Title: Mechanisms underlying cellular responses of cells from haemopoietic tissue to low
dose/low LET radiation

Dr. Munira Kadhim
Faculty of Health and Life Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK
Email: mkadhim@brookes.ac.uk

RELEVANCE STATEMENT

The above studies will provide fundamental mechanistic information relating genetic predisposition to important low dose phenomena, and will aid in the development of Department of Energy policy, as well as radiation risk policy for the public and the workplace. We believe the proposed studies accurately reflect the goals of the DOE low dose program. To accurately define the risks associated with human exposure to relevant environmental doses of low LET ionizing radiation, it is necessary to completely understand the biological effects at very low doses (i.e., less than 0.1 Gy), including the lowest possible dose, that of a single electron track traversal. At such low doses, a range of studies have shown responses in biological systems which are not related to the direct interaction of radiation tracks with DNA. The role of these "nontargeted" responses in critical tissues is poorly understood and little is known regarding the underlying mechanisms. Although critical for dosimetry and risk assessment, the role of individual genetic susceptibility in radiation risk is not satisfactorily defined at present.
The aim of the proposed grant is to critically evaluate radiation-induced genomic instability and bystander responses in key stem cell populations from haemopoietic tissue. Using stem cells from two mouse strains (CBA/H and C57BL/6J) known to differ in their susceptibility to radiation effects, we plan to carefully dissect the role of genetic predisposition on two non-targeted radiation responses in these models; the bystander effect and genomic instability, which we believe are closely related. We will specifically focus on the effects of low doses of low LET radiation, down to doses approaching a single electron traversal. Using conventional X-ray and we will be able to assess the role of genetic variation under various conditions at a range of doses down to the very low dose of 0.01Gy. Irradiations will be carried out using facilities in routine operation for such studies. Mechanistic studies of instability in different cell lineages will include the role of cytokines which have been shown to be involved in bystander signaling and the initiation of instability. These studies also aim to uncover protein mediators of the bystander responses using advanced proteomic screening of factors released from irradiated. Integral to these studies will be an assessment of the role of genetic susceptibility in these responses, using CBA/H and C57BL/6J mice.

Here, we are providing a report on the work that was conducted Oxford Brookes University for the above project reporting period. Products from this project have been recently delivered at several international meeting, such as: 56th Annual Meeting of the Radiation Research Society, Hawaii, September 2010; the Department of Energy annual workshop, USA, May 2011 and most recently 14th International Congress for Radiation Research, Poland, August 2011.

PROJECT DESCRIPTION

Introduction

There is strong evidence for genetic basis of susceptibility to radiation-induced genomic instability in both human and mouse cells (Kadhim, 2003). Differences in radio-sensitivity in response to low doses of high LET and high doses of low LET radiation are known between several mouse strains. However, it is vitally important to study the biological effects at very low doses of low LET ionizing radiation to better understand the risk associated with human exposure to relevant environmental doses of radiation. Several evidences suggest that the cellular response to ionizing radiation involves the recognition of the radiation-induced damage and the initiation of signal transduction cascade(s), which lead to cell cycle arrest, damage repair, and a decision to survive, or die by apoptosis. Of all the types of DNA damage, DNA double strand break (DSB) is the greatest threat to the integrity of the genome. Important progress has been made in elucidating the DNA DSB checkpoint pathway. This pathway is constituted of sensors which recognise the presence of DNA DSBs, signal transducers which amplify the DNA damage signal and effectors which induce cell cycle delay and programmed cell death. The earliest signalling molecules, which are known to initiate the transduction cascade at damage sites, are protein kinases ataxia telangiectasia mutated (ATM) and ATM- and Rad-3-related (ATR). At the molecular level, activation of the DNA damage checkpoints is based on a phosphorylation cascade that starts with the activation of ATM and ATR, and members of the PIKK (phosphatidyl-inositol 3 kinase like protein kinase) family at the site of lesions (Abraham, 2004).In the current study two such common inbred strains, CBA/CaH and C57BL/6J, were selected to examine their sensitivity to the lowest possible dose (< 0.1 Gy) of low LET X-rays. Therefore, an analysis of the expression and activation of ATM, ATR and their downstream target genes (effectors) in CBA/CaH and C57BL/6J haemopoietic stem cell, with and without exposure to ionising radiation (Low Let X-rays), could help to understand the role of the different genetic susceptibility observed in these two strains of mice. Following exposure to both high LET radiation and high doses of low LET radiation, reviewed in (Kadhim, 2003) and (Morgan and Sowa, 2007), it has been demonstrated that genetic background can influence the expression of genomic instability. Following alpha-particle irradiation (high LET), we have previously demonstrated that primary haemopoietic stem cells derived from C57BL/6J and CBA/H mouse strains differ in their levels of radiation-induced genomic instability (Watson et

al., 1997, Kadhim, 2003).However, the relationship between genetics and delayed instability response has not been fully characterised at low, environmentally-relevant doses of low LET radiation.

Material and Methods

CBA/CaH and C57BL/6J in-bred strains of mouse bred at the MRC were used. In addition to the data previously obtained from the CBA/CaH strain, results presented here are those obtained from the C57BL/6J strain. As shown in figure 1, femoral bone marrow cells were isolated from 10-12 week old male CBA/CaH and C57BL/6J mice. The cells were exposed to 0, 0.01, 0.1, 1 and 3 Gy X-rays (250 keV). Immediately after irradiation/sham-irradiation, cells were cultured in 25cm2 Falcon culture flasks containing modified α Eagles media supplemented with 25% horse sera, antibiotics and sources of pre-tested conditioned media (Lorimore et al., 1990) to permit measurement of GI by chromosomal analysis 2 and 15 population doublings (pd) post-irradiation.

Liquid culture metaphase harvests were carried out at 2 and 15 population doublings (days 2 and 7) chromosomal instability. Whole cell extracts were analysed for the background expression and activation of ATM and ATR; immediately, 2 and 24 hours post-irradiation. Cells were lysed at a concentration of approximately 1 x 107 cells/ml in cell lysis buffer (20 mM HEPES, pH 7.20, 50 mM NaCl, 10 mM EDTA, 2 mM EGTA, 0.5 nonidet P-40 (NP-40), 0.5 mM PMSF, 10 µg/ml Leupeptine, 0.5 mM DTT, 10 mM Na2MoO4 • H2O, 10 mM Na3VO4, 100mM NaF) to eliminate the red blood cell population. For immunoblot analysis, lysates were first adjusted to contain equal amounts of proteins (20 µg total proteins) using the Bradford assay and were then dissolved in SDS-PAGE sample buffer prior to separation by 7.5% SDS polyacrylamide gel. Analysis was performed with ECL detection kit (Amersham). The following antibodies were used: mouse ATM and ATR (Santa Cruz Biotechnology, Inc) diluted 1:200. Apoptosis analysis was carried out on the cells, these were double stained with fluorescein isothiocyanate (FITC)-labelled annexin V and propidium iodide (PI) in a binding buffer (HEPES [N-2hydroxyethylpiperazine-N'-2-ethanesulfonic acid]-buffered saline solution supplemented with 0.25 mM CaCl2). A minimum of 10.000 cells within the gated region were analyzed by flow cytometry. For each group, supernatants were combined in Amicon® Ultra-15 filter columns (Millipore, Watford, UK) in order to separate out proteins greater than 5kDa in molecular weight. The columns were centrifuged at 4000g for 45 min at room temperature. Cytokine analysis of the supernatants was performed using Quantikine kits for detection of total TGF-ß (latent + active forms of TGF-β) and active TGF-β1, and TNF-α (R&D Systems, MN, USA). Cell supernatant samples were plated onto the appropriate ELISA plate with relative controls and standards as per manufacturer's instructions.

