 cultured FDC-P1 cells. Between two and seven months after cell injection, 76% of all DU-implanted animals developed leukemia (median latency 148 days; latency 31–210 days). The development of myeloid leukemia in DBA-/ male mice is shown in Table 1. A small percentage of control animals (12%) that carried no metal implants but had been injected with FDC-P1 cells, developed leukemia (latency 91–259 days). This observation was similar to that described previously for control animals injected with FDC-P1 cells [12–14]. In contrast to animals implanted with DU and then injected with FDC-P1 cells, mice (un-implanted) that were injected with DU-incubated FDC-P1 cells did not exhibit a significant increase in the development of leukemia.

Development of leukemia in DU-implanted mice and correlation with tissue uranium content at the time of FDC-P1 cell injection

Two additional experiments were done with 15 mice per group. Mice were implanted with DU pellets (6 pellets/mouse) and at 60 days post-pellet implantation, the mice were intravenously injected with 10^6 FDC-P1 cells. The cumulative incidence of DU-induced leukemia from these two experiments (total 30 mice/group) is illustrated in Fig. 1. These data confirm the initial results that DU-implanted mice develop leukemia and also demonstrate the asynchronous nature of the development of the leukemia in these mice. As a control for metal-implantation, 30 animals were implanted with

Table 1. Incidence of leukemia in DBA/2 mice implanted with DU and injected with FDC-P1 cells

Implant[a]	Number of animals implanted	Number of leukemic animals[b]	Latency (days)[c]
None (+FDC-P1)	25	4	91–259 (180)
DU (6 pellets + FDC-P1))	25	19*	31–210 (148)
None[d] (+DU-treated FDC-P1 cells)	25	3	85–272 (188)

[a]Animals were implanted with 6 pellets of DU in the hind limb muscle (three per leg) and injected with FDC-P1 cells at 60 days post-metal implantation.
[b]Evidence of leukemia by necropsy, confirmed by histology.
[c]Interval between FDC-P1 cell injection and death from leukemia.
[d]FDC-P1 cells were incubated with DU-UO_2 for 24 h prior to injection into un-implanted DBA/2 mice.
*Indicates statistical significance.

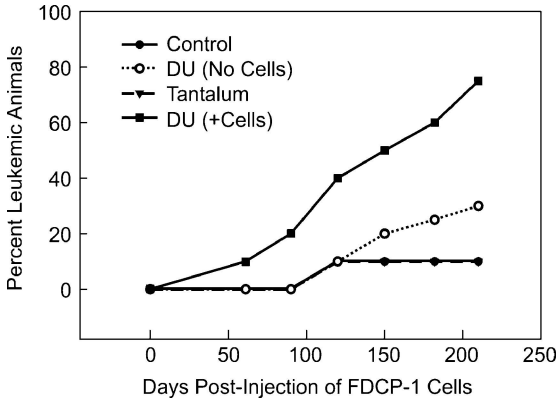

Fig. 1. Cumulative Incidence of leukemia in DBA male mice implanted with DU pellets (6, 3 per hind limb) and intravenously injected with 10^6 FDC-P1 cells at 60 days post-pellet implantation. Control (no implants) and tantalum (6 pellets, 3 per hind limb) implanted animals were done for comparison. Data are control (●), DU (no cells) (○); tantalum (▼), DU (+ cells) (■). Pooled data are from two experiments with 15 mice per group per experiment.

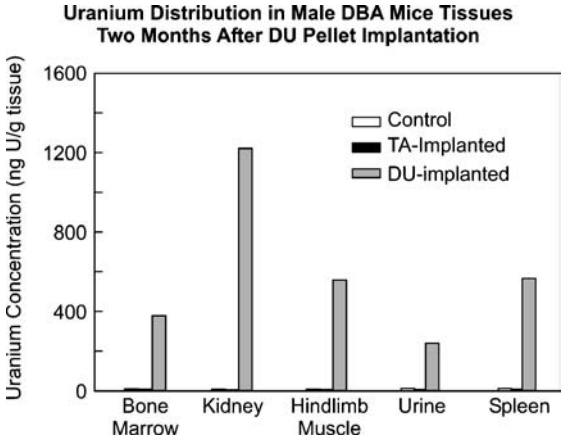

Fig. 2. Uranium concentrations measured in tissues dissected from mice euthanized 60 days after implantation of either DU (6 pellets), TA (6 pellets), or no pellets (control). Bars represent the mean from 10 mice.

