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Mice heterozygous for the ATM gene are more sensitive to both X-ray and heavy ion exposure than are wildtypes

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Abstract

Previous studies have shown that the eyes of ATM heterozygous mice exposed to low-LET radiation (X-rays) are significantly more susceptible to the development of cataracts than are those of wildtype mice. The findings, as well as others, run counter to the assumption underpinning current radiation safety guidelines, that individuals are all equally sensitive to the biological effects of radiation. A question, highly relevant to human space activities is whether or not, in similar fashion there may exist a genetic predisposition to high-LET radiation damage.

Mice haplodeficient for the ATM gene and wildtypes were exposed to 325 mGy of 1 GeV/amu ^{56}Fe ions at the AGS facility of Brookhaven National Laboratory. The fluence was equivalent to 1 ion per lens epithelial cell nuclear area. Controls consisted of irradiated wildtype as well as unirradiated wildtype and heterozygous mice. Prevalence analyses for stage 0.5–3.0 cataracts indicated that not only cataract onset but also progression were accelerated in the mice haplo-deficient for the ATM gene.

The data show that heterozygosity for the ATM gene predisposes the eye to the cataractogenic influence of heavy ions and suggest that ATM heterozygotes in the human population may also be radiosensitive. This may have to be considered in the selection of individuals who will be exposed to both HZE particles and low-LET radiation as they may be predisposed to increased late normal tissue damage.

Keywords: Heavy ions; X-rays; Radiation cataract; ATM heterozygotes; Radiation sensitivity; HZE particles

1. Introduction

Of the major impediments to extended manned forays into deep space the unique radiation environment ranks as perhaps the greatest challenge. Numerous predictions suggest that high-LET radiation from HZE particles represent a potentially mission-compromising problem. It is critical to make an effort to fully understand the biological consequences of exposure to such radiation. A question of some moment is whether or not there is a possibility of a genetically based radiosensitivity for high-LET induced damage.

Previous studies have shown that the eyes of *atm* heterozygous mice exposed to low-LET radiation (X-rays) are more susceptible to the development of cataracts than are those of wildtype mice (Worgul et al., 2002). The findings, as well as others, run counter to the assumption underpinning current radiation safety guidelines, that individuals are all equally sensitive to the biological effects of radiation. A question, highly relevant to human space activities is whether or not, in similar fashion there may exist a genetic predisposition to the development of cataracts from high-LET

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radiation exposure. Since the ATM gene controls, among other things, DNA repair and checkpoint events, we wondered whether or not the difference seen between wildtype and atm heterozygotes when exposed to X-rays would be as apparent or as large for a high-LET radiation such as ^{56}Fe ions. Iron ions are a particularly relevant radiation to use because they constitute a major component of the heavy ion flux in cosmic rays and they have been extensively studied in the context of their cataractogenic potential (Brenner et al., 1993; Worgul et al., 1993).

As indicated above current radiation safety guidelines are predicated on the assumption that the human population is homogeneous as far as radiosensitivity is concerned. There is, however, some evidence to the contrary. For example, a few percent of cancer patients receiving radiation therapy show unexpected normal tissue damage (Hall et al., 1998), and there is the small unexplained group of survivors of the A-bomb who developed breast cancer early in life (Tokunaga et al., 1994). It has long been suspected that there might be a genetic component to this radiosensitivity.

A well-known but relatively rare autosomal recessive disorder, ataxia telangiectasia (A-T), has been shown to be associated with increased radiation sensitivity when mutations in both alleles of the ATM gene are present. In addition to elevated radiation sensitivity, individuals homozygous for *atm* express varied neuropathies, immunological anomalies and cancer predisposition (Swift, 1990). More common are individuals with mutations in only one allele (*atm* +/−). These comprise 1–3% of the human population (Swift et al., 1991). Recent studies have shown that while phenotypically indistinguishable from the rest of the population, individuals heterozygous for *atm* gene may have increased risk of developing cancer (Bay et al., 1999; Broeks et al., 2000). There are preliminary reports suggesting that radiotherapy patients, heterozygous for the ATM gene, may be at greater risk of developing late normal tissue damage (Hall et al., 1998; Iannuzzi et al., 2002). Experimental studies have also shown an increased sensitivity to low-LET radiation manifested by decreased survival (Barlow et al., 1999) and an increase in cataract development (Worgul et al., 2002). A question remains regarding the influence of atm haplodeficiency on susceptibility to high-LET radiation damage.