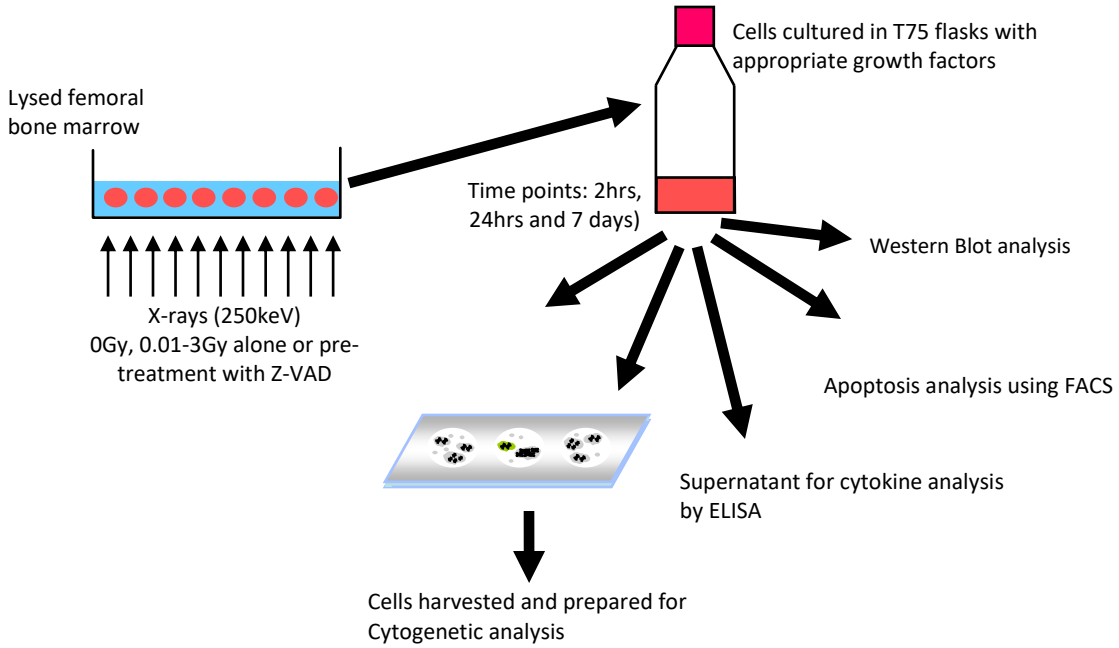


Figure 1 Schematic diagram for the methods and endpoints utilised.

Results

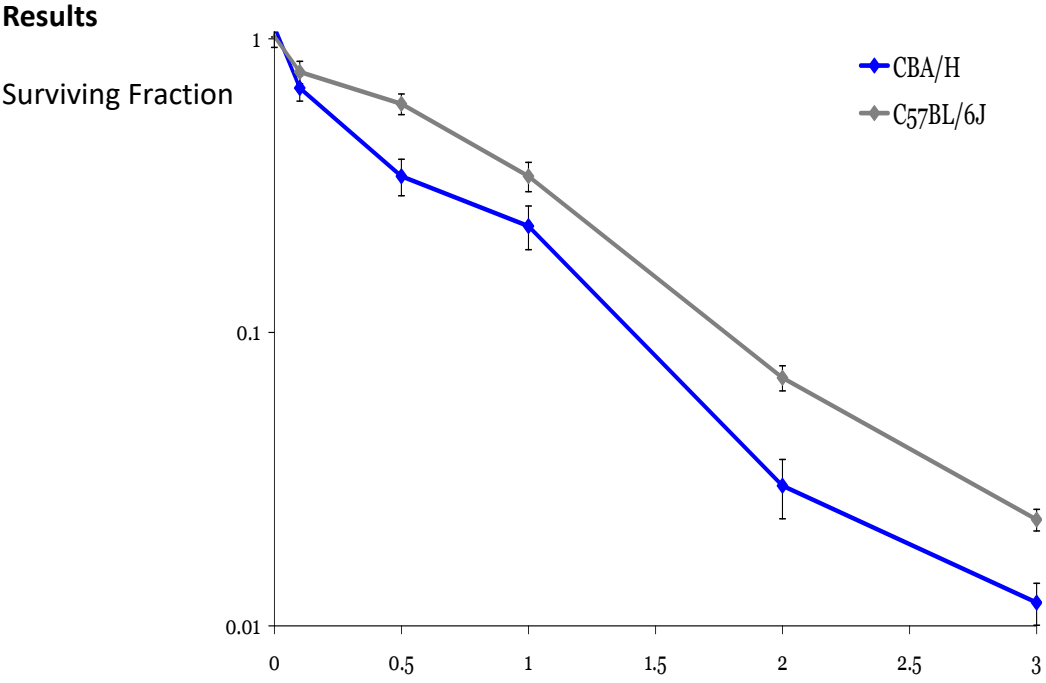


Figure 2a Clonogenic survival

Table 1 Target Cell Number

	Mouse Strain		
Stem cells	CBA/H	C57BL/6J	Fold difference
CD34+	0.27%	1.23%	4.6
LY6+	0.07%	0.50%	7.1
CD34+/LY6+	0.08%	0.15%	1.9

Overall, survival was significantly reduced in CBA/CaH compared to C57BL/6J (see Figure 2a) after low LET X-rays (p=0.013). Surviving fractions are corrected to respective control values. Error bars represent +/- SEM of 3 experiments. Potential target cell number are 2 – 7 x greater in C57BL/6J compared to CBA (Table 1).