the inert metal, tantalum (TA) (6 pellets/mouse), for comparison. The same percentage of TA implanted mice as control (non-implanted) mice developed leukemia (12%) (Fig. 1). A small, but statistically significant percentage (30%) of DU implanted mice that were not injected with FDC-P1 cells developed leukemia within 210 days post-DU implantation (latency 93–210 days).

Necropsy of leukemic animals (DU-implanted and control) revealed the enlargement of mesenteric lymph nodes, spleen, liver, concomitant with histological changes indicative of leukemia. Hematology at necropsy also revealed that leukemic mice from both DU-implanted and control groups, had elevated white blood cell counts, ranging from 6000 to 133,000 per ul (median 49,000; normal range: 5000–85,000). Blast cells accounted for 17–88% of the cell population; the remainder was promyelocytes, myelocytes, or immature monocytes. There was also an increase in mature neutrophilic granulocytes upon necropsy, enlargement of the spleen ranging from (160–2100 mg: median: 395 mg: normal 80–100 mg) and enlarged mesenteric, intrathorasic, and peripheral lymph nodes. Cytocentruifuge preparation of bone marrow, spleen, and lymph nodes showed an increased percentage of blast cells. Histological examination of the leukemic mice (31/40 DU implanted; 5/40 control) demonstrated extensive infiltration of immature, mitotically active cells in the portal regions of the liver (79% of specimens) and sinusoidal infiltration in 45% of specimens. DU-induced leukemia was also associated with significant hepatosplenomegaly in contrast to leukemia in control animals which did demonstrate extreme hepatosplenomegaly.

To determine what the tissue uranium content was at 60 days post-DU pellet implantation, 10 mice were similarly implanted with DU or TA. At 60 days post-DU pellet implantation, animals were euthanized and the kidney, bone marrow, spleen, hind muscle, and urine were obtained for uranium analysis using ICPSM. At 60 days following implantation, the highest concentration of uranium was in kidney (Fig. 2) of the tissues tested. In kidney uranium concentration reached 1196 ng U/g kidney. Bone marrow, hind limb, and spleen also exhibited a 42 to 61-fold increase in tissue uranium content. Significant accumulations were also observed in urine. The concentration of uranium was significantly higher in these tissues in comparison to TA-implanted or control mice.

FDC-P1 origin of leukemic cells and their tumorigenicity

Karyotypic analysis of cells from mesenteric lymph nodes was done to verify the FDC-P1 origin of the leukemic cells obtained from DU and control-leukemic animals. Cultured FDC-P1 cells contain 24 acrocentric and 8 metacentric chromosomes. Karyotypic preparations of cells isolated from grossly enlarged mesenteric lymph nodes from 10 animals from DU-induced leukemia group, showed metaphases with 8 metacentric chromosomes. This finding demonstrates that the mitotic cells originated from the injected FDC-P1 cells.

To assess for tumorigenicity of the FDC-P1 cells obtained from leukemic animals, a transplantability test was done. Isolated FDC-P1 Cells from enlarged lymph nodes (10^6 cells) from each of 10 leukemic animals were transplanted into 10 non-implanted DBA/2 male mice. All animals developed palpable tumors at the site of injection within 2–3 weeks after injection. Necropsy revealed large subcutaneous tumors and a variable enlargement of lymph nodes, spleen, and liver. Karyotypes were performed in six cases and confirmed the FDC-P1 origin of the tumors. In contrast, the injection of

cultured FDC-P1 cells (10^6 cells) obtained from the stock culture of FDC-P1 cells into DBA/2 male mice did not yield the induction of any subcutaneous tumors within the observation period of 12 months.

