

## Mechanisms Underlying Cellular Responses to Low Doses/Low LET Ionizing Radiation in Primary Haemopoietic Cells.

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Because the human population is genetically heterogeneous, it is important to understand the role that heterogeneity may play in radiation response. Exposure to ionizing radiation can lead to a suite of changes, including increased mutation rate, delayed reproductive cell death, and delayed chromosomal aberrations, all of which are manifestations of the complex genomic instability (GI) phenotype. Following exposure to either high LET radiation or high doses of low LET radiation, it has been demonstrated that genetic background can influence the expression of GI (Kadhim 2003). Following high LET alpha particle irradiation, we have previously demonstrated that primary haemopoietic stem cells derived from C57BL/6J (C57) and CBA/H (CBA) mouse strains differ in their levels of radiation-induced chromosomal instability, with CBA being more sensitive to the induction of GI (Watson *et al.* 1997). The relationship between genetics and instability has not been fully characterized at low, environmentally-relevant doses of low LET radiation.

The aim of this project is to investigate this relationship. In the current study, CBA/H and C57BL/6J mice were used and several phenotypic endpoints associated with response to irradiation were measured in bone marrow stem cells. The cells were exposed to low X-ray doses from 0.01 to 0.1 Gy and a high dose 3 Gy for comparison. CFU-A derived colonies from the irradiated and control groups were collected for:

- 1) clonogenic survival assessment
- 2) delayed cytogenetic aberrations (chromosome instability) analysis using both conventional and molecular cytogenetics techniques such as SKY
- 3) induction of apoptosis using established techniques
- 4) cytokine analysis from supernatants collected from these cultures

Further, in order to understand the role of the different genetic susceptibilities observed in these two strains of mice, cell pellets from the irradiated as well as the control groups were collected for the analysis of the expression and activation of ATM, ATR, and p53, which is a substrate of ATM. Moreover, p53 can induce expression of genes that induce apoptosis. For this reason, it is also important to analyse the expression of genes such as bcl-2, bcl-x<sub>L</sub>, and bax.

Through analysis of early and delayed responses of murine haemopoietic stem cells to varying doses of low LET irradiation, we observed distinct differences in response between the two common inbred mouse strains CBA/H and C57BL/6J. The frequency of delayed chromosomal aberrations was significantly elevated in both strains after low doses, but not at the higher doses. In the C57BL/6J derived cells, the aberrant cells had

multiple aberrations and thereby contributed more heavily to the sum of total aberrations scored. In contrast the CBA/H derived samples had very few such cells present. An inverse relationship between apoptosis and instability was evident in both strains; however it was more prominent in C57BL/6J.

Collectively, these results suggest a model where in erroneous recognition of DNA damage leads to cell death and a consequent reduction in aberrant cells and cells with more than one aberration (eg. CBA/H). Conversely, recognition of damage and a subsequent abnormal repair or apoptosis response might lead to increased survival, decreased apoptosis, increased number of aberrant metaphases and increased numbers of metaphases with more than one aberration (eg. C57BL/6J). Proteins involved in the phosphorylation of H2AX, as well as having roles in DNA repair and apoptosis (eg. ATM, ATR, and p53) are attractive candidates for such genetic discrepancies. Experiments are underway to analyse protein expression and activation level differences between the two strains, as well as discrepancies in their processing of telomeric ends.