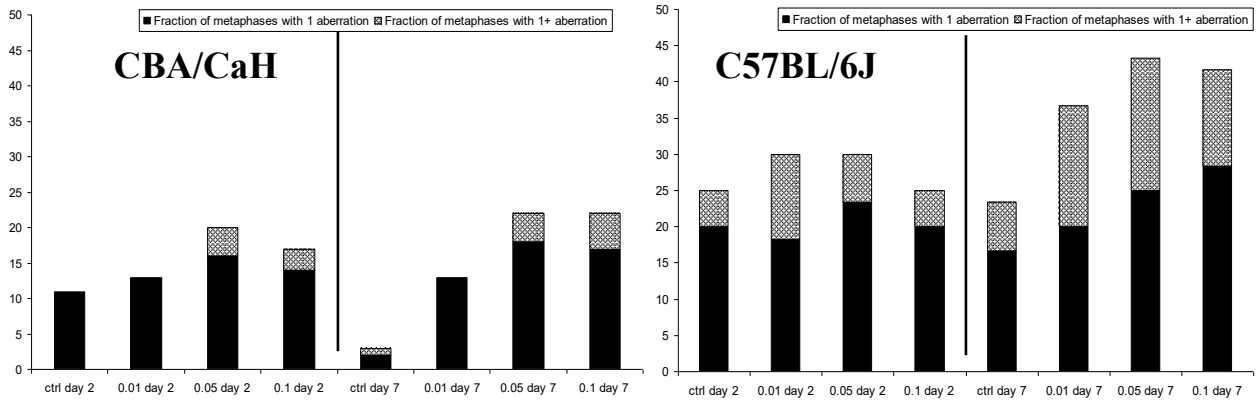


Figure 3 Chromosomal instability analysis

Very low doses of low LET radiation induce chromosome/genetic instability in CBA/CaH and
C57BL/6J mouse haemopoietic stem cells (figure 3)

Chromosome instability was induced with very low doses of low LET X-rays in stem cells derived from CBA/CaH and C57BL/6J mice, but the magnitude of induction demonstrated some strain specificity. The level of instability in CBA/CaH mouse strain at 2 and 15 pd post irradiation were similar following 0.01 Gy exposure with a significant

increase after exposure to 0.05 and 0.1 Gy. In contrast, the chromosomal instability in C57BL/6J was demonstrated to be notably higher at 15 pd post-irradiation compared to the initial damage with no obvious dose dependency. The percentage of heavily damaged cells was greater in C57BL/6J than in CBA/CaH. A large fraction of the difference may be due to the contribution of heavily damaged cells.

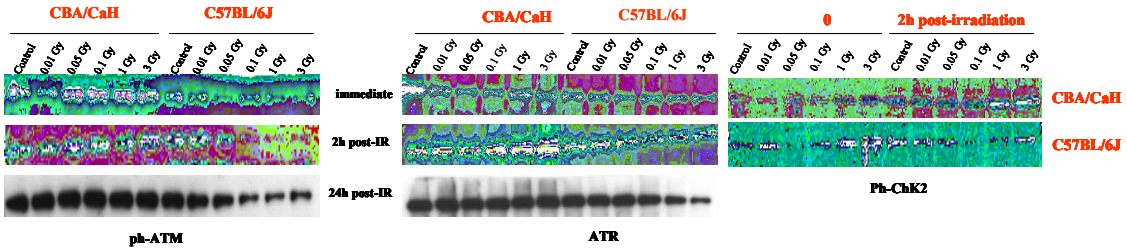


Figure 4a

Western blot analysis demonstrated the ATM was constitutively activated to similar levels in non-irradiated CBA/CaH and C57BL/6J cells. Following exposure to very low doses (0.01Gy-0.05Gy) of X-rays, the ATR expression was not significantly modified in either mouse strain. In contrast, ATM activation was higher in CBA/CaH than in C57BL/6J up to 24 hours post-irradiation (figure 4a). Furthermore, the expression of Chk2 (Thr-68) phosphorylation, which is largely dependent on ATM activation, was shown to be maximal after 3Gy at 2 hours post-irradiation in CBA/CaH. To the contrary at the same time point in C57BL/6J cells, Chk2 activity was diminished.

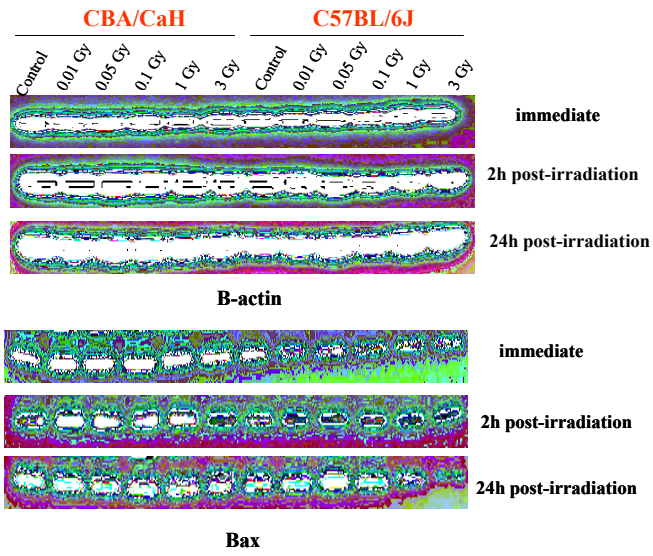


Figure 4b

Bax expression was subtly unregulated from the control in CBA/CaH compared to C57BL/6J up to 2 hours post-irradiation. In comparison, its expression was significantly down-regulated in C57BL/6J after exposure to high dose of X-rays at 24 hours post-irradiation (figure 4b).

Levels of TGF-β and TNF-α were measured in ICCM (irradiated conditioned cell medium) from 0, 0.01, 0.1, 1 and 3 Gy irradiated CBA and C57 haemopoietic cells at 2h, 24h and 7 days following X-ray exposure. For CBA there was a small (but not statistically significant) increase in TGF-β levels (figure 5a) after 2h for the 1 and 3Gy samples. Levels of TGF-β declined at 24h but were elevated above control levels in all irradiated groups at day 7, mainly following a dose-dependent increase (with the exception of 0.1Gy). Levels of TGF-β in all C57 samples were far lower than those for CBA, showing no increase above control levels for all time points (figure 5C). There were no detectable levels of TNF-α at the 2h time point for either strain (figure 5B and 5D). In CBA there was detectable TNF-α at 24h in the 0.1 - 3 Gy groups, following a dose-increase trend. At 7 days post-irradiation TNF-α was barely detectable in the CBA supernatant samples. In contrast, at the 24h time point C57 showed a marked decrease in TNF-α levels in all irradiated groups when compared to the sham irradiated control (figure 5D).