Altered growth properties in vitro *of the leukemic cells*

The growth properties of FDC-P1 cells obtained from leukemic animals were assessed [Table 2]. The *in vitro* growth properties of cultured FDC-P1 cells (prior to injection) were assessed initially for comparison. Cultured FDC-P1 cells formed large, compact colonies in agar when cultured with IL-3 as a growth stimulant; however, if IL-3 was not supplied, no colonies would form. In contrast, the lymph node cells isolated from leukemic animals exhibited a significant qualitative enhancement in growth capability. The lymph node cells isolated from leukemic animals spontaneously developed large colonies in agar without any stimulation from IL-3.

Effects of different DU doses on leukemia induction

Figure 3 illustrates the dependence of leukemia induction on the dose of DU implanted. DU doses consisting of 2, 6, and 8 DU pellets each resulted in leukemia development with the 6 and 8 pellet dose resulting in a similar pattern of leukemia development (76–80% within 210 days). In contrast, a dose of 2 DU pellet resulted in development of leukemia in 30% of the animals within the same time period. Data demonstrate that there was no difference between the median latent period to leukemia development in animals implanted with either 8 or 6 pellets (161 and 164 days respectively). In contrast the dose of 2 DU pellets exhibited a median leukemia latency

Table 2. Assessment of *in vitro* growth patterns of leukemia cells obtained from lymph nodes of DU-implanted mice with primary leukemia: comparison to control mice with leukemia

Number of animals	Colon morphology	Colonies per 1000 cultured cells +IL3	Colonies per 1000 cultured cells −IL3	Success rate establishing in continuous cell lines
	DU-implanted animals with leukemia			
8	Large, compact	51–299	47–262	95–100%
2	Variable	10–21	2–12	33–72%
	Control animals with leukemia			
6	Large, compact	40–186	33–191	90–99%
4	Variable	2–18	1–6	18–59%

Compilation of results obtained from agar cultures of 10^2–10^6 lymph node cells in the presence or absence of 400 U of IL3.

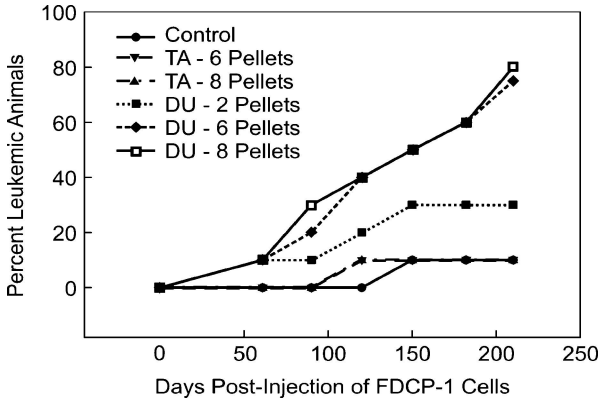

Fig. 3. Effect of DU Dose on cumulative incidence of leukemia in DBA male mice. Animals were implanted with DU pellets 2 (■), 6 (◇), or 8 (□) in the hind limbs and intravenously injected with10^6 FDC-P1 cells at 60 days post-pellet implantation. Control (no implants) mice and mice implanted with tantalum 6 (▼) and 8 (▲) pellets were done for comparison. Pooled data are from two experiments with 15 mice per group per experiment.

of 184 days. Un-implanted control animals exhibited a significantly reduced leukemia rate as was observed in previous experiments (12% within 210 days). Animals implanted with TA pellets, either 6 or 8 pellets, did not exhibit a significant difference in leukemia development from control animals. To evaluate the relationship between different DU doses and leukemia induction, the uranium content of the bone marrow at various times after implantation of different doses of DU was measured (Fig. 4). As was shown previously, there was significant distribution of uranium to bone marrow at the time of FDC-P1 cell injection (2 months/60 days after DU