Cataractogenesis was chosen as a model to test for radiation sensitivity in ATM gene deficient vs. wildtype mice. The radiation cataract model has enjoyed a long history as a means to determine radiation damage in a late responding tissue both for low- and high-LET radiations. Its amenability to non-invasive assessment over extended periods and the predictability of the pathology underscore its utility as a means to monitor radiation exposure.

2. Materials and methods

Descendants of breeding ATM knockouts kindly provided by Dr. Philip Leder (Harvard University) were used throughout the study. The genetic background of the mice is a cross of the 129SvEv and Black Swiss strains. The ATM gene was disrupted by inserting a neo cassette within an exon at a site corresponding to nucleotide number 5460 of the human *atm*. There was no presence of full length or truncated protein in the ATM knockout mice (Elson et al., 1996). The homozygous *atm* deficient mice display many characteristics associated with A-T, such as retarded growth, defective lymphocytic differentiation, neurological dysfunction and hypersensitivity to ionizing radiation. Most *atm* −/− mice develop thymic lymphomas and rarely live beyond 5 months. Also pup yield for homozygotes appear non-Mendelian ostensibly due to reduced embryo survivability. Heterozygotes on the other hand appear healthy. It is on the heterozygotes that we focused our attention since 1–3% of the human population are ATM heterozygotes and are clinically indistinguishable from the general population.

Prior to irradiation at 28 days (+/−0.5 day) postpartum the mice were genotyped as described in another report (Elson et al., 1996). Each treatment group had approximately equal numbers of males and females.

2.1. Low-LET radiation

In a previous series of experiments animals were anesthetized (5 mg/kg xylazine + 50 mg/kg ketamine) and one eye of each animal was irradiated, while the other was shielded. Shielding was accomplished by using a collimator on the X-ray machine, as well as a contoured 5 mm thick lead covering for the contralateral eye. Measurements indicate that the shielded eye receives less than 2% of the dose delivered to the treated eye. The shielded eye served as an intra-animal control. In addition mice with both eyes left un-irradiated but otherwise treated in the same manner as those exposed, served as inter-animal and group controls. A subsequent analysis showed no difference in cataract formation between the intra-animal and inter-animal controls. The treated eyes were exposed to 250 kVp X-rays at a dose-rate of 0.5 Gy/min.

2.2. High-LET radiation

Together with those of wildtypes, the eyes of AT heterozygous knockout mice were exposed to 325 mGy of 1 GeV/amu ^{56}Fe ions administered at a dose rate of 500 mGy/min at the alternating gradient synchrotron (AGS) facility of Brookhaven National Laboratory. The dose chosen yielded a fluence equivalent to an average 1 ion per nuclear area (100 μm^2). The aim was to

approach unity with the number of nuclei traversed and minimize the number of multiply hit nuclei. Ten mice constituted each group. Animals were anesthetized with ketamine/xylazine IP and placed in positional cradles to stabilize the head position during irradiation. Four animals were irradiated simultaneously. The head only of each mouse was irradiated using a trilaminar collimator to protect the mice body. The laminations consisted of 3.3 in acrylic, 2.9 in aluminum and 3.5 in of high-density polyethylene. The upstream acrylic layer slows down the primary ions with low Z material to minimize fragmentation. The aluminum layer attenuates the primary particles. The polyethylene layer attenuates low Z fragments (protons and alpha particles) arising upstream. Four equidistant 1 cm diameter apertures that are collimated are positioned in front of the beam giving a 1 cm diameter field at full width half max at the head of the mice. At this depth, the average energy and stopping power in water of the primary iron ions were approximately 1 GeV and a LET of 148 keV/μm. Contralateral eye shielding was impossible so whole head exposures were delivered. Both eyes were situated in the Bragg plateau (residual range ~15 cm). Controls consisted of irradiated wildtypes as well as unirradiated wildtypes and heterozygotes. A full description of the dosimetry methods and characterization of the 1 GeV/n Fe beam at the AGS has been published previously (Zeitlin et al., 1997).

Following both radiations each mouse was examined weekly, using conventional slit-lamp biomicroscopy as described below until the mouse died or developed a 3+ cataract stage. The animals were coded so that the radiation dose and genotype were unknown to the observers.