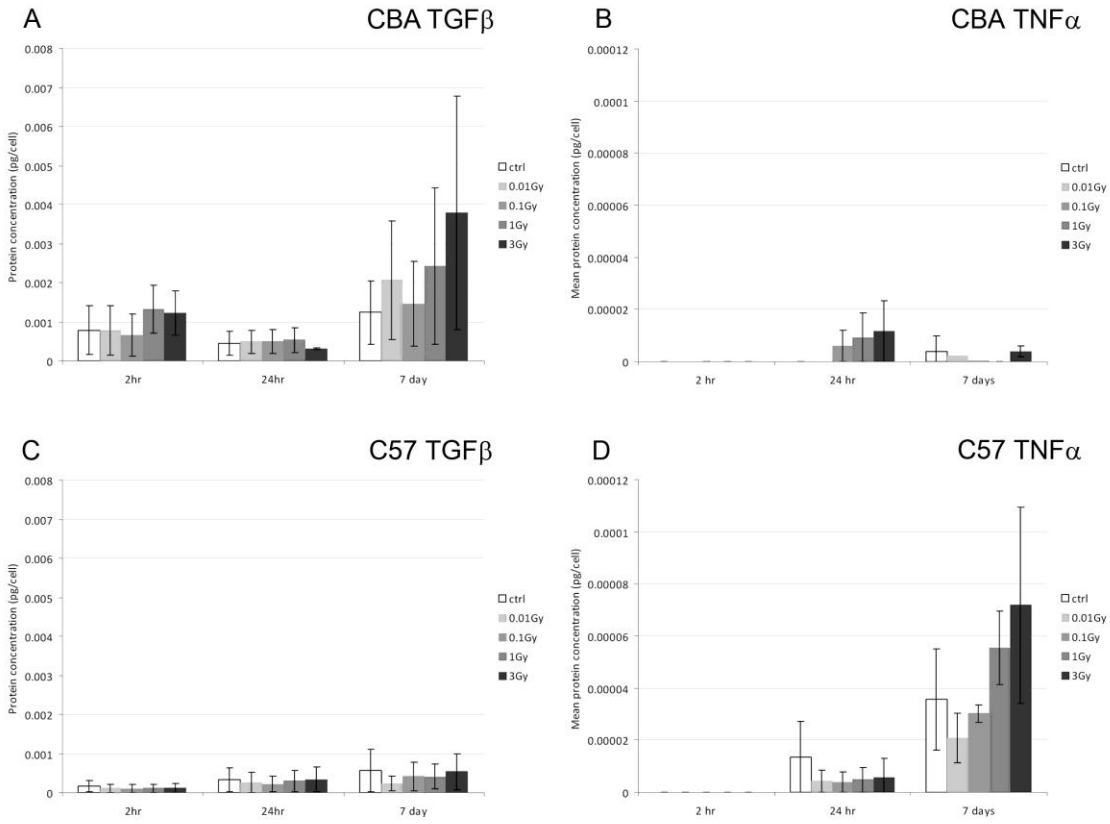


Figure 5a-d Total levels of cytokines (pg/cell) of TGFb and TNFa in CBA and C57 at 2h, 24h and 7d post X-irradiation (0-3Gy). Error bars ± SEM. A: Levels of TGFb/cell in CBA, B: Levels of TNFa/cell in CBA, C: Levels of TGFb/cell in C57, D: Levels of TNFa/cell in C57.

Whilst on day 7, levels of C57 TNF-α were highest, with the 1 and 3 Gy samples showing an increase above control, however, levels in the 0.01 Gy and 0.1 Gy C57 7d samples fell below the sham control level. The 0.01 Gy sample had levels approximately half to that of the concentration of the corresponding control group.

Overall, there were marked strain specific differences in the selected cytokines observed, with TGF-β levels in CBA samples higher than those for C57 samples at all time points, whilst C57 samples showed a greater TNF-α response at day 7, though CBA did show a small peak of TNF-α at 24h. Due to variability in concentration measurements differences in the levels of TGF-β and TNF-α observed changes did not reach statistical significance.

Chromsomal instability experiments were repeated during the reporting period with several alterations to X-ray dose exposure with the pre-treatment with the chemical ZVAD to determine the effect of caspase inhibition on chromosomal instability (figure 6).

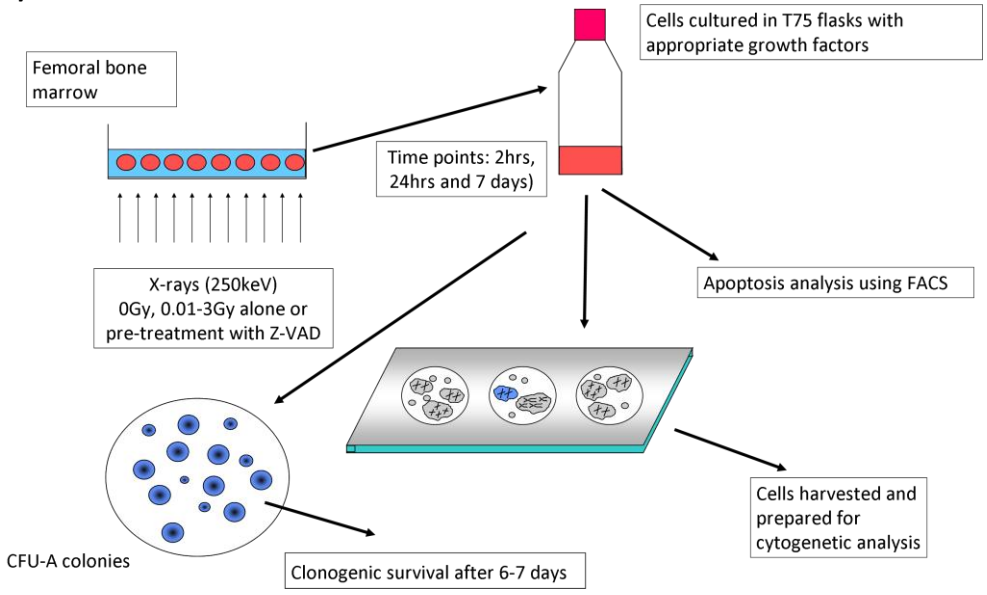


Figure 6 Schematic diagram for methods and endpoints utilised (2).

Material and Methods

Femoral bone marrow was removed from male mice from both strains- CBA/CaH and C57BL/6J, the single cell suspension was lysed and cells were prepared for X-irradiation. Cells were either sham/irradiated alone or in the presence of caspase inhibitor Z-VAD. Those groups to have caspase inhibited had a final concentration of 50μM Z-VAD and were incubated for thirty minutes at 37°C, 5%CO_2 prior to sham/irradiation. Sham/irradiations (doses 0Gy, 0.01Gy, 0.1Gy 1Gy and 3Gy) were carried and in universal tubes using a 250keV X-ray source.