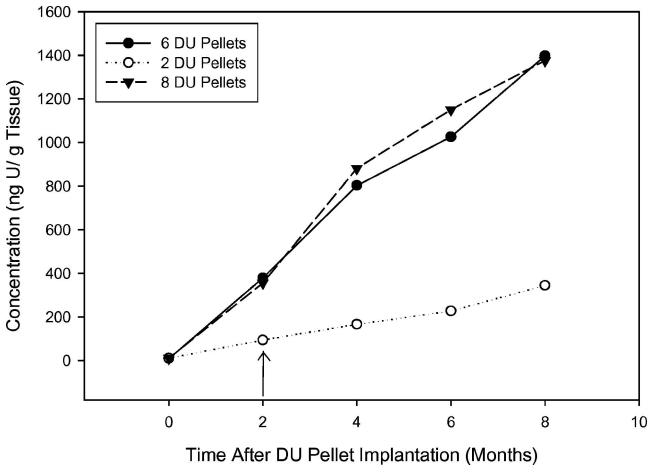

Fig. 4. Uranium concentrations measured in bone marrow at various times after DU pellet implantation: Effect of DU pellet number. Animals were implanted with 2 (◇), 6 (●), or 8 (▼) DU pellets in the hind limbs. Animals were euthanized at 2, 4, 6, and 8 months after pellet implantation. Uranium measurements were also done at time zero. Pooled data are from 10 animals per group per time point.

Implantation). Uranium in the 6- and 8- pellet group exhibited a higher elevation in bone marrow uranium content in contrast to the 2- pellet group. There was however, no difference in the uranium tissue concentration between the 6 and 8 pellet groups at all time points. The concentration of uranium in bone marrow of animals implanted with 2 DU pellets showed an increase over time although the magnitude of the increase was significantly less that that for the 6- or 8-pellets groups.

Effect of variation in number of FDC-P1 cells injected

To investigate the influence of injected FDC-P1 cell numbers on the development of leukemia, groups of DU-Implanted (6 pellets) DBA/2 mice were injected with varying numbers of FDC-P1 cells ranging from 10^4–10^8 cells per mouse. Figures 4 and 5 show that, with increasing numbers of cells injected; there was some shortening of the median latent period to leukemia development. The most significant difference observed was between animals injected with 10^4 *versus* 10^8 FDC-P1 cells (median latency 182 and 148 days, respectively).

Discussion

This is the first report describing the consistent development of leukemic transformation of FDC-P1 cells when injected intravenously into DU-implanted male mice. Karyotypic analysis confirmed that the leukemic cells had originated from the injected cells. The abnormal growth properties of the leukemic cells in vitro and in vivo confirmed the pathological evidence indicating that the FDC-P1 cells had been malignantly transformed. While our results show that internal exposure to embedded DU significantly increased leukemia induction, the mechanisms are largely unknown. Previous studies by Duhrsen and Metcalf demonstrated that interleukin-3 (IL-3)-dependent FDC-P1 cells undergo leukemic transformation when injected into sublethally irradiated (^{60}CO) DBA/2 mice [12–15]. Transformation was related to aberrant activation of growth-regulatory genes by insertion of intracisternal A-particle (IAP) genomes [15]. Their data also demonstrated a role of the irradiated host in the leukemogenesis process, although the host-mediated mechanisms are still not fully clarified [12–14].

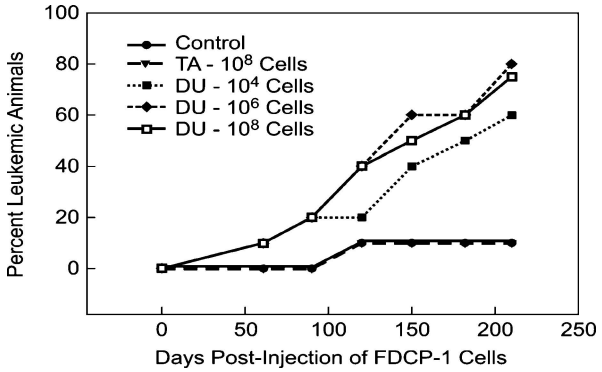

Fig. 5. Effect of FDC-P1 cell number on cumulative incidence of leukemia in DBA male mice. Animals were implanted with DU pellets (6, 3 per hind limb) and intravenously injected with 10^4 (■), 10^6 (◇), or 10^8 (□) FDC-P1 cells at 60 days post-pellet implantation. For comparison, animals were implanted with tantalum pellets (6, 3 per hind limb) and were intravenously injected with 10^8 (▼) FDC-P1 cells at 60 days post-pellet implantation. Control (no implants) mice were done for comparison. Pooled data are from two experiments with 15 mice per group per experiment.