2.3. Cataract analyses

Cataract assessment employed a frequently used modification of the well-defined and widely used Merriam/Focht radiation cataract scoring method (Merriam and Focht, 1962). The technique involves using a conventional slit-lamp biomicroscope to follow changes in lens transparency. The earliest changes consist of vacuoles or diffuse opacities around the suture in the central posterior subcapsular region – while not pathognomonic a hallmark of radiogenic cataract onset. These are gauged as 1+. If fewer than four vacuoles are noted, a 0.5+ cataract is scored. Continued cataract development leads to progression of the posterior changes and the involvement of the anterior subepithelial region, the 2+ stage. Fewer than four vacuoles observed anteriorly are recorded as a 1.5+ cataract. A 3+ stage is scored when the anterior opacities progress and the density of the cataract posteriorly prevents the slit beam from passing into the vitreous. If however, the entire cortex is involved, yet the posterior capsule can still be discerned, a 2.5+ cataract is noted. A 4+-cataract stage is one with complete anterior opacification preventing visualization of the remainder of the lens. If the opacity has not become severe enough to prevent passage of the slit beam into the posterior region, yet detailed visualization is impossible, a 3.5+ stage is scored. The pluses after the score indicate the reality that a particular score at some given examination time reflects a cataract stage which was reached during the interval between the previous exam and the current one.

The data were analyzed using the Kaplan–Meier technique (Kaplan and Meier, 1957) to make non-parametric maximum likelihood (NPML) estimates of grade-specific cataract prevalence as a function of time after exposure. The method used successfully in previous studies is detailed elsewhere (Worgul et al., 1989, 1993, 2002; Brenner et al., 1991, 1993).

3. Results

Throughout this study the development of radiation cataracts qualitatively manifested the pathogenic sequence characteristic of radiogenic cataracts in all mammalian species including man. Following a latent period dependent on dose the initial opacities appeared in the posterior subcapsular region (posterior superficial cortex) of the lens. At a rate also correlated with dose, the posterior cortex became more involved and anterior cortical changes appeared and progressed. Those animals whose lenses reached a stage wherein the cortex was in an advanced state of opacification (3.0+) were euthanized.

Fig. 1 illustrates the development of Grade 1 cataracts following 1.0, 2.0 and 8.0 Gy of X-rays. Examining the data between +/− and +/+ it is apparent that in a dose dependent manner the heterozygotes exhibit increased sensitivity relative to their wildtype litter-mates. The highest dose (8 Gy) exhibited almost no difference (ostensibly due to a saturation effect) while the greatest difference was manifest in the lowest dose group (1 Gy). Interestingly the two unirradiated controls groups (representing heterozygotes and wildtypes) also show a disparity. The heterozygotes clearly develop "naturally occurring" cataracts earlier than do the wildtypes.

Due to experimental constraints mice were exposed to only a single dose of 325 mGy iron ions. Fig. 2(a)–(f) illustrate the results. The time required for prevalence to reach 50% (T_{50}) as an endpoint for each stage indicated that not only cataract onset but also progression were accelerated in the mice haplo-deficient for the ATM gene. For example, the T_{50} for definitive cataract onset (stage 1) in the atm heterozygotes was 10 weeks whereas 17 weeks were required for the wildtypes. Similarly, at the conclusion of the experiment (35 weeks),