Post-irradiation cells were put into liquid culture with the appropriate growth factors and conditioned medium.
At the relevant time points; 2 hours, 24 hours and 7 days cells were removed for the flowing endpoints; apoptosis analysis by FACS, western blotting analysis, chromosomal analysis. Supernatant was also collected for cytokine analysis by ELISA. Gene array analysis was carried out on cell pellet samples from X-ray exposed alone (no ZVAD) cells, these were re-suspended to have RNA extracted for gene array.

Lysis Procedure

1) Single cell suspensions were put into 50ml tubes and centrifuged @1700rpm, 10mins@4^{0}C.
2) Supernatant was discarded.
3) Pellets were re-suspended in 5ml filtered lysis buffer (8.26g NH_4Cl, 1g KHCO, 0.037G EDTA/1 litre double distilled water), for 8mins@room temp, tubes were flicked every 1-2 mins.
4) Tubes were topped up with MEM alpha media, and inverted 3-4 times
5) Samples were centrifuged @1700rpm, 10mins@4^{0}C.
6) Supernatant was discarded.
7) Pellets were re-suspended in 10ml MEM alpha media.
8) Cell counts were performed, and tubes placed on ice.

Apoptosis Analysis

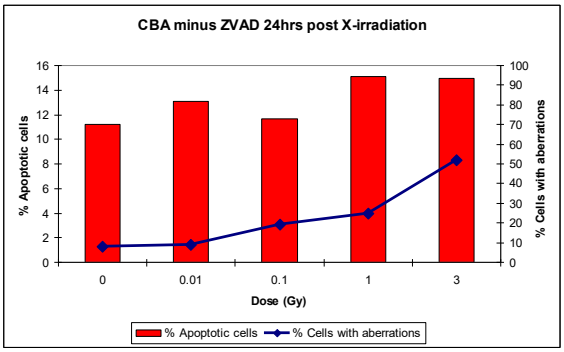
For apoptosis analysis, cells were double stained with fluorescein isothiocyanate (FITC)-labelled annexin V and propidium iodide (PI). Briefly, 5 x 105 cells were re-suspended in 0.5 ml binding buffer (HEPES [N-2hydroxyethylpiperazine-N'-2-ethanesulfonic acid]-buffered saline solution supplemented with 0.25 mM CaCl2). Twelve microliters FITC-annexin V (Pharmingen, San Diego, CA) reagent was added to the cells and the mixtures were gently mixed and incubated for 15 minutes at room temperature in the dark. Immediately before the analysis by flow cytometry, 5µl of PI were added to each sample. A minimum of 10.000 cells within the gated region were analyzed.

Chromosomal analysis

To each sample of bone marrow suspension, 2µl/ml demecolcine was added and then incubated for 1 hour at 37°C, 5% CO2.
Post-incubation, cells were centrifuged for 10 minutes at 1200rpm. The supernatant was discarded and pellets were re-suspended with 5ml 0.075M potassium chloride, drop by drop. Tubes were gently inverted and incubated at room temperature for 20 minutes. Samples were centrifuged for 10 minutes at 1000rpm, supernatant was discarded and pellets re-suspended in 5ml 3:1 Carnoy's fixative (methanol: acetic acid) added drop by

drop and samples placed in fridge overnight prior to preparing slides for chromosome analysis. Metaphase spreads were prepared on slides, stained with Giemsa solid stained then mounted. Slides were scored using Zeiss Axioscop light microscope.

Results



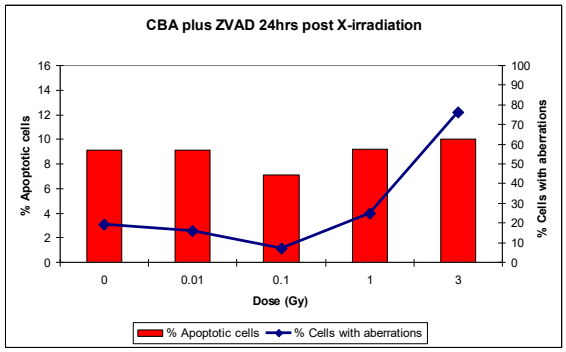
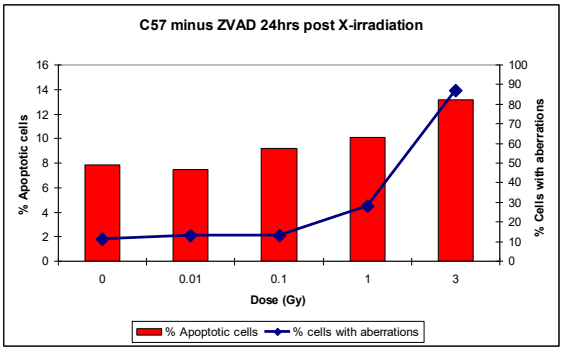


Figure 7a Figure 7b

Inverse relationship between chromosomal instability and apoptosis in CBA bone marrow cells 24hrs post irradiation alone and pre-treatment with ZVAD.

Initial analysis of the level of apoptosis in CBA/CaH, indicates an induction with all doses of X-irradiation, for chromosomal analysis, an increase in the proportion of cells with aberrations can be observed at doses at 0.01Gy (figure 7a). Following the treatment of cells, prior to X-irradiation, with Z-VAD, the level of apoptosis in CBA/CaH cells (figure 7b) was greatly decreased at all doses and included control levels of apoptosis. However, this reduction has led to an increase in the level of chromosomal instability, although not at all doses.



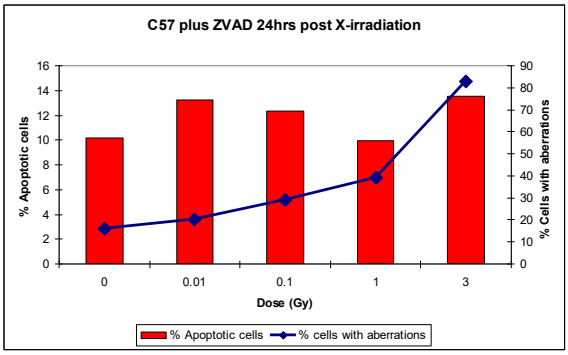


Figure 8a Figure 8b

Inverse relationship between chromosomal instability and apoptosis in C57 bone marrow cells 24hrs post irradiation alone and pre-treatment with ZVAD.

In C57BL/6J (figure 8a) the percentage of apoptotic cells is increased at doses above 0.01Gy X-irradiation, for chromosomal instability, an induction can be seen in cells exposed above 1Gy. In the presence of Z-VAD (figure 8b), there was no observed reduction in the level of apoptosis in C57BL/6J cells. The level of chromosomal instability however was increased and at all irradiated doses and remained very much higher than that of CBA/CaH at all doses.

Overall, following irradiation alone, with the absence of the caspase inhibitor Z-VAD, levels of apoptosis are much higher in CBA/CaH compared to C57BL/6J, however, the converse can be said of chromosomal instability which has a higher level of induction in C57BL/6H cells.