The involvement of internally embedded uranium to the development of leukemia has not been previously addressed although there are numerous reports concerning uranium exposure and carcinogenesis including leukemogenesis. External uranium exposure and leukemogenesis has been widely reviewed by the National Research Council Committee on Biological Effects of Ionizing Radiation which has prepared several reports (BEIR IV, VI) [18]. Their conclusions indicate that external exposure to uranium in industrial workers cannot be definitively linked to leukemia induction due to multiple confounders in the studies the committee considered (BEIR IV, VI). In contrast, Harrison and colleagues observed acute myeloid leukemia in CBA mice following intraperitoneal injection of uranium-233 [19]. Their findings support other studies that indicate that internally deposited alpha particle emitters, like thorotrast, can cause leukemia development [20]. However, differences in the mode of uranium exposure and the alpha particle tissue dose (microdosimetry) make direct comparisons between our data and their findings difficult.

To rule out the possibility that the malignant disease was due to selection *in vivo* of a transformed autonomous subclone pre-existing in the population of FDC-P1 cells, several points should be considered. First, subcutaneous tumor development has never been observed after subcutaneous injection of cultured FDC-P1 cells into un-implanted control mice. Second, IL-3-growth dependence of the cultured FDC-P1 cells was verified in vitro at frequent intervals throughout the study. Autonomous subclones were never detected. Third, DU implantation into mice increased the percentage of animals developing leukemia from 12% in control animals to 68–75% in DU implanted mice. This finding strongly suggests that the DU-altered host environment played a role in inducing leukemic transformation. Lastly, the latent period between FDC-P1 cell injection and death from leukemia was variable (60–210 days). This type of variable pattern of disease development in mice is in contrast to the pattern seen in mice subcutaneously injected with tumor cells in which synchronous tumor development is observed. Similarly in

the present studies when primary leukemia cells were transplanted into a secondary recipient, synchronous neoplastic disease development was observed. These data confirm that the FDC-P1 cells had been transformed to the tumorigenic phenotype.

Development of leukemia in a small percentage of non-implanted control mice after injection of FDC-P1 cells dose not necessarily support the argument that cultured FDC-P1 cells are tumorigenic. Subcutaneous injection of FDC-P1 cells failed to produce tumors. Leukemic cells, obtained from both DU-implanted and non-implanted control animals with leukemia, exhibited similar growth characteristics suggesting that the observed respective malignancies were due to transformation of FDC-P1 cells and not due to proliferations of unaltered parental-type FDC-P1 cells. A small percentage of Ta- implanted mice also developed leukemia; however the number exhibiting leukemia was the same as non-implanted controls. It appears that the process of metal implantation per se does not induce leukemia. Additionally, while heavy metal exposure is linked to solid tumor carcinogenesis, leukemia induction is much less common following exposure to heavy metals like cadmium, nickel, or chromium [21–23].

Long-term exposure to embedded DU results in a dramatic increase in tissue uranium content. At the time of FDC-P1 cell injection, kidney, spleen, bone marrow, and urine exhibited a 41 to 82 fold increase in tissue uranium content. Whether the high uranium content directly affected FDC-P1 cells or whether uranium induced a host-specific effect that caused the FDC-P1 cell transformation, is unknown. The current study does not provide definitive data to support a direct uranium-induced FDC-P1 cell transformation *in vivo.* Our laboratory has previously demonstrated that exposure to DU compounds can transform immortalized human cells to the tumorigenic phenotype and that DU is mutagenic and genotoxic *in vitro* and *in vivo*. [1–2, 4, 7–8]. Results in this study showed that *in vitro* exposure of FDC-P1 cells to DU-uranium oxide prior to injection into mice did not result in any increase in leukemia development nor did short-term uranium exposure result in IL-3-independent growth of FDC-P1 cells. It is also possible that a significant alteration in the host tissue environment induced by uranium may have played a critical role in the transformation of FDC-P1 cells. Uranium exposure *in vivo* and *in vitro* induces the expression of a variety of genes [4, 10]; genetic changes such as these in the host may play a significant role in the FDC-P1 transformation process. Others have demonstrated that indirect radiation leukemogenesis in DBA/2 is partially due to aberrant activation of growth-regulatory genes by insertion of intracisternal A-particle (IAP) genomes [15]. Further studies are ongoing which will address the questions of altered growth characteristics of the FDC-P1 cells.