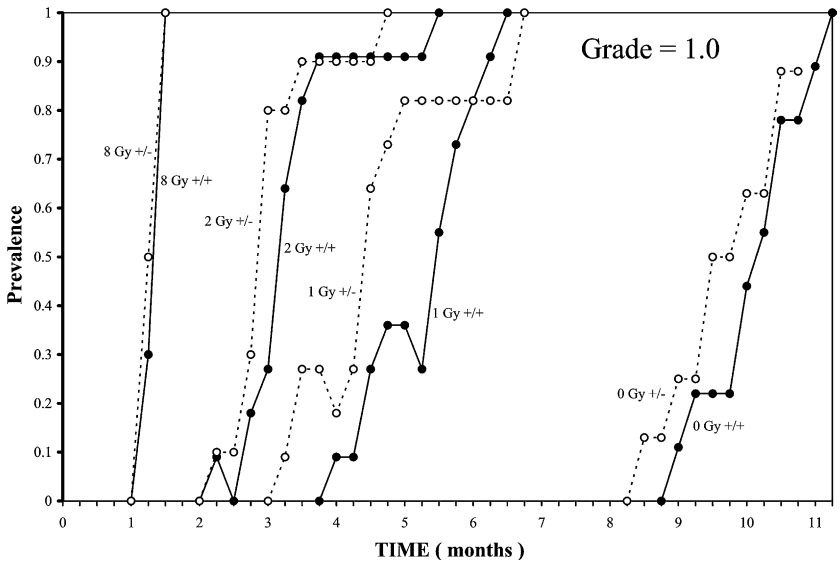

Fig. 1. Grade 1 cataract prevalence for as a function of time after exposure to 0, 1.0, 2.0, and 8.0 Gy of 250 kVp X-rays comparing wildtype mice animals with those heterozygous for the ATM gene. Note the tendency for the enhanced sensitivity to increase as the dose decreases.

40% of the lenses of allele-deficient mice had progressed to stage 3 (near fully opaque and obviously visually debilitating), while only one lens (5%) from the wildtype irradiated eyes reached that level of severity.

4. Discussion

The earlier low-LET data show unquestionably that heterozygotes for the atm gene are more sensitive to the development of radiation cataracts (Worgul et al., 2002). The expanded data from the heavy ion studies show a similar predilection. The ^{56}Fe results suggest that onset and progression of the cataract of cataract is significantly accelerated. Although a single heavy ion dose precludes generating valid RBE data comparing T_{50} values for the stage 1 low- and high-LET results there is a suggestion that the RBE for the heterozygotes may be higher than for the wildtypes. This of course deserves further investigative attention. Of additional interest is the mechanistic basis of the increased sensitivity to high-LET radiation.

According to current models, the ATM protein is a sensor protein which detects DNA double strand breaks and regulates directly multiple cell signaling pathways involved in the response to DNA damage and repair. Primary targets for the ATM protein are the p53, Chk1 and Chk2/hCds1 kinases, cAbl, RPA, RAD 51 and BRCA1, BRCA2 complex (Abraham, 2001; Khanna et al., 2001). This intimates that the increased radiosensitivity is due to failed DNA repair. Such a situation is consistent with the results of the low-LET studies but is more difficult to relate to the high-LET outcome wherein DNA repair is thought to be less efficient to begin with. The lack of sparing effect and the dose dependent existence of an inverse dose-rate effect for heavy ion cataractogenesis are thought to reside in some way in the inability for lens cells to effectively repair genomic damage. These issues are less clear-cut than at first blush. The unique energy deposition characteristics of heavy ions and the variability in nuclear size in epithelial cells may together or alone play a role in the observed sensitivity in the atm haploinsufficient animal. Our decision to try and achieve unity in the number of ions and nuclei traversed directly suffers from both ion distribution and nuclear size statistical uncertainties which can conceivably have effects unique to the fluence and the system itself. The delta ray penumbra has been implicated as a basis for the inverse dose rate effect observed in rodents following heavy ion exposure (Worgul, 1988). Perhaps the low-LET radiation also plays a role in the increased sensitivity of lens epithelial cells to heavy ion radiation.

These data assume additional significance in the light of a recent publication (Cucinotta et al., 2001) linking an increased risk of cataracts for astronauts with higher lens doses (>8 mSv) of space radiation relative to other astronauts with lower lens doses (<8 mSv). In addition, there was a significant association between cataracts and high-inclinations or lunar missions, with 35 of the 39 cases observed after space flight occurring in astronauts participating in these missions. It is speculated that the cause is the much higher flux of heavy ions associated with high inclination and lunar missions, with lower inclination missions having a large fraction of the dose from low-LET trapped protons. Although the report emphasizes nuclear cataracts the data on the prevalence of posterior subcapsular (PSC) and cortical lens