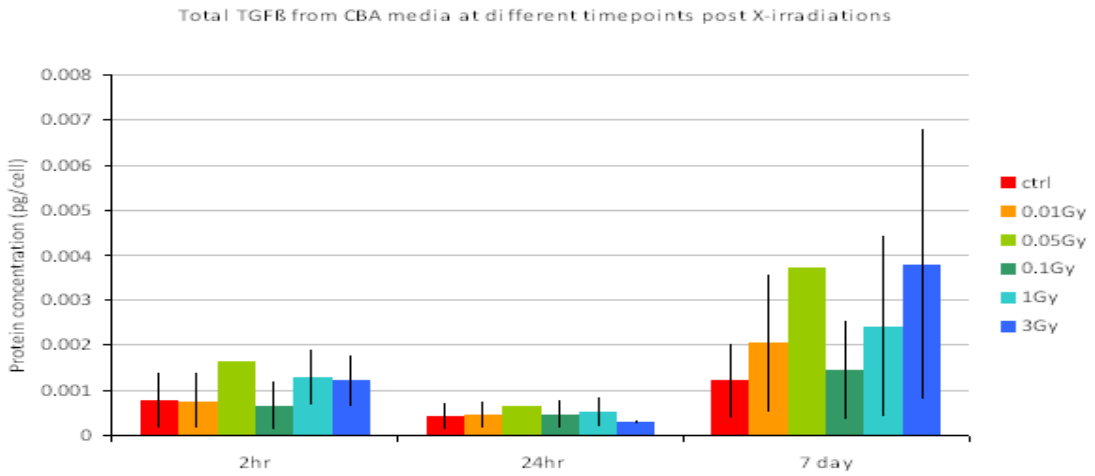


Figure 9a Total level of TGF-beta following exposure of CBA cells to doses of X-ray from 0.01-3Gy X-ray.

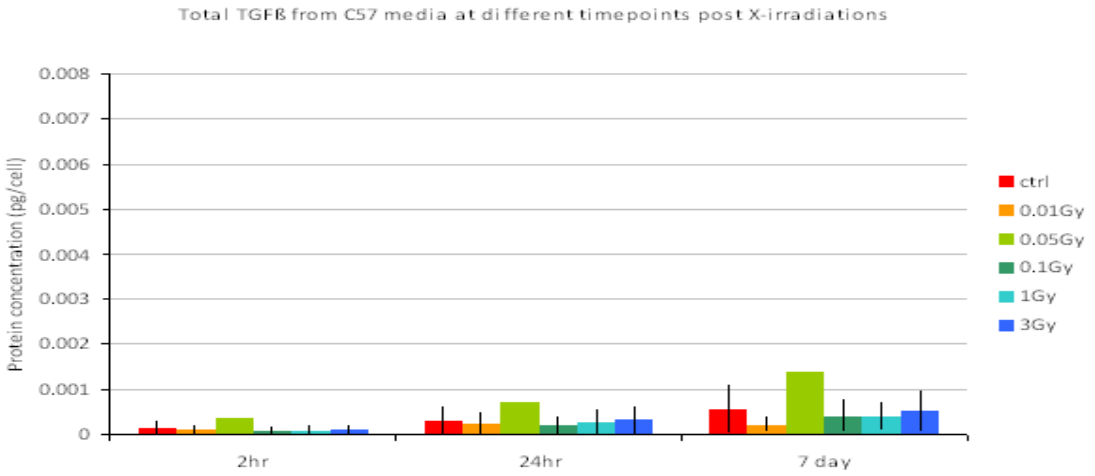


Figure 9b Total level of TGF-beta following exposure of C57 cells to doses of X-ray from 0.01-3Gy X-ray.

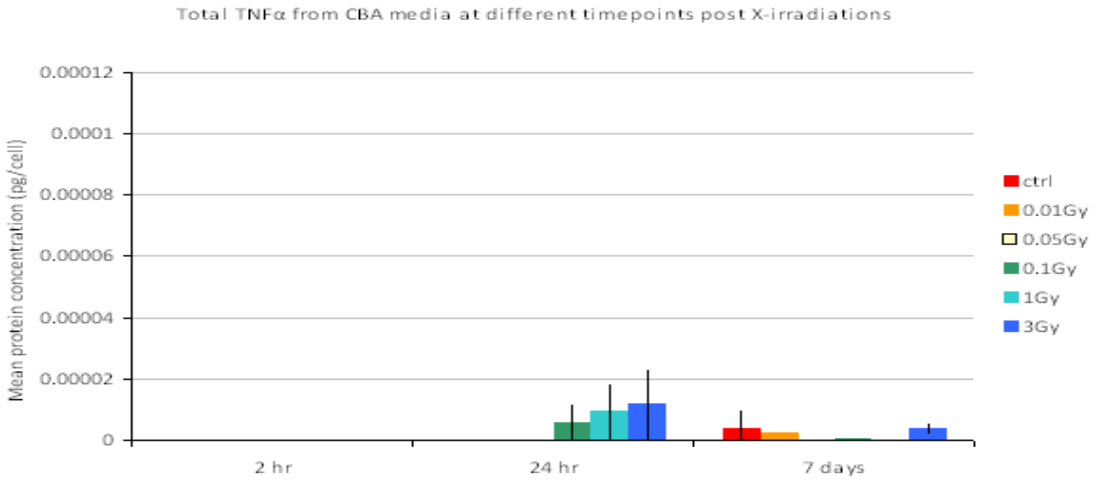


Figure 9c Total level of TNF-alpha following exposure of CBA cells to doses of X-ray from 0.01-3Gy X-ray.

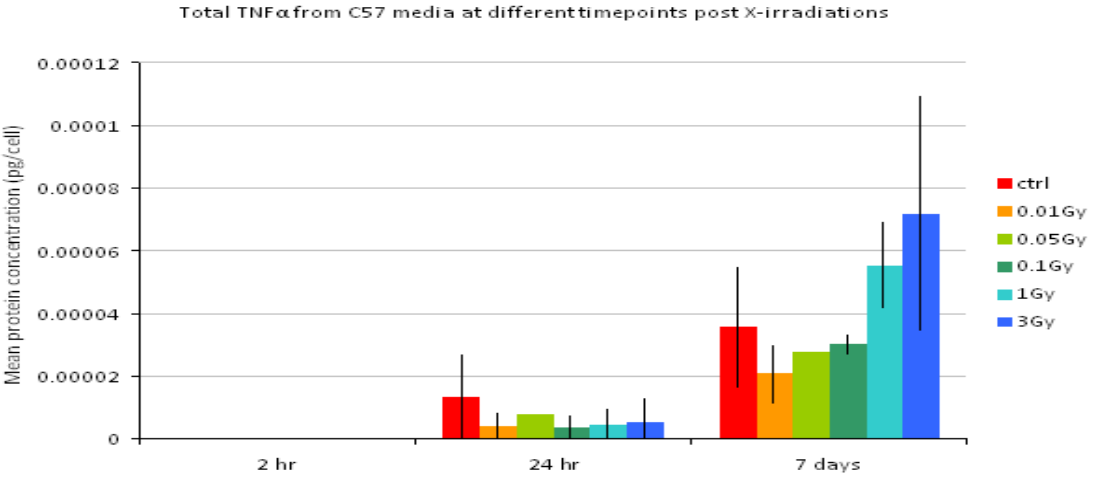


Figure 9d Total level of TNF-alpha following exposure of CBA cells to doses of X-ray from 0.01-3Gy X-ray.