The data do suggest that high numbers of FDC-P1 cells are a prerequisite for subsequent leukemic transformation. Injection of varying numbers of FDC-P1 cells was paralleled by a difference in leukemic latent periods and for a 10^4-fold difference in injected cell numbers, a 25-day difference in the median latency was observed. It is a reasonable assumption that the transformation event primarily depends on the presence of a crucial number of cells, therefore this time difference is more pronounced than expected based on the proliferative behavior of FDC-P1 cells *in vitro*. In culture FDC-P1 cells have a doubling time of 19 h so a 10^4-fold difference in cell numbers should result in a 10-day difference in reaching a certain threshold of cells. It is possible that the FDC-P1 cells are suboptimally stimulated *in vivo* or that the continual uranium exposure affected cell growth and proliferation. Results with immortalized human cells continually cultured (>15 days) in minimally toxic concentrations of DU compounds, demonstrated that long-term DU exposure decreased the cells proliferative rate and inhibited cell cycle [1, 9]. It is likely therefore, that the presence of uranium could have negatively affected the stimulation of FDC-P1 cells *in vivo.*

There was somewhat of a DU dose-dependent affect on leukemia development. Exposure to embedded DU doses of 8, 6, and 2 pellets all demonstrated a significant increase in leukemia development in comparison to non-implanted control mice. Although there was no statistical difference in the percentages of animals developing leukemia when either 8 or 6 DU pellets were implanted; animals that carried only 2 DU pellets demonstrated a reduction in the percentage of animals exhibiting leukemia. It appears that the tissue uranium content at the time of FDC-P1 cell injection can impact on the leukemic process and there maybe a threshold uranium level that is necessary to affect leukemia development. Further support for uranium-induced host effects that directly impact leukemia development is derived from the experiments demonstrating that embedded DU could even induce leukemia in mice that were not injected with FDC-P1 cells.

The results indicate that a uranium-altered environment plays an important role in the pathogenesis of DU-induced leukemia. It is unclear to what extent the internalization of DU *in vivo* induces specific host environment changes that enhance the development of leukemia or whether the *in vivo* uranium exposure directly affects the FDC-P1 cells. It is tempting to speculate that one of the effects on leukemic transformation in DU exposed animals is that tissues with high uranium content may support a rapid accumulation of FDC-P1 cells. These cells subsequently have a fixed probability and susceptibility of undergoing secondary changes leading to leukemic transformation. Furthermore an analysis of the cellular and molecular events underlying leukemic transformation needs to be completed. *In vitro* analysis of the leukemic cells should be able to address questions regarding growth parameter changes and gene or chromosomal alterations.