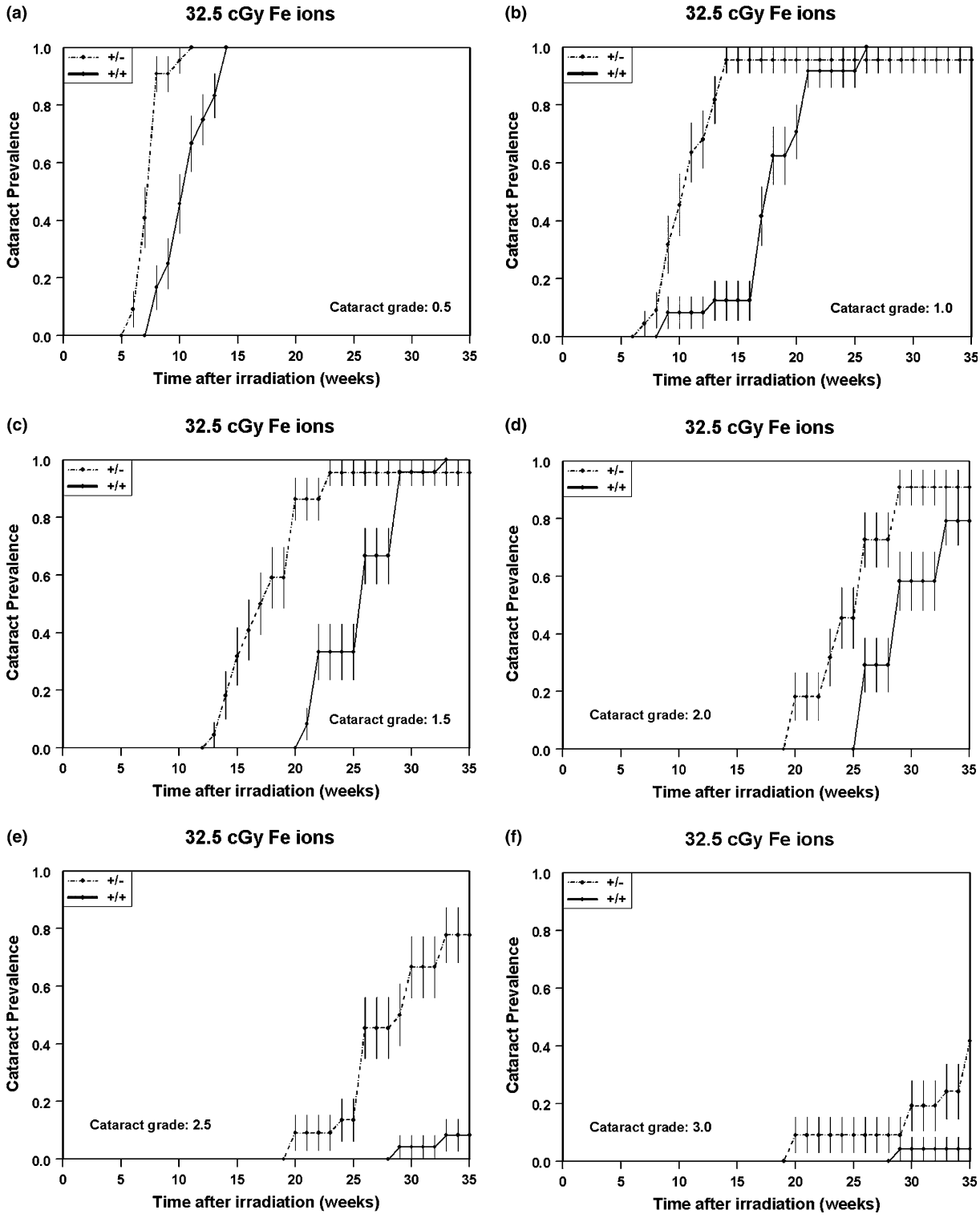

Fig. 2. (a)–(f). Prevalence for different grades of cataract cataracts in wildtype mice and in animals heterozygous for the ATM gene following a dose of 325 mGy of 1 GeV/amu ^{56}Fe ions. The heterozygous animals reach each cataract stage earlier and progress faster than wildtype animals. The vertical bars are standard errors, calculated using Greenwood's formula (13).

opacities provide more highly suggestive evidence to support a radiogenic etiology. As shown in the present ATM study and consistently throughout the history of radiation cataract research, opacification in these areas although not pathognomonic are highly, and definably, characteristic of radiation cataractogenesis. In fact, the Merriam/Focht radiation cataract scoring system is fully defined by opacities appearing in these regions (Merriam

and Focht, 1957). In the case of the astronauts, among the 295 flight personnel examined, of the 48 recorded cataracts, 40 involved opacities of the cortical and/or PSC variety. Overall, three of the 295 astronauts followed developed vision-impairing cataracts requiring surgery relatively early in life, even though the accumulated doses were quite low; it is unfortunate that the report does not indicate whether these three cases were of a type consistent with a radiation etiology, but the number possibly suggests a genetically predisposed radiosensitivity in some individuals. This position is underscored by the track record of rodent studies in predicting heavy ion effects on human cataract.

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