Cytokines TGF-ß and TNF-α measured in supernatant using Quantikine ELISA kits.
Strain specific differences were suggested from the data, with CBA showing overall higher TGF-ß levels at each time point, with notable elevation at 7d/15p.d post-irradiation (figure 9a). TNF-α levels were higher in C57 samples, with the highest concentrations observed at 7d/15p.d post-irradiation (figure 9d).
Strain specific changes in cytokines occur following X-irradiation. Results suggest a genetic difference in the induction of different types and levels of cytokines

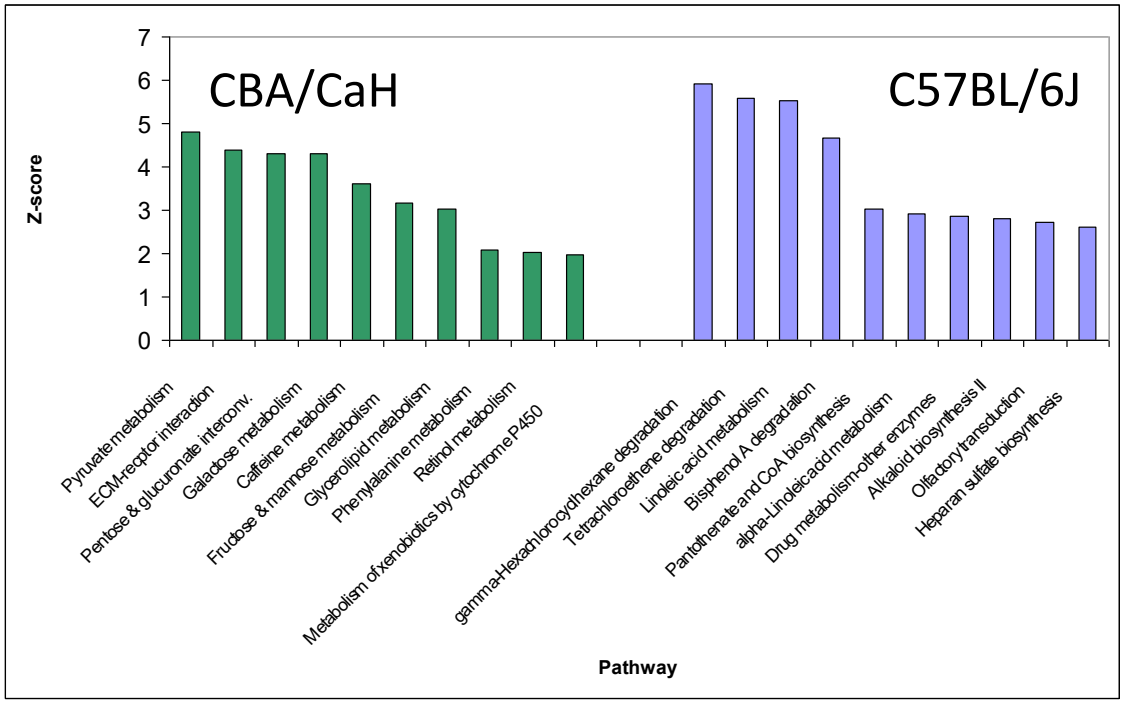


Figure 10a Gene pathways showing high scores in changes in expression levels 2hrs post-low dose (0.1Gy) X-ray.

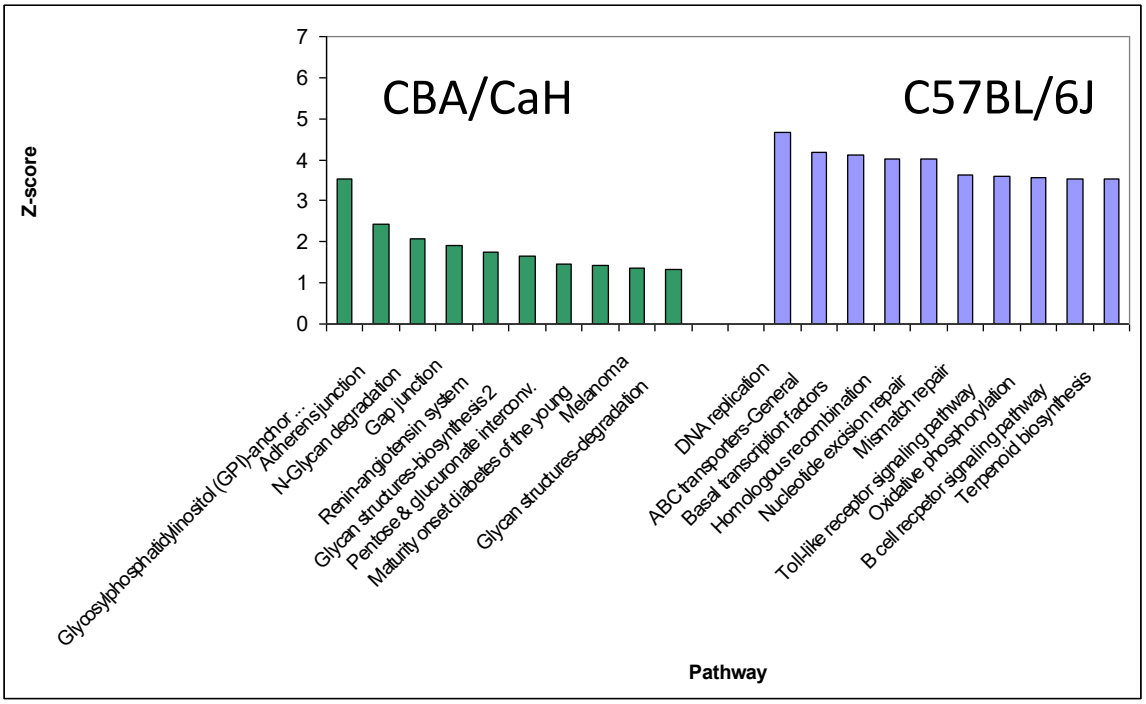


Figure 10b Gene pathways showing high scores in delayed changes in expression levels at 7 days/15 p.d. post-low dose (0.1Gy) X-ray.