References

1. Miller AC, Blakely WF, Livengood D, Whittaker T, Xu J, Ejnik JW, Hamilton MM, Parlette E, St. John T, Gerstenberg HM, Hsu H: Transformation of human osteoblast cells to the tumorigenic phenotype by depleted uranium-uranyl chloride. Environ Health Perspect 106: 465–471, 1998
2. Miller AC, Fuciarelli AF, Jackson WE, Ejnik EJ, Emond C, Strocko S, Hogan J, Page N, Pellmar T: Urinary and serum mutagenicity studies with rats implanted with depleted uranium or tantalum pellets. Mutagenesis 13: 101–106, 1998
3. Andrew SP, Caligiuri RD, Eiselstein LE: A review of penetration mechanisms and dynamic properties of tungsten and depleted uranium penetrators. In: A. Crowson, E.S. Chen (eds). Tungsten and Tungsten Alloys: Recent Advances, Plenum Press, NY, 1991
4. Miller AC, Whittaker T, Hogan J, McBride S, Benson K: Oncogenes as biomarkers for low dose radiation-induced health effects. Can Detect Prev 20: 235–236, 1996
5. Pellmar TC, Fuciarelli AF, Ejnik JW, Hamilton M, Hogan J, Strocko S, Emond C, Mottaz HM, Landauer MR: Distribution of uranium in rats implanted with depleted uranium pellets. Toxicol Sci 49: 29–39, 1999
6. Pellmar TC, Kaiser DO, Emond C, Hogan JB: Electrophysiological changes in hippocampal slices isolated from rats embedded with depleted uranium fragments. Neurotoxicology 20: 785–792, 1999
7. Miller AC, Xu J, Mog S, McKinney L, Page N: Neoplastic transformation of human osteoblast cells to the tumorigenic phenotype by heavy metal-tungsten alloy particles: Induction of genotoxic effects. Carcinogenesis 22: 115–125, 2001
8. Miller AC, Brooks K, Stewart M, Shi L, McClain D, Page N: Genomic instability in human osteoblast cells after exposure to depleted uranium: Delayed lethality and micronucleus formation. J Environ Radioact 64: 247–259, 2003
9. Miller AC, Xu J, Stewart M, Brooks K, Hodge S, Shi L, Page N, McClain D: Observation of radiation specific damage in human cells exposed to depleted uranium: Dicentric frequency and neoplastic transformation as endpoints. Radiat Protect Dosim 99: 275–278, 2002
10. Miller AC, Brooks K, Smith J, Page N: Effect of militarily relevant heavy metals, depleted uranium and heavy metal tungsten alloy on gene expression in human liver carcinoma cells [HePG2]. Molec Cell Biochem 255: 247–256, 2004
11. Mould RF: Depleted uranium and radiation-induced lung cancer and leukemia. Br Radiol 74: 677–683, 2001
12. Duhrsen D, Metcalf D: A model system for leukemic transformation of immortalized hemopoietic cells in irradiated recipient mice. Leukemia 2: 329–333, 1988
13. Duhrsen D, Metcalf D: Factors influencing the time and site of leukemic transformation of factor-dependent cells after injection into irradiated recipient mice. Int J Can 44: 1074–1081, 1989
14. Duhrsen D, Metcalf D. Effects of irradiation of recipient mice on the behavior and leukemogenic potential of factor-dependent hematopoietic cell lines. Blood 75: 190–197, 1990
15. Blumenstein M, Hossfield DK, Duhrsen U: Indirect radiation leukemogenesis in DBA/2 mice: Increased expression of B2 repeats in FDC-P1 cells transformed by intracisternal A-particle transposition. Ann Hematol 76: 53–60, 1998
16. Ejnik JW, Carmichael AJ, Hamilton MM, McDiarmid M, Squibb K, Boyd P, Tardiff W: Determination of the isotopic composition of uranium in urine by inductively coupled plasma mass spectrometry. Health Phys 78: 143–146, 2000
17. Danesi PR, Bleise A, Burkart W, Cabianca T, Campbell MJ, Makarewicz M, Moreno J, Tuniz C, Hotchkis M: Isotopic composition and origin of uranium and plutonium in selected soil samples collected in Kosovo. J Environ Radioact 64: 121–131, 2003
18. National Research Council Committee on Biological Effects of Ionizing Radiation: Health Effects of Exposure to Radon – BEIR VI, National Academy Press, Washington, DC, 1999
19. Ellender M. Harrison JD, Pottinger H, Thomas JM: Induction of osteosarcoma and acute myeloid leukaemia in CBA/H mice by the alpha-emitting nuclides, uranium-233, plutonium-239, and amercium-241. Int J Radiat Biol 77: 41–52, 2001
20. Harrison JD, Muirhead CR, Harrison: Quantitative comparisons of cancer induction in humans by internally deposited radionuclides and external radiation. Int J Radiat Biol 79: 1–13, 2003
21. Waalkes MP, Rehm S, Sass B, Ward JM: Induction of tumors of the hematopoietic system by cadmium in rats. IARC Sci Pub 118: 401–404, 1992
22. Costa M: Molecular mechanisms of nickel carcinogenesis. J Biol Chem 383: 961–967, 2002
23. Shi X: Molecular mechanisms of Cr[VI]-induced carcinogenesis. Mol Cell Biochem 234: 293–300, 2002