Table 2a 2hrs post-irradiation

Genes upregulation more in C57	Genes upregulated less in C57
P53	Cn
NK-kβ	MAPK
Bcl-xl	Ap

Table 2b 7 day/15p.d. post-irradiation

Genes upregulation more in C57	Genes upregulated less in C57	
None identified	Fas	P53
	CASP7	RAK
	Cn	CASP8
	Cyt C	Calpain
	NK-kβ	ENDO-G

Tables 2a and 2b demonstrate the differences in the regulation of genes between the two strains of mice following exposure to low (0.1Gy) X-ray.

Discussion

The current study demonstrates that chromosomal instability was induced in CBA/CaH and C57BL/6J strains of mice following very low doses of low LET irradiation (< 0.1 Gy). Although instability was significantly induced in cultures obtained from CBA/CaH and C57BL/6J at 2 and 15 population doublings, after very low doses (< 0.1 Gy), we also observed qualitative differences relating to more heavily damaged cells after exposure to 0.05Gy and 0.1Gy than 0.01Gy in both strains (Fig. 2a). Phosphorylated ATM in both CBA/CaH and C57BL/6J showed an active pathway in these cells. However, following exposure to low dose X-ray, ATM was significantly more active in CBA/CaH than in C57BL/6J. To support these differences in ATM activity observed in both strains of mice, the first ATM downstream target gene Chk2 was investigated. Chk2 activity results were concordant with the ATM phosphorylation trend observed and demonstrated that ATM is required for the phosphorylation of Chk2 kinase. These data suggest that C57BL/6J mice are less effective in repairing the damage caused by ionizing radiation and support cytogenetic analysis which showed a greater percentage of heavily damaged cells in C57BL/6J compared to CBA/CaH. The defective ATM activity in C57BL/6J erroneously repairs the damage, leading to decreased apoptosis (especially at high doses of ionizing radiation and by Bax down-regulation), increased survival and chromosomal instability. Collectively, these findings suggest a model wherein normal recognition and repair of DNA damage leads to cell death and a consequent reduction in aberrant cells especially with more than one aberration (e.g. CBA/CaH.) Conversely, normal recognition of damage but abnormal repair might lead to increased survival, decreased apoptosis and increased aberrant metaphases with more than one aberration (e.g. C57BL/6J).

The delayed response, at 7 days post X-irradiation, has demonstrated a clear inverse relationship between high levels of chromosomal instability and low levels of apoptosis in C57BL/6J (figures 3 and 4).

Apoptosis results suggest the early response (24 hours post-irradiation) determines the late effect of radiation in both mouse strains. Radiation also induced an up-regulation of Bax (pro-apoptotic protein) in CBA/CaH up to 2 hours post-irradiation but a significant down-regulation in C57BL/6J cells 24 hours post-irradiation especially after high dose of X-rays. We speculate that Bax is more involved in the apoptosis signal compared to Bcl-2 in both strains.

The defective ATM activity in C57BL/6J erroneously repairs the damage, leading to decreased apoptosis (especially at high doses of ionizing radiation and by Bax down-regulation), increased survival and chromosomal instability. Collectively, these findings suggest a model wherein normal recognition and repair of DNA damage leads to cell death and a consequent reduction in aberrant cells especially with more than one aberration (e.g. CBA/CaH.) Conversely, normal recognition of damage but abnormal repair might lead to increased survival, decreased apoptosis and increased aberrant metaphases with more than one aberration (e.g. C57BL/6J).

Further to the delayed response data, results from initial time points of 24 hours post X-irradiation indicate that there is an inverse relationship between apoptosis and chromosomal instability. Furthermore, differences observed in the magnitude of damage induction indicate the there is some species specificity. This suggests that genetic factors may play a key role in the damage response to radiation.

Bone marrow from CBA analysed at the initial time point (2 population doublings post irradiation) showed a slight increase in mean chromosomal aberrations per cell. This type of damage was significantly induced at 15 Pd which suggests that chromosomal instability was induced in CBA bone marrow tissue following exposure to very low dose of X-ray. As for bone marrow from C57 mice, initial chromosomal damage above control was observed, and again at the delayed time point.

This suggests that regardless of genetics, at low doses (0.01Gy) of X-rays, chromosomal instability can be induced.

Data from apoptosis analysis in bone marrow of CBA mice showed an increase in the number of apoptotic cells, while for C57, there was no evidence of apoptosis induction during the first 24hrs post-irradiation with 0.01Gy X-rays.

Overall, at low dose (0.01Gy), CBA mice were more sensitive to the induction of apoptosis, compared to C57 mice. In contrast, chromosomal instability was induced significantly following 0.01 Gy of x-ray in bone marrow of C57 mice**.** These results indicate subtle genetic differences between strains with an inverse relationship between induction of delayed chromosomal instability and apoptotic pathway function.

Reported genetic differences at low dose of Low LET reported here may have implications for risk assessment in heterogeneous populations.

Through out all experiments data for chromosomal analysis has consistently suggested a potential inverse relationship between chromosomal damage and apoptosis at the delayed time-point in the C57 strain of mouse, based on theses results at low doses, we suggest that there is a genetic component affecting the threshold for the induction of

apoptosis and chromosomal instability, and also that apoptosis is largely responsible for the removal of heavily damaged cells.
In the more recent experiments, differences in non-targeted effects may be linked to strain specific differences in cytokine levels indicating the use of alternative signalling pathways. Whilst gene array data at low dose radiation shows strain specific metabolic and degradation pathway changes suggesting some genetic dependence.

In conclusion, low dose low LET X-irradiation induced delayed GI in both CBA/CaH and C57BL/6J haemopoeitc tissue. Using several biological approaches some key strain-specific differences have been identified. This work has shown that genetic background plays a significant role in the signalling mechanisms underlying induction and perpetuation of genomic instability following radiation exposure Higher apoptosis levels were observed for CBA than C57, CBA preferentially used TGF and C57 TNF responses. This highlights a link between initial signalling events and longer term effects, which warrants deeper investigation to identify possible points for exploitation e.g. in targeting therapy/ameliorating abscopal effects.

Conclusion and Mechanistic Considerations

These results, in combination with differences in potential numbers of stem cells and the immune/metabolomic/repair status of haemopoietic stem cells post-irradiation in the two mouse strains, suggest that there are two main types of response to irradiation: 1. responses associated with survival of damaged cells and 2. responses associated with elimination of these cells. It is possible that these phenotypic differences including molecular signalling are influenced by the genotype of stem cells and/or the haemopoietic microenvironment (i.e. stromal cells). These data suggest interesting possibilities for future research in this area